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Application of multivariate statistical analysis to assess browning susceptibility in sweet potatoes (*Ipomoea batatas* (l.) Lam.) cultivars, based on chemical and enzymatic determinations

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Abstract

The selection of a sweet potato cultivar for minimal processing must be performed considering the nutritional value and the lower susceptibility to browning development, which will result in greater stability of the vegetable colour. The aim of this work was to evaluate browning susceptibility in four sections of two sweet potato cultivars combining chemical and enzymatic determinations with colour variables, by applying multivariate statistical techniques. Each cultivar had a characteristic browning pattern; in white cultivar colour changes were represented by changes in variable b^* while in the red cultivar these changes responded to variations in variable a^*.The regions with major colour changes ($\Delta E^*>6$) after 24 hours also had high levels of phenolic compounds (658±98 mg chlorogenic acid/kg fresh tissue) and high oxidative enzymes activities. Principal Component Analysis indicated that three regions in white cultivar and two in red cultivar had low browning susceptibility. Partial Least Square analysis indicated that colour changes (ΔE^*) were highly associated with Polyphenoloxidase activity and phenolic compounds. By comparing both cultivars analyzed, the white cultivar presented 3 regions with low browning susceptibility and therefore would be more suitable for minimally processing. This low susceptibility would be related to low phenol content and lower enzyme activities.

Introduction

Worldwide, there are about 50 genera and over 1000 species of sweet potato (*Ipomoea batatas* (L.) Lam.) (Woolfe, 1992). Genetic selection, natural hybridizations and mutations resulted in a large number of cultivars that differ in their morphology, texture, colour of peel and pulp, nutritional quality and sensorial attributes. Within horticultural crops of economic importance, production of sweet potato ranks fifth in developing countries (FAO, 2014). Consumption of minimally processed products increased in recent years because of their convenience (Rico *et al.,* 2007). Minimally processed sweet potatoes are of interest for the consumers, because the peeling operation is avoided and the product could be fully used, however it could promote the development of enzymatic browning phenomena. Therefore the selection of cultivars that are less susceptible to browning could be an effective strategy to overcome this drawback (Moretti *et al.,* 2002).

Enzymatic browning is usually associated with the activities of the enzymes polyphenol oxidase (PPO, EC 1.14.18.1), peroxidase (POD, EC 1.11.1.7) and polyphenols, however there is not always a direct association between these variables and the development of browning (Cantos *et al.,* 2002).Polyphenols are ubiquitously distributed in plants and are considered secondary metabolites. Their synthesis is mainly regulated by the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5), which may be increased in response to tissue injury generating trans-cinnamic acid, a precursor for chlorogenic acid. In sweet potatoes phenolic compounds such as chlorogenic acid and its isomers (4,5-dicaffeoylquinic acids, 3,5-dicaffeoylquinic and 3,4-dicaffeoylquinic) have antioxidant activity (Padda and Picha, 2008; Zheng and Clifford, 2008).

Both, the content of phenolic compounds and the expression of enzymes involved on its metabolism, can vary depending on the cultivar and the section of the root tissue examined. The differential expression of enzymes directly or indirectly involved in damage repair, and the concentration of substrates in differential proportions depend on the tissue. The faster responses to cut injury (polyphenol production and increase enzymes activities) in sweet potatoes tissues taken beneath the proximal surface than the responses observed in the internal tissue layers were found (Tanaka and Uritani, 1977; Uritani, 1999). This

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could be related to the differential responsiveness of each tissue section to damage, which in turn might result in differential susceptibility to browning development. Sweet potatoes cultivars differ in the colour of its flesh and peel, which is mainly related to the contents of different pigments, such as anthocyanins and carotenoids (Padda and Picha, 2008). However, even cultivars with similar flesh colour, may differ in the content of phenolic compounds, phenolic profile and antioxidant activity (Walter and Purcell, 1979).

The selection of the cultivar for processing must be performed taking into account the nutritional value and the lower susceptibility to browning development, which will result in greater stability of the vegetable colour. For this reason, the study of the characteristics of cultivars, as well as their susceptibility to browning development, are necessary to choose a suitable material for minimal processing. Colour changes in food are instrumentally measured in CIELAB coordinates L*, a*, b* and combinations of these. There is no general consensus about the best colour variable that describes browning, particularly in the case of products with heterogeneous colour. Some authors suggest the use of ΔE*, L* or hue, as seen in apples, pears and potatoes. However, it is important to determine the variable that best fits each product to achieve a correct description of the phenomenon (Piagentini *et al.,* 2012)

Therefore, colour changes due to browning phenomenon are complex since they do not exclusively depend on one variable. Many metabolites could participate in browning development, and the interactions between these could lead to different degrees of browning susceptibility, therefore, a deep understanding of the results is the first basic step for any proper data treatment. Statistics offers a number of helpful tools, such as multivariate methods, which consider inter correlation between variables, allowing a more complete interpretation of data (Oliveri and Forina, 2012). One of the multivariate methods that is widely used for food composition analysis is Principal Component Analysis (PCA). This is an exploratory method and can be employed for feature and noise reduction purposes and constitutes the basis for other more complex pattern recognition techniques. The Partial Least Square (PLS) is another technique that generalizes and combines PCA and linear regression analysis. It is particularly useful when it is desired to predict a set of dependent variables (e.g flavor or colour changes) from a set relatively large and possibly correlated of predictor variables (e.g chemical variables), and has been applied for objective and sensory evaluation of wines (San-Juan *et al.,* 2011).

The objective of the present work was to evaluate the susceptibility to browning in different tissue sections of two sweet potatoes cultivars of the Northeast Region of Argentina. To achieve this objective the analysis of chemicals variables (PPO, POD and PAL activities and the contents of total phenolic compounds, chlorogenic acid, flavonoids, ascorbic acid and antioxidant activity) and colour changes (evaluated with the variables L*,a*, b* and ΔE^*), was conducted through univariate and multivariate statistical analysis.

