ANTI-BROWNING EFFECT OF THE COMBINATION OF ASCORBIC, CITRIC AND TARTARIC ACIDS ON THE QUALITY OF MINIMALLY PROCESSED YACÓN (*SMALLANTHUS SONCHIFOLIUS*)

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Yacón (*Smallanthus sonchifolius*) presents significant amounts of phenolic compounds and peroxidases, turning the roots very susceptible to enzymatic browning reaction and related losses. To limit it, this study evaluated the efficiency of the anti-browning agents ascorbic, citric and tartaric acids in combination, with controlling these reactions, preservation of phenolic compounds and peculiar sensory characteristics. Mixture design was used, with 13 assays and 5 replicates of the center point; the final concentration of each mixture was 0.2 mol. L^{-1}. Sensory (descriptive) and physicochemical (total phenolic content, peroxidase activity, color and texture profile) analysis were performed periodically. Sensory analysis indicated that minimally processed yacón treated with anti-browning agents did not present variation ($p>0.05$) on color, yacón flavor, sweet taste, sweet aroma and moisture appearance. For acid taste, acid aroma and astringency, the greater the presence of citric acid, the greater the perception. Brightness and yacón aroma had higher scores in the presence of tartaric acid. For physicochemical analysis, the presence of the agents, independent of concentration allowed to preserve the phenolic compounds, control peroxidase activity and therefore to maintain the light color of the root for 17 days. Regarding instrumental texture, there was no alteration ($p>0.05$) during the 17 days of storage. Thus, it is concluded that the use of acids isolated or in mixture presented the same efficiency on controlling enzymatic browning; however, to obtain minimally processed yacón with more attributes similar to fresh yacón, a lower concentration of citric acid can be used.

KEYWORDS: FRESH-CUT VEGETABLES; SENSORY ANALYSIS; ENZYMATIC BROWNING; NON-THERMAL PRESERVATION TECHNOLOGIES; PHENOLIC COMPOUND.

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1. INTRODUCTION

Yacón (*Smallanthus sonchifolius*) root presents a significant amount of phenolic compounds with antioxidant potential, chelating ability and that modulate the activity of several enzyme systems, mainly to act as elements in the diet, which promote health against chemical and physical stressors factors for the organism (Cheynier 2012, Vasconcelos e al. 2015). Among them, chlorogenic acid, caffeic acid derivatives, and altraric acid were isolated in yacón (Pereira et al. 2016).

Other components in significant quantity in the root are oxidative enzymes from the group of polyphenol oxidase (PPO), particularly peroxidases (POD). The presence of both enzymes, as well as the phenolic compounds (reaction substrate), turns yacón roots quite susceptible to enzymatic browning reaction and, consequently, related nutritional and sensory losses (López-López et al. 2013; Pereira et al. 2013).

During minimal processing, the number of phenolic compounds can be drastically reduced by oxidative enzymes that catalyze the oxidation of these compounds to quinones, which in turn polymerize to form dark-colored pigments, called melanoidins (Jiang et al. 2016). To limit the root oxidation phenomenon, various chemical treatments are used. They differ by their action depending on the chemicals used: antioxidant agent, chelating agent, firming agent and acidifying agent (Ioannou and Ghoul 2013).

According to previous studies of minimally processed yacón, were found the use of anti-browning agents with different forms of action, separately employed, such as sodium bisulfite, ethylenediaminetetraacetic acid (EDTA), sodium metabisulfite, cysteine hydrochloride, ascorbic acid, citric acid, tartaric acid and others (Reis et al. 2011; Pereira et al. 2013; Rodrigues et al. 2013; Romero et al. 2014; Vasconcelos et al. 2015). However, ascorbic, citric and tartaric acids appear to be more efficient in promoting control of enzymatic reactions, with the maintenance of the root natural color during storage, without affecting negatively their sensory attributes, suggesting that these agents combine technological and sensory characteristics towards a qualitatively better product.

Citric and ascorbic acids possess the ability to reduce quinones formed by the action of oxidases preventing, therefore, the formation of dark pigments, and can act as inhibitors of oxidative enzymes, by lowering pH, and sequestering the ion in the oxidase prosthetic group (Robles-Sánchez et al. 2013). It is known that these acids work in synergy to preserve the color also enhancing other antioxidants. Also, the use of ascorbic acid as an antioxidant is completely safe for human consumption, cheap, well accepted by consumers and can increase the content of vitamin C (Saba and Sogvar, 2016). Regarding tartaric acid, the references addressing the mechanism of action and effect of this agent in the oxidative reactions are insufficient.

Therefore, considering the results presented by ascorbic, citric and tartaric acids in maintaining the physicochemical and sensory quality of minimally processed yacón, this study intended to evaluate the effect of these anti-browning agents combined, to control the oxidative reactions and, consequently, to preserve the phenolic compounds and its sensory characteristics.

2. MATERIALS AND METHODS

2.1 MINIMAL PROCESSING OF YACÓN

The yacón roots used as raw material, approximately 50 kg, came from CEASA (Belo Horizonte, MG). Anti-browning agents - ascorbic acid, citric acid and tartaric acid - were all from Sigma Aldrich® and polyethylene packaging impermeable to gases was obtained in the local trade. Ascorbic, citric and tartaric acids and their concentrations were defined based on a previous study (Vasconcelos et al. 2015).

Firstly, only roots without physical injuries were selected and cleansed with abundant water at room temperature (≈ 20 ºC). After this, they were manually peeled and cut into cylindrical slices with a thickness of approximately 1.5 cm. The slices were immersed in an aqueous solution of Sodium Dichloro-s-Triazinetrione (Sumaveg, Suma®; 200 mg. L^{-1}) for 15 minutes, and then drained on perforated trays. Next, the slices were immersed in an aqueous solution of Sumaveg, 3 mg. L^{-1}, for 15 minutes, and drained on perforated trays. Approximately 50 yacón slices were immersed in 1 liter of water containing 0.2 mol of anti-browning agent or mixture of agents, for 5 minutes, then removed and drained (2 minutes) again. The slices were packed in polyethylene bags impermeable to gases, without changing the atmosphere, and then stored at 5.5 ± 0.3 °C (Frost Free Metalfrio, model VB995, Germany). All immersion steps were performed on an ice bath (3 ± 0.2 °C).

2.2 EXPERIMENTAL DESIGN

Mixture design was used to evaluate the effect of ascorbic acid, citric acid and tartaric acid to control enzymatic browning of minimally processed yacón. The amount of acid added was 3% relative to the volume of water used for soaking the roots. Thus, taking tartaric acid as a basis for calculating the lowest molecular weight, the final concentration of each solution was 0.2 mol. L^{-1}, whether it comprises only acid or a mixture. The mixture design for three components totalized 13 assays, and the central point (assay 13), repeated 5 times as shown in Table 1

TABLE 1. MIXTURE DESIGN OF ASCORBIC, CITRIC AND TARTARIC ACIDS.

Assay	Coded			Concentration (mol. L^{-1})		
	Citric	Ascorbic	Tartaric	Citric	Ascorbic	Tartaric
1	1.00	0.00	0.00	0.200	0.000	0.000
2	0.00	1.00	0.00	0.000	0.200	0.000
3	0.00	0.00	1.00	0.000	0.000	0.200
4	0.50	0.50	0.00	0.100	0.100	0.000
5	0.50	0.00	0.50	0.100	0.000	0.100
6	0.00	0.50	0.50	0.000	0.100	0.100
7	0.33	0.67	0.00	0.067	0.133	0.000
8	0.33	0.00	0.67	0.067	0.000	0.133
9	0.00	0.33	0.67	0.000	0.067	0.133
10	0.00	0.67	0.33	0.000	0.133	0.067
11	0.67	0.33	0.00	0.133	0.067	0.000
12	0.67	0.00	0.33	0.133	0.000	0.067
13	0.33	0.33	0.33	0.067	0.067	0.067

Slices of minimally processed yacón were evaluated sensory and physiochemically. In the descriptive sensory evaluation, carried out at time 0, i.e., immediately after processing, a randomized block design with five replications was used, referring to the central point of the mixture, with the blocks represented by assessors.