Materials and Methods

Plant material

Two cultivars of sweet potatoes, called 'white' (WC) and 'red' (RC) were harvested in Chaco, Argentina between the months of April and May of 2013. Roots (5 kg of each) with no bump or bruises, uniform size and without signs of sprouting were chosen. These were washed with tap water and peeled with a sharp knife, removing the outer tissue to a depth of 2 – 4 mm from the surface, called region 1 (R1). Subsequently, the peeled sweet potatoes were immediately divided into the following sections: surface (R2) tissue (2 – 4 cm of the tissue located immediately under the peel), (R3) peeled ends (proximal and distal ends 3 cm of each) and (R4) central region (the remaining material). Surface colour changes were evaluated during 24 hours in 100 g of the material. The remaining material was immediately frozen at -20°C until analysis. Chemical and enzymatic variables were performed in triplicate.

Surface colour

Surface colour measurements were performed using a digital colorimeter CR400 Minolta (Osaka, Japan) with diffuse illumination/0° viewing angle - pulsed Xenon lamp equivalent to D65 – Temperature close to 6000 K, recording the L^*, a^* and b^* variables at room temperature. Total and individual colour differences (ΔE^*, ΔL^*, $\Delta a^*$and Δb^*) at different times were calculated by $\Delta E_x^* = ((L_x^* - L_0^*)^2 + (a_x^* - a_0^*)^2 + (b_x^* - b_0^*)^2)^{1/2} = ((\Delta L_x^*)^2 + (\Delta a_x^*)^2 + (\Delta b_x^*)^2)^{1/2}$, where L_0^*, a_0^*, b_0^* are the variables measured immediately after cutting the sweet potato (fresh sweet potato) and the subscript ´x´ indicates the time of the experiment. The determinations were carried out in 10 samples taken from each region and for samples R1 were done in the internal section. Readings were taken at the beginning of the experiment and after 1, 2, 4, 8, 12 and 24 hours of exposure to air at room temperature.

Ascorbic acid (AA)

The determination of AA was performed by high performance liquid chromatography Shimadzu LC-20 A (Tokyo, Japan) in an extract prepared with phosphoric acid filtered through a nylon membrane of 0.45 µm before the injection (Sgroppo et al., 2010). Mobile phase was acidified water (pH=2.5 with H2SO4), flow rate 0.8 mL min-1, Hypersil ODS column 250 x 4.6 mm, 5 µm particle size (Thermo Scientific, Walthman, MA, USA) and detection was performed at 260 nm with DAD/UV-visible detector Shimadzu, SPD-20MA (Tokyo, Japan). A standard curve using ascorbic acid (Sigma, USA) was prepared and the results were expressed as milligrams of AA per kilogram of fresh weight (mg AA/kg FW).

Total phenolic content

Total phenolic compounds were determined on a methanol extract following the methodology of Padda and Picha (2008) with slight modifications; 5 g of tissue were homogenized with 30 mL of 80% methanol using a manual processor. The mixture was incubated at 80°C for 20 min and then filtered through filter paper and the total phenolic content was determined using Folin Ciocalteau reagent. The absorbance was determined at 750 nm. Results were expressed as miligrams of chlorogenic acid per kilogram of fresh weight (mg chlorogenic acid/kg FW).

Chlorogenic acid

The extract was prepared as previously described for total phenols and filtrated through 0.45 µm nylon membrane before injection into HPLC Shimadzu LC-20 A (Tokyo, Japan). The mobile phase was 1% (v/v) formic acid/methanol (70:30) (pH = 2.5), with Hypersil ODS column 250 x 4.6 mm, 5 µm particle size (Thermo Scientific, Walthman, MA, USA), flow rate 0.8 mL min-1 and detection at 320 nm with UV-visible detector Shimadzu, SPD-20 MA (Tokyo, Japan). Results were expressed as milligrams chlorogenic acid per kg FW (mg chlorogenic acid/kg FW) (Ojeda *et al.,* 2014).

Flavonoid content

Five grams of tissue were homogenized with 30 mL of ethanol 96%, magnetically stirred at 4°C/10 min and filtered. The determination of total flavonoids was performed according to the method of Subhasree *et al.,* (2009). Briefly, 500 µL of ethanolic extract, 500 µL of ethanol and 25 µL of 5% Na_2NO_2 were mixed in a test tube and allowed to react for 5 min. Then 600 µL of 10% $AlCl_3$ were added and after 6 min, 500 µL of 1M NaOH were finally added; the absorbance was measured at 415 nm. The results were expressed in grams of catechin per kg of FW (g catechin/kg FW).

Antioxidant activity (AOA)

The antioxidant activity (AOA) determined with DPPH • quantifies the scavenging ability against free radical of the compounds present in a given extract. Ten grams of tissue were homogenized with 30 mL of methanol 80% (Zheng and Clifford, 2008), magnetically stirred at 4°C/10 min, filtered and centrifuged (4000 g/ 5 min). The AOA was determined by mixing 0,2 mL of properly diluted extract with 3 mL of a 0.076 mM DPPH • solution in methanol , the reaction mixture was left in the dark for two hours and the absorbance was read at 517 nm, the inactivation percentage was calculated as previously described (Ojeda *et al.,* 2014). Results were expressed in grams of chlorogenic acid equivalents per kilogram of FW (g chlorogenic acid eq/kg FW).

Crude enzyme activities

Five grams of tissue were homogenized with 20 mL of cold acetone and vacuum-filtered, the residue was left overnight in a vacuum desiccator. A sample of 0.2 g of acetone powder was resuspended in a solution of 10 mL of 0.1 M phosphate buffer (pH = 6.0) and 10 mL of 1 M NaCl 2 mM phenylmethylsulfonyl fluoride (PMSF) under magnetic stirring in ice bath for 45 min. The product was centrifuged (4000 g/10 min) and filtered; this product was named as enzyme extract.