The physicochemical determinations were carried out at time 0 and 3, 6, 9, 12, 15 and 17 days of storage, as defined by Vasconcelos et al. (2015). The results were submitted to analysis of variance (ANOVA), according to the experimental mixture model with 5 repetitions at the central point.

2.3 DESCRIPTIVE SENSORY ANALYSIS OF MINIMALLY PROCESSED YACÓN

Initially, the Federal University of Viçosa students were recruited for descriptive sensory analysis, through appropriate questionnaires to verify good health, time availability, ability to work with unstructured scales and familiarity with sensory terms. All steps occurred at the Sensory Analysis Laboratory of Federal University of Viçosa - UFV, where facilities include individual cabins with lighting control. The Federal University of Viçosa's Ethics Committee of Research with Human

Beings (number 452,899) authorized this project.

The selection of consumers happened using a Brazilian cheese type “requeijão cremoso” (Viçosa®), diluted with 10% of skim milk as used in Minim et al. (2010). In the selection, the assessors underwent a series of four triangular tests, in order to verify the ability of discrimination of the samples. Eleven assessors were chosen, with the percentage of success equal or greater than 75%.

The selected assessors underwent several training sessions using the list of attributes defined in Vasconcelos et al. (2015) and the same reference material from the unstructured scale, except the characteristic sensory attributes of anti-browning with sulfur (cysteine and sodium metabisulfite).

Descriptive terms and references used by the sensory team are described in Table 2.

TABLE 2. TERMINOLOGY USED IN THE SENSORY DESCRIPTIVE EVALUATION OF MINIMALLY PROCESSED YACÓN

Terms	Definition	Reference
Color	Tonality of yellow color ranging from light yellow to dark yellow	0 cm = lemon juice Tang® 4 %; 7.5 cm = pineapple juice Tang® 4 %; 15 cm = orange juice Tang® 4 %.
Brightness	Brightness intensity on the sample surface	0 cm = yacón fresh dry in paper; 15 cm = yacón fresh after 30 min immersed in water.
Sweet taste	Sweet stimulated by sugars such as sucrose, fructose and glucose	Sucrose solution: 2 cm = 2 %; 5 cm = 5 %; 10 cm de se as de 15 cm= 10 %.
Acid taste	Sweet stimulated by acids such as citric acid, malic acid and others	Latic acid solution: 2 cm = 0.05 %; 5 cm = 0.08 %; 15 cm = 0.15 %.
Astringency	Substance that produces the feeling of “tie the mouth”	5 cm = 2 sachet of Boldo /200 mL boiled water; 15 cm = 0.33 % grape juice Bela Ischia®.
Yacón flavor	Characteristic flavor of yacón	15 cm = yacón fresh.
Yacón aroma	Characteristic aroma of yacón	15 cm = yacón fresh.
Sweet aroma	Aroma characteristic of the presence of sugars	0 cm = water; 15 cm = saturated solution of solution.
Acid aroma	Aroma characteristic of the presence of acids	0 cm = water; 15 cm = 2 % lemon juice Tang®.
Apparent moisture	Force and sound to which the sample breaks	3 cm = carrot; 7.5 cm = apple.

The validation of assessors consisted of an analysis of minimally processed yacón in a randomized block design with three replications (Cochran and Cox 1981). Untreated yacón slices (control) were evaluated and treated with ascorbic acid, which was presented in the same session, in three replications. Attributes of more contrasting characteristics between the samples analyzed were evaluated: color, brightness, acid taste, acid aroma and astringency. The assessors used an evaluation form containing descriptive terms and an unstructured scale of 15 centimeters. The results were submitted to analysis of variance (ANOVA) with two sources of variation (assay and repetition) for the data of each assessor in each descriptive term evaluated.

Those who presented good discriminatory power ($pF_{assay} \leq 0.50$) and repeatability of results ($pF_{repetition} \geq 0.05$) in all descriptive terms evaluated, according to the methodology proposed by Damasio and Costell (1991), were eligible for the final evaluation of the samples.

The evaluation of the samples from yacón roots treated with the mixture of acidic agents occurred only at time 0, i.e. immediately after processing. Each assessor received the samples in disposable plates, coded with three-digit random numbers. The presentation was also random, and in each session, the assessor evaluates a maximum of 3 samples. The assessors received a glass of water at room temperature (20 ± 2 °C) and the evaluation form with the descriptive terms and unstructured scale of 15 cm along with the samples.

2.4 PHYSICAL-CHEMICAL ANALYSIS OF MINIMALLY PROCESSED YACÓN

2.4.1 Total Phenolic Compounds in Minimally Processed Yacón

Yacón extracts containing phenolic compounds were obtained as described by Bloor (2001), with a few adaptations. The analyses to quantify such compounds were performed according to the methodology proposed by Singleton et al. (1999). For obtaining the phenolic extract, 1 g of the minimally processed yacón slices was mechanically homogenized in Ultra Turrax IKA T18 basic, 18,000 revolutions/min, with 10 mL of methanol: water (60:40 v/v) solution and, next, the mixture was centrifuged at 7,000×g for 10 min (Hitachi, model himac CR21, Japan). The supernatant was transferred to test tubes, and the remaining volume completed with distilled water to obtain a concentration of pulp ≈ 0.066 g mL^{-1}. One milliliter of this extract was collected and added to 1 mL of Folin-Ciocalteu reagent from Sigma Aldrich® (USA) (diluted 5 times in water) and 1 mL sodium carbonate solution (7.5 g.100 mL^{-1}) and then left to react for 30 min at room temperature (≈23 °C). After this, the absorbance at 765 nm was read (spectrophotometer Shimadzu UV–vis, Kyoto, Japan). A calibration curve using gallic acid standard (six concentrations varying from 0 to 500 mg L^{-1}) was elaborated. Results were expressed in mg of gallic acid equivalents (GAE) per g of yacón.

2.4.2 Peroxidase (POD) Activity in Minimally Processed Yacón

Peroxidase activity assessment was done based on the method devised by Kar and Misha (1976). To obtain the crude extract for peroxidase quantification, 1 g of minimally processed yacón was added to 10 mL of potassium phosphate buffer (0.1 M; pH 6.8) also containing 0.1 mM EDTA, 1 mM of phenylmethanesulfonyl fluoride (PMSF) and 1 % of polyvinylpolypyrrolidone (PVPP - w/v). This mixture was mechanically homogenized in Ultra Turrax IKA T18 basic (18,000 revolutions/min) for 1min and then filtered using four layers of gauze. The filtrate was centrifuged at 12000×g for 15 min, at 4 °C (Thermo scientific, model Heraeus Fresco 21, Germany). After centrifugation, the supernatant was removed for subsequent use. This procedure was entirely carried out at a controlled temperature, using ice/water baths (≈3 °C). For the absorbance readings, 2.9 mL of potassium phosphate buffer (25 mM; pH 6.8), with the addition of 20 mM of guaiacol and 20 mM of hydrogen peroxide, was added with 100 µL of supernatant in a glass cuvette placed in a Shimadzu UV–vis (Kyoto, Japan) spectrophotometer during 2 min, during which, absorbances at 420 nm were recorded. The enzymatic activity (EA; expressed in) calculation of the peroxidase was estimated using Eq. 1:

$$\mathrm{EA} = \frac{\Delta \mathrm{A}}{\varepsilon} \times \frac{\mathrm{V}}{\mathrm{m}_{\mathrm{prot}}} \times \mathrm{D} \qquad \text{(Eq. 1)}$$

where V = 3 mL is the quantity of the reaction media used for the absorbance reading; ΔA = average absorbance after a time t = 2 min of reading; ε (26.6 mM^{-1} cm^{-1}) = molar extinction coefficient of tetraguaiacol, the oxidation product of guaiacol (Verma and Dubey 2003); m_{prot} = mass of proteins contained in the sample; D = dilution factor.