For PPO activity determination 1 mL of enzyme extract was mixed with 1 mL of 40 mM catechol (Biopack, Argentina) in 10 mM phosphate buffer (pH = 7.0). The reaction mixture was incubated at 25°C, and absorbance was read every 15 s for 2 min at 420 nm. Enzyme activity was expressed as variation in units of absorbance, $\Delta UA420\ min^{-1}\ mg\ protein^{-1}$, where $\Delta UA\ min^{-1}$ represents variations in absorbance units per minute (Chikezie, 2006).

The peroxidase (POD) activity was quantified by mixing 1 mL of enzyme extract with 1 mL of 40 mM guaiacol (Fluka AG, Switzerland) in 50% ethanol solution and 1 mL of 25 mM hydrogen peroxide. The reaction mixture was incubated at 25°C (Fehrmann and Diamond, 1967). Readings were taken every 15 s for 5 min at 470 nm. Enzyme activity was expressed as $\Delta UA470\ min^{-1}\ mg\ protein^{-1.}$

For PAL activity, acetone powder (0.4 g) was dissolved in 20 mL of 0.1 M borate buffer (pH = 8.8) containing polyvinylpyrrolidone 1% (w/v) and 5 mM β-mercaptoethanol, with constant stirring in ice bath for 20 min (Walton and Sondheimer, 1968).

Table 1. Colour variables in 4 regions of the studied sweet potatoes cultivars

	White Cultivar (WC)				Red Cultivar (RC)			
L*	R1	R2	R3	R4	R1	R2	R3	R4
0	77.41±3.06^{b,C}	78.8±2.29^{bc,A}	73.8±4.46^{a,B}	80.18±2.12^{c,C}	77±1.29^{b,A}	76.9±3.19^{bc,A}	73.53±2.44^{a,C}	78.37±2.44^{c,A}
1	72.66±4.84^{a,B}	77.91±3.98^{b,A}	73.59±2.87^{a,A,B}	80.29±2.49^{b,BC}	78.4±2.92^{b,B}	78.39±2.46^{b,AB}	72.71±2.02^{a,BC}	79.26±2.01^{b,B}
2	72.66±4.93^{a,B}	79.21±3.4^{b,A}	72.88±3.92^{a,AB}	80.21±2.35^{b,C}	78.48±1.94^{b,B}	78.62±3.26^{b,A}	70.66±2.02^{a,BC}	80.2±2.02^{b,BC}
4	72.24±4.87^{b,B}	79.19±3.58^{c,A}	70.97±4.5^{a,A}	80.25±1.97^{c,C}	79.06±2.03^{b,B}	79.49±1.64^{b,BC}	70.35±2.35^{a,BC}	80.27±2.35^{b,BC}
8	72.52±4.31^{a,B}	78.19±3.21^{b,A}	69.99±4.92^{a,AB}	78.16±3.75^{b,AB}	79.16±2.13^{b,B}	79.9±2.15^{b,BC}	71.65±2.04^{a,AB}	80.64±2.04^{b,C}
12	72.06±3.7^{a,B}	77.8±3.71^{b,A}	72.09±4.6^{a,AB}	78.02±2.43^{b,AB}	77.32±3.27^{b,AB}	81.1±1.7^{b,C}	71.48±1.94^{a,AB}	80,69±1.94^{b,C}
24	68.33±4.81^{a,A}	78.27±2.68^{b,A}	71.39±3.37^{a,A}	76.87±2.1^{b,A}	77.06±2.37^{b,AB}	79.37±2.24^{b,ABC}	69.13±1.16^{a,A}	80,86±1.16^{b,C}
a*								
0	-2.56±0.67^{a,A}	-2.52±0.53^{b,A}	-1.98±0.726^{c,A}	-2.51±0.33^{a,A}	-2.58±0.53^{b,A}	-2.64±0.54^{b,A}	-2.07±0.63^{c,A}	-2.99±0.37^{a,A}
1	-1.78±0.77^{b,B}	-1.42±0.73^{a,B}	-0.73±0.619^{c,B}	-1.46±0.91^{a,B}	-1.96±0.9^{b,B}	-2.51±0.65^{ab,AB}	-1.26±0.77^{c,B}	-2.88±0.51^{a,AB}
2	-1.62±0.83^{a,B}	-1.51±0.54^{a,BC}	-0.87±0.484^{b,B}	-1.38±0.64^{a,B}	-1.73±0.71^{b,B}	-2.68±0.67^{a,AB}	-1.24±0.66^{c,BC}	-2.65±0.57^{a,BC}
4	-1.59±0.89^{b,B}	-1.51±0.57^{a,BC}	-0.64±0.407^{c,BC}	-1.58±0.72^{a,B}	-1.47±0.71^{b,BC}	-2.45±0.61^{ab,B}	1.05±0.92^{c,BC}	-2.54±0.51^{a,CD}
8	-1.51±0.69^{a,B}	-1.23±0.5^{a,CD}	-0.57±0.43^{b,BC}	-1.03±0.63^{a,C}	-1.18±0.67^{b,CD}	-2.25±0.54^{a,B}	1.32±0.88^{c,BC}	-2.22±0.47^{a,DE}
12	0.59±0.22^{a,B}	-0.72±0.52^{a,D}	-0.54±0.22^{b,BC}	-0.95±0.28^{a,C}	-0.83±0.53^{b,DE}	-2.19±0.3^{a,B}	2.17±0.97^{c,CD}	-2.02±0.48^{a,E}
24	1.62±0.63^{b,C}	-0.4±0.37^{a,D}	1.72±0.65^{c,C}	0.6± 0.35^{a,b,D}	1.05±0.58^{b,E}	-1.4±0.27^{a,C}	2.34±0.72^{c,D}	-1.68±0.39^{a,F}
b*								
0	24.46±2.15^{ab,B}	25.71±1.992^{b,D}	23.98±3.06^{a,C}	23.59±2.12^{a,D}	29.6±2.9^{c,D,E}	27.74±2.9^{b,D,E}	29.79±2.83^{c,D}	25.63±2.08^{a,C}
1	24.31±2.62^{a,B}	23.19±1.513^{a,C}	23.63±2.92^{a,C}	23.35±2.42^{a,D}	30.1±2.67^{c,E}	28.08±2.67^{b,E}	29.23±2.81^{bc,D}	25.69±2.77^{a,C}
2	24.46±2.34^{b,B}	22.52±1.27^{a,B,C}	22.8±2.76^{a,C}	21.83±1.91^{a,C}	28.53±2.65^{c,CD}	26.6±2.65^{ab,CD}	28.39±3.39^{bc,CD}	25.54±2.71^{a,C}
4	23.31±2.52^{b,B}	21.82±1.33^{a,B}	19.96±2.71^{a,B}	20.54±1.15^{a,B}	27.95±2.26^{b,C}	26.38±2.26^{b,C}	27.76±2.34^{b,C}	24.08±2.15^{a,B}
8	21.41±2.58^{b,A}	19.20±1.97^{a,A}	19.8±2.65^{a,A,B}	18.76±0.95^{a,A}	25.82±1.91^{c,B}	24.47±1.91^{bc,B}	25.51±2.57^{b,B}	22.21±1.76^{a,A}
12	21.31±2.29^{b,A}	18.68±2.08^{a,A}	19.75±2.54^{a,AB}	18.63±1.36^{ab,AB}	24.07±1.29^{a,A}	22.56±1.29^{a,A}	24.46±2.2^{a,A,B}	22.43±1.92^{a,A}
24	20.14±2.78^{b,A}	18.63±2.58^{ab,A}	18.58±2.62^{a,A}	18.51±0.89^{ab,A}	23.12±2.49^{a,A}	21.76±2.49^{a,A}	22,76±1.75^{a,A}	21.9±1.39^{a,A}

a,b,c Values in the same column for each colour variable followed by a different superscript are significantly different $p<0.05$(between regions for the same cultivar along time). Each value is the mean of ten replicates.
A,B,C,D Values in the same column for each variable followed by a different superscript are significantly different $p<0.05$(between regions for the same cultivar at the same time). Each value is the mean of ten replicates.

The product was centrifuged (4000 g/5 min) and the supernatant was filtered. For the determination of PAL activity, 1 mL of extract was mixed with 2 mL of 0.1 M borate buffer (pH = 8.8) and 1 mL of 100 mM L-phenylalanine (Biopack, Argentina). The reaction mixture was incubated at 37°C, and readings were taken at 0, 60, 120, 180 and 240 min. Results were expressed as ΔUA290 h^{-1} mg $protein^{-1}$.

Total protein content in the enzymatic extracts was determined by the method proposed by Lowry *et al.,* (1951) using bovine albumin as a standard.

Statistical analysis

The significant differences between the results in the four regions were evaluated, by an analysis of variance (ANOVA) followed by Tukey test with a significance level of $p<0.05$. Multiple linear regression was applied to obtain an expression of ΔL*24 as a function of all chemical variables. The relationship between chemical and colour variables was studied by multivariate analysis of the normalized variables. First, inter-relation between chemical and colour variables was evaluated with principal component analysis (PCA). Then, the partial least square (PLS) technique was applied to select the chemical variable that would better predict the colour changes. All statistical analyses were made with the software INFOSTAT 2009 (Cordoba, Argentina).

Results and Discussion

Evaluation of colour variables

Colour is a characteristic of food and is essential to its identification and quality judgment. Colour variables are characterized by having a high correlation with the visually observed colour in fruits and vegetables and were used in studies of maturation, preservation and storage. Among these variables L^* is frequently used as an indicator of browning, although some authors indicated that a^* variable would be also suitable (Piagentini *et al.,* 2012). Combinations of these, like ΔE^*, could also be used to indicate colour differences.

In WC, the internal area of R1 had an initial L^* value of 77.41 ± 3.06 which decreased significantly ($p<0.05$) during the 24 hours of the experiment. However, for RC region R1, no significant changes were evidenced in L^* variable (Table 1) as well as in R4 (central zone) and R2 (surface tissue) for both cultivars. Moreover, when analysing the values of L^* in region R3 (peeled ends) for both cultivars, a large variability without definite trends was observed during 24 hours of the experiment (Table 1). This would be related to the heterogeneity of the sample, since the proximal and distal ends differ in their phenolic content (Tanaka and Uritani 1977; Jung *et al.,* 2011).

Although L^* variable has been proposed by several authors to assess colour changes induced by browning phenomena (Piagentini *et al*., 2012), McConnell *et al.* (2005) did not find significant changes for minimally processed orange fleshed sweet potatoes. Changes in a^* variable from $a^*<0$ (green) to $a^*>0$(red), as previously mentioned, could also be used to evaluate browning. When a^* variable was measured, a significant increase was observed in

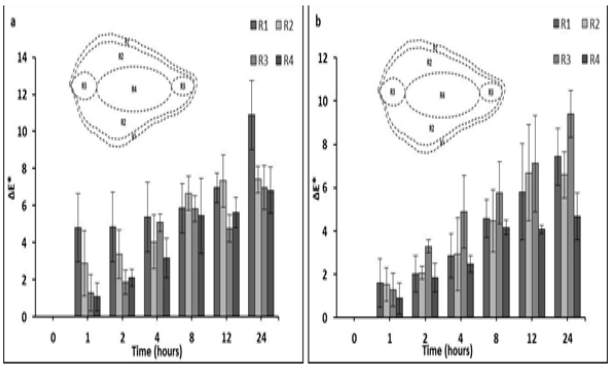

Figure 1. ΔE^* variables in four regions of two sweet potato cultivars a) white cultivar (WC) and b) red cultivar (RC). Each value is the mean of three replicates, and error bars indicate the standard deviation. A scheme of the 4 analyzed regions (R1, R2, R3 and R4) is also presented

all regions of cultivars, being particularly remarkable for regions R1 and R3 of both cultivars. The region R1 presented a significant ($p<0.05$) increase, from -2.56 ± 0.67 to 1.62 ± 0.63 in WC at the end of the experiment. Also in the RC, the values were positive after 24 hours of storage (1.05± 0.58).

The tissue of region R3 in WC had initial values of -1.98 ± 0.72 for a^* variable, and a marked increase after 12 hours of the experiment was observed. This same region in RC showed more noticeable changes from the second hour of the experiment (Table 1). Regions R2 and R4 (which represent about 80% of the root) of both cultivars also showed an increasing trend in the value of a^*, keeping negative values for both regions in the two cultivars, except for R4 after 24 hours (Table 1).

The b^* variable showed a significant decrease ($p<0.05$) for all regions in both cultivars being particularly pronounced for regions R1 and R2. Regions R2 of both cultivars showed the largest decreases in this variable after 24 hours (Table 1). The ΔE^* variable is frequently used to indicate differences between two colours and Limbo and Piergiovanni, (2006) proposed it as a quick and simple method to describe enzymatic browning in pre-peeled potatoes. One of the scales used to quantify the colour differences is: $0.2<\Delta E^*<0.5$: slight differences; $2<\Delta E^*<3$: slightly perceptible differences; $3<\Delta E^*<6$: perceptible differences; $6<\Delta E^*<12$: strong differences.

When ΔE^* was evaluated a significant increase ($p<0.05$) for all regions of both cultivars was observed. In R1 region of the WC, ΔE^* values were greater than 4 after one hour of exposition to air and gradually increased to values greater than 10 at the end of the experiment (Figure 1a). However, in R1 region of the RC, ΔE^* values were originally lower than 2 (Figure 1b) and increased up to values higher than 6, indicating the presence of strong colour differences when compared to the original colour. During the first two hours of the experiment regions R3 and R4 of WC, had ΔE^* values < 2 and subsequently had a significant increase ($p<0.05$), reaching a value of almost 6 at the eighth hour of the experiment without further changes ($p>0.05$). The surface region (R2) had ΔE^* values slightly higher during the first 8 hours (Figure 1a). In RC the results for regions R2 and R4 were similar to those found in WC. A gradual increase in the variable ΔE^* was observed for regions R2 and R4 during the first eight hours of the experiment, reaching values close to 6. The region R3 of this cultivar showed a significant increase ($p<0.05$) from the start of the experiment, reaching $\Delta E^*> 8$ at the end (Figure 1b). According to colour changes in different regions of the sweet potato, it was observed that in WC, three of the regions (R2, R3 and R4) reached ΔE^* values in the range corresponding to "detectable colour differences" after 24 hours, meanwhile in the first few hours after cutting they were only "slightly perceptible". Regions R2 and R4 of RC had similar values of ΔE^* as WC.

Crude enzyme activities

Several studies pointed out that PPO is a key enzyme in the development of enzymatic browning of fruits and vegetables (Severini *et al.,* 2003). On the other hand, Walter and Purcell, (1980) had previously reported that the activity of this enzyme would not be critical in the development of browning in sweet potatoes, and the latter would be more related to the initial phenolic content. In the present work the maximum value for the PPO activity (Table 2) was found in region R1 of WC being nearly 2.5 times higher than that found in RC. The values found in regions R2 and R3 of WC were significantly lower ($p<0.05$) than those in the peel (R1) of this cultivar (Table 2), while regions R1, R2 and R3 of RC showed similar values. The region R4 in both cultivars had similar values for this enzyme activity. It is noteworthy that PPO activity in WC was lower than that found in RC for all regions except for the region of the peel (R1).

Some authors suggested that POD enzyme could also be involved in enzymatic browning, particularly in plants having high activity of this enzyme (Aquino-Bolaños and Mercado-Silva, 2004). POD expression is relatively high in the periderm, since this enzyme is involved in tissue repair through lignin synthesis (Uritani, 1999). In this work for both cultivars studied, POD activity was much higher than that of PPO for all regions (Table 2). POD activities in the peel region (R1) showed values almost 10 times higher than

Table 2. Chemical and enzymatic determinations in 4 regions of the studied sweet potatoes cultivars

Cultivar/ Region	Total Phenolics (g/kg)	Cholorogenic Acid (mg/kg)	Flavonoids (g/kg)	AOA (g/kg)	AA (mg/kg)	POD (ΔUA_{470} /min mg protein)	PPO (ΔUA_{420} /min mg protein)	PAL (ΔUA_{290} /h mg protein)
White Cultivar								
R1	2.02 ± 0.29^{b,A}	560 ± 75.9^{c,A}	1.13 ± 0.3^{b,A}	11.61 ± 2.26^{b,A}	0.41 ± 0.03^{a,A}	32.24 ± 2.95^{c,B}	0.335 ± 0.066^{b,B}	0.211 ± 0.012^{c,B}
R2	0.46 ± 0.06^{a,A}	30.56 ± 2.89^{a,A}	0.12 ± 0.018^{a,A}	1.11 ± 0.09^{a,A}	0.3 ± 0.04^{a,A}	1.37 ± 0.44^{ab,A}	0.068 ± 0.010^{a,A}	0.069 ± 0.008^{a,B}
R3	0.63 ±0.16^{a,A}	46.05 ± 4.65^{a,A}	0.16 ± 0.04^{a,A}	1.31 ± 0.16^{a,A}	0.21 ±0.01^{a,A}	1.86 ± 0.62^{b,A}	0.067 ± 0.016^{a,A}	0.116 ± 0.020^{b,B}
R4	0.43 ± 0.02^{a,A}	77.07 ± 7.36^{b,A}	0.12 ± 0.01^{a,A}	0.98 ± 0.07^{a,A}	14.15 ± 2.82^{b,A}	3.26 ± 0.47^{b,A}	0.082 ± 0.025^{a,A}	0.073 ± 0.018^{a,A}
Red Cultivar								
R1	2.49 ± 0.49^{c,A}	756 ± 120^{d,B}	1.35 ±0.32^{c,A}	12.53 ± 2.54^{c,A}	3.28 ± 1.67^{b,B}	23.76 ± 5.92^{c,A}	0.135 ± 0.018^{b,A}	0.185 ± 0.005^{d,A}
R2	0.77 ± 0.14^{a,B}	206 ± 18.5^{c,B}	0.3 ± 0.09^{a,B}	3.95 ± 0.95^{a,B}	1.21 ± 0.27^{a,B}	2.12 ± 0.67^{a,A}	0.127 ± 0.035^{b,B}	0.045 ± 0.003^{a,A}
R3	1.97 ± 0.33^{b,B}	95.8 ± 5.12^{a,B}	1 ± 0.27^{b,B}	8.58 ± 0.38^{b,B}	1.22 ± 0.33^{a,B}	2.89 ± 0.69^{a,B}	0.178 ± 0.021^{b,c,B}	0.083 ± 0.007^{c,A}
R4	0.83 ± 0.09^{a,B}	139 ± 14.1^{b,B}	0.29 ± 0.02^{a,B}	3.22 ± 0.32^{a,B}	22.27 ± 5.21^{c,B}	3.67 ± 0.66^{b,A}	0.092 ± 0.006^{b,A}	0.053 ± 0.007^{b,A}

^{a,b,c} Values in the same column for each variable followed by a different superscript are significantly different $p<0.05$ (between regions for the same cultivar). Each value is the mean of three replicates.
^{A,B} Values in the same column for each variable followed by a different superscript are significantly different $p<0.05$ (between cultivars for the same region). Each value is the mean of three replicates.
AOA: antioxidant activity; PPO: Polyphenoloxidase; POD: Peroxidase; PAL: Peroxidase; AA: Ascorbic acid.

in other regions, being significantly higher for WC ($p<0.05$) than RC (Table 2), as Castillo-León et al., (2002) found in sweet potatoes produced in Sweden. The central region (R4) of both cultivars had values almost two times higher than those found in R3 and R2 (Table 2).

Another enzyme that could be related to the phenomenon of browning is PAL, however its participation would be indirectly. This enzyme has a cytoplasmic localization and initiates the biosynthetic pathway of monolignols by deamination of L-phenylalanine to trans-cynamic acid, a precursor of various phenylpropanoids such as lignins, coumarins, flavonoids, furanocoumarins, etc. In sweet potatoes, this pathway leads to the production of chlorogenic acid, one of the main substrates for PPO enzyme (Padda and Picha, 2008; Zheng and Clifford, 2008).

Maximum values for PAL activity were found in the R1 region, being 0.211 ± 0.012 ΔUA290 h^{-1} mg $protein^{-1}$ for WC and 0.185 ± 0.005 ΔUA290 h^{-1}mg $protein^{-1}$ in RC (Table 2). This could be associated with the fact that the periderm of sweet potatoes is a tissue with relatively high baseline levels of PAL and other enzymes related with phenolic metabolism (Tanaka and Uritani, 1977), besides of being adapted to actively respond to mechanical damage (McClure, 1960). In the remaining regions, WC showed higher values of enzyme activity than those found in RC. Region R3 of both cultivars had slightly higher activities than those in regions R2 y R4, and this may be related to an increased activity of phenolic metabolism (Walter and Schadel, 1981).

Phenolic compounds

The phenolic compounds, chlorogenic acid, caffeic acid and cafeilamyde, could be used as substrates by enzymes PPO and POD. These compounds are major substrates of PPO enzyme in sweet potatoes and their oxidized derivates could form quinones, which react with other molecules to give brown-coloured polymers (Marshall *et al.,* 2000). The analysis of phenolic composition in different regions of the root tissue indicated maximum values of phenolic compounds in region R1 of both cultivars. The WC had phenolic contents significantly ($p<0.05$) lower than those in RC for all regions. The lowest value was found in region R2 of RC (0.77 ± 0.14 g chlorogenic acid/kg FW), while the maximum value was found in the peel (R1) (2.49 ± 0.49 g chlorogenic acid/kg FW) of RC (Table 2). Son et al. (1991) reported that in the periderm (3 mm from the surface) of five sweet potatoes the phenolic compounds accounted for 0.92% of the fresh weight, a value three times higher than the observed in internal sections.

Flavonoids in sweet potatoes, flavones in WC and anthocyanins in RC, are characterized by a high antioxidant potential. The interest in these compounds is due to the protective effect that they might have against different diseases. No significant differences ($p>0.05$) in the flavonoids content of region R1 of both cultivars were found. In the other regions studied, WC had lower values than RC ($p <0.05$), showing a similar pattern ($r=0.96$) to that observed for phenolic compounds content. Red cultivar ends (R3) had a value of 1 ± 0.27 g catechin/kg FW, which was more than four times higher than regions R2 and R4 (Table 2).

In sweet potatoes it has been reported the presence of chlorogenic acid and some derivates such as quinic acid esters with one or four residues of trans-cinnamic acids (caffeic, ferulic and p-coumaric

acid) (Zheng and Clifford, 2008). The chlorogenic acid content had a similar pattern to that observed in phenolic compounds for all regions of both cultivars. In WC the maximum value was found in region R1 and the minimum value in regions R2 and R3 (Table 2).

In RC the maximum values were also found in region R1 and the minimum values at the ends (R3). This might indicate that in the end region (R3) browning potential would be associated with other phenolic compounds in addition to chlorogenic acid. The average concentration of chlorogenic acid in regions R2 and R4 was 172 ± 16.3 mg chlorogenic acid/ kg FW. Other authors indicated chlorogenic acid levels in a range of 25.9 to 422.4 µg g^{-1} dry weight (Padda and Picha, 2008; Rautenbach *et al.*, 2010).

Ascorbic acid (AA)

Ascorbic acid is recognized as an important antioxidant compound in certain fruits and vegetables, however; in some sweet potatoes cultivars its participation in antioxidant activity might be low, due to the high antioxidant activity of the previously mentioned phenolic compounds. The presence of AA in the vegetable tissue might have a role in browning due to its reducing effect on the quinones. In sweet potatoes AA content is dependent on the cultivar, so while RC could have on average 20 mg ascorbic acid/ kg FW (FAO, 2014; Sgroppo *et al.,* 2010); WC has a relatively low content (Ojeda *et al.,* 2014). The AA contents were always more than four times higher in RC than in WC and the central region R4 of RC had values almost twice higher (Table 2). The values were similar to those reported by other authors for Caribbean (Aina *et al.,* 2009) and South African (Rautenbach *et al.,* 2010) cultivars.

Antioxidant activity

The antioxidant activity (AOA) determined with DPPH• reagent quantifies the uptake capacity of free radical by compounds present in a given extract (methanolic, ethanolic, aqueous, etc.). It should be noted that the DPPH• do not react with flavonoids lacking hydroxyl groups in B ring, or with aromatic acids containing a single hydroxyl group (Roginsky and Lissi, 2005). The AOA values, showed a strong correlation with the content of phenols and flavonoids (r=0.98). In all cases, the AOA values found in WC were lower than in RC. However, there were no significant differences in these values in region R1 between cultivars. Region R3 in RC had AOA values almost seven times higher than those found in WC. The regions R4 and R2 of RC showed values almost three times higher than those found in WC (Table 2).

Analysis of the chemical and colour variables to evaluate browning susceptibility

Chlorogenic acid content showed strong correlation among POD (r=0.92) and PAL (r=0.90) activities, being lower for PPO (r=0.64), while correlation values (r) between total phenolic compounds and enzyme activities were between r=0.78 and r=0.70, being this correlation lower for PPO. This would be consistent with reports of other authors who propose that PPO activity is not limiting for the development of browning in some vegetables (Walter and Purcell, 1980; Cantos *et al.,* 2002) Correlation coefficients 'r' for flavonoids and the three enzymes were ≥0.80.

In the tested sweet potatoes cultivars, the antioxidant activity was mainly due to the content of total flavonoids (r=0.98) and in a lower proportion to chlorogenic acid (r=0.79). Both compounds were evaluated as total phenols, which have a recognized antioxidant activity.

The colour variable differences were calculated as follows: ΔL*24= (L*24 – L*0); Δa*24=(a*24 – a*0) and Δb*24=(b*24 – b*0).

When multiple linear regression analysis for ΔL*24was made with all chemical variables in regions R2, R3 and R4 of WC, the following expression was found:

ΔL*24= 18.04 + 5.25 AA -36.93 Chlorogenic acid + 13.16 Flavonoids - 5.6 Total phenolics + 77.64 AOA + 4.11 PAL – 4.63 PPO + 6.12 POD with r2=0.88.

It can be noted that changes in ΔL*24 were highly dependent on chlorogenic acid content and antioxidant activity, so the values of those variables could be used to evaluate the susceptibility to browning. Principal component analysis (PCA) and partial least squares regression (PLS) were applied to evaluate possible interactions among phenolic compounds, oxidative (PPO and POD) and synthesis (PAL) related enzymes and the other variables such as antioxidant activity, ascorbic acid content and colour variables.

In WC, PCA of colour variables indicated that the greatest variations in ΔE*24 which mainly respond to changes in Δb*24, appeared in region R1. The variable ΔL*24 was placed opposite to the mentioned variables (because it decreased during browning) and the region R2 had the largest changes. It is noteworthy that the vectors of all variables had a modulus >1, suggesting a good explanation of the experimental variation of these variables in the two main components considered.

When PCA was performed for chemical variables in WC these were grouped on the right side of the graph indicating a positive correlation between these variables, except for the ascorbic acid content their vectors modules have greater variations in the PC1 axis. On the right side of the graph it was also located in region R1, which is consistent with the fact that these variables had maximum values in R1. This region also presented considerably browning signs by visual evaluation. The AA content showed greater variation in PC2 axis and its contents was important in the inner region (R4).

When PCA was performed with both, chemical and colour variables, it was observed that on the right side of the graph the chemical variables (except AA) and ΔE*24 were highly correlated (r> 0.95) and region R1 was located near them. The first component explained 83.4% of variability, while the second explained 11.7%. On the left side of the graph were located AA content and ΔL*24, as well as regions R2, R3 and R4. As can be noted in Figure 2a, the region R1 has different characteristics than the others thus it should be treated separately. Moreover, when considering the suitability for minimal processing of this cultivar in terms of browning susceptibility, the analysis should be assessed in regions R2, R3 and R4.

PLS analysis was applied, and the obtained results were similar to those found in the PCA confirming the idea that to assess colour changes the most suitable colour variable would be ΔL*24, which was strongly associated to phenolic compounds content compounds and enzyme activities (Figure 2b). The multiple regression analysis of the variable ΔL* in RC was carried out considering all chemical variables for regions R2 and R4, resulting the following expression:

ΔL*24= 1.95 – 0.04 AA + 0.11 Chlorogenic acid - 0.10 Flavonoids + 1.21 Total phenolic -0.13 AOA + 0.95 PAL – 0.006 PPO - 0.34 POD with r2 = 0.46. This r2 indicates that browning susceptibility should be studied by other techniques in these two regions. However, it is noticeable that the total phenolic compounds and PAL activity were the variables with more effect on the equation. By applying PCA to the colour variables, results indicate that the greater variations in the variable ΔE*24, which mainly respond to changes in Δa*24, were presented in the regions R1 and R3. The ΔL*24 variable was placed opposite to the mentioned variables and R4 region had the main changes. The vectors of all evaluated variables had moduli >1, suggesting a good explanation of the experimental variance of these variables. The PCA for all the chemical variables

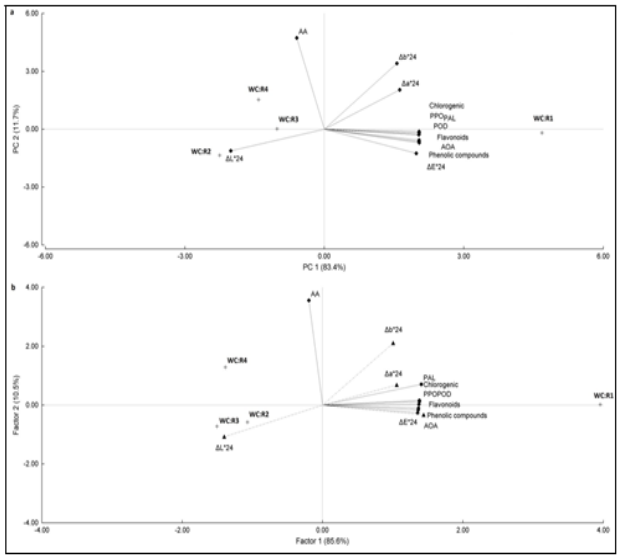

Figure 2. Chemical and colour variables in white cultivar a) PCA plot b) PLS plot

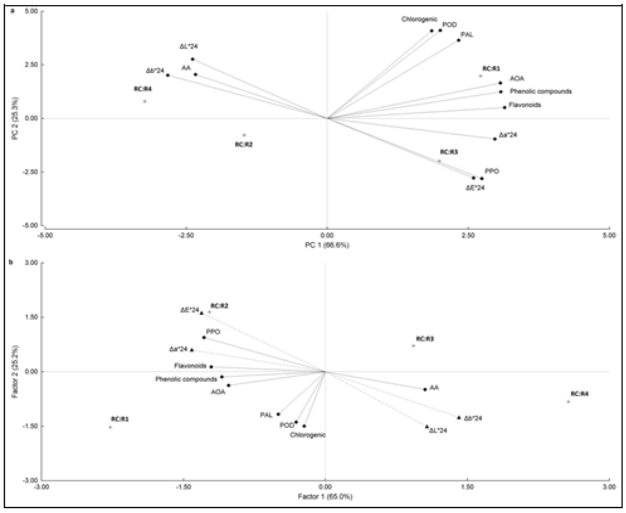

Figure 3. Chemical and colour variables in red cultivar. a). PCA plot b)PLS plot

were grouped very closely, indicating a positive correlation between them, except for ascorbic acid content and PPO activity. The moduli of their vectors have greater variations in the PC1 axis, while the vectors for AA and PPO showed considerable variations in PC2. These two components explained 93.9% of the variance.

On the right side of the graph R1 and R3 regions were located, in coincidence with the fact that the variables showed maximum values in these regions. Both regions had marked signs of browning when visually evaluated. In the PCA plot with both, chemical and colour variables, all the chemical variables (except for AA) were located at the right side of the graph, where ΔE*24 was also located. The later was mainly correlated with PPO activity (r=0.96). Furthermore, R3 region was the one with larger variations for these two variables. R2 and R4

regions were located on the left side of the graph; in these major changes in ΔL^*24 and Δb^*24 variables were observed, being also the regions with the highest values of AA. The Δb^*24 variable was significantly correlated (r=0.95) with the content of AA then, the latter could be used as a predictor of susceptibility to browning (Figure 3a).

Regions R1 and R3 had different characteristics to that of R2 and R4, thus in order to predict browning susceptibility, R1 and R3 should be excluded for proper analysis of the other regions. The PC1 explained 66.6% of variance, while the PC2 explained 25.3% of it (Figure 3a).

The PLS analysis was performed in regions R2 and R4. For region R4 the variables that best described colour changes were ΔE^*24 and Δb^*24, whose values could be associated with the activity of the enzyme PPO. Since the variable ΔE^*24 had a strong negative correlation with ΔL^*24 (r=-0.91), its results could be inferred from the latter (Figure 3b).

Conclusion

Browning development in different regions of two cultivars of sweet potatoes (WC and RC) was dependent on the cultivar indicating a differential susceptibility. Each cultivar had a characteristic browning pattern; in white cultivar colour changes were represented by changes in variable b* while in the red cultivar these changes responded to variations in variable a*. The expression obtained by multiple regression explained the colour changes in terms of the chemical variables analysed, having a better fit for the WC.

The different behaviour of the tested cultivars can be related to the content of phenolic compounds and the activities of the enzymes found in each of the analysed regions. Multivariate analysis techniques allowed to establish correlations between chemical and colour variables, thereby reducing the number of variables to be evaluated for studying the phenomena of browning. By comparing both analysed cultivars, the white cultivar presented three regions with low browning susceptibility and therefore would be more suitable for minimally processing. This low susceptibility would be related to low phenol content and lower enzyme activities.

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