Protein quantification was performed using the methodology described by Bradford (1976). A calibration curve using bovine serum albumin (BSA) as standard (five concentrations varying from 20 to 100 µg. mL^{-1}) was built. One milliliter of Coomassie brilliant blue reagent solution was used. After 10 min of reaction, the absorbance of the complex was measured at 595 nm through a spectrophotometer. After finishing calibration curves, 0.1 mL of the crude extract was added to the reagent solution; the absorbance of the formed complex was also measured at 565 nm after 20 min of reaction, to determine extract protein concentration through the calibration curve.

2.4.3 Color Evaluation of Minimally Processed Yacón

Instrumental color analyses (colorimeter Chroma Meter CR-400, Konica Minolta, Japan) were performed based on the CIE-Lab system, using the color space (L*a*b*) and illuminant D65. Measurements were done directly on the yacón slices, at random points, with two readings for each slice. With the average values of L*, a*, and b*, browning index (BI), chroma, and hue angle (hue°) were calculated as explained in detail elsewhere (Pathare et al. 2012, Li et al. 2014).

2.4.4 Instrumental Texture Profile Analyses in Minimally Processed Yacón

Texture profile analyses (TPA) were carried out using a universal mechanical testing machine (Instron Series 3367, USA), equipped with Blue Hill 2.0 software (Instron, United States, 2005). Yacón cylindrical slices with ≈1.5 cm in height and 3 cm in diameter were centrally placed under an aluminum cylinder probe (diameter = 5 mm), which penetrated the sample to a depth of 4mm (≈27 % of the sample's height), in a double compression cycle, at a constant speed of 1 $mm.s^{-1}$. The attributes hardness (N), fracturability (N), cohesiveness (dimensionless), chewiness (J), elasticity (dimensionless), elasticity (N), and adhesiveness (J) were calculated from the force-deformation relationships data generated during the test (Chen and Opara 2013). Each analysis was done on five yacón slices of each treatment, soon after processing and on the last day of 5 °C storage.

2.5 STATISTICAL ANALYSIS

The data collected in the descriptive sensory analysis were evaluated using ANOVA with two sources of variation (assay and assessor) and interaction Assay*Assessor for each sensory attribute. For the sensory attributes that presented significant interaction ($p \leq 0.05$) the magnitude of these interactions was observed using graphics of attributes intensity through Assay by Assessor data demonstrating significant interactions were removed from the analysis of the respective attribute, and the ANOVA was recalculated. After that, the sensory attributes that demonstrated $p \leq 0.05$ were assessed using equations of multiple linear regression, quadratic, special cubic and special quadratic along with the preparation of contour lines as the conditions studied.

Correlations between all the sensory attributes were studied using the Pearson correlation coefficient (r) at a 5% probability.

For the physicochemical analysis, the sources of variation were verified: Assay, Time and interaction Assay*Time. For the results with $p \leq 0.05$ equations of multiple linear regression, quadratic, special cubic and special quadratic were used, to analyze the effects of the independent variables (x_1, x_2, x_3) of the process on the responses (Y_i). The response surface graphs were drawn according to the levels studied, using multiple regression equations. The response can be written as function (f) of x: $Y_i = f(x_1, x_2, x_3)$.

Statistical analyses were performed in the statistical program SAS (Statistical Analysis System - SAS Institute Inc., North Carolina, USA, 1989) version 9.4 licensed by the Federal University of Viçosa.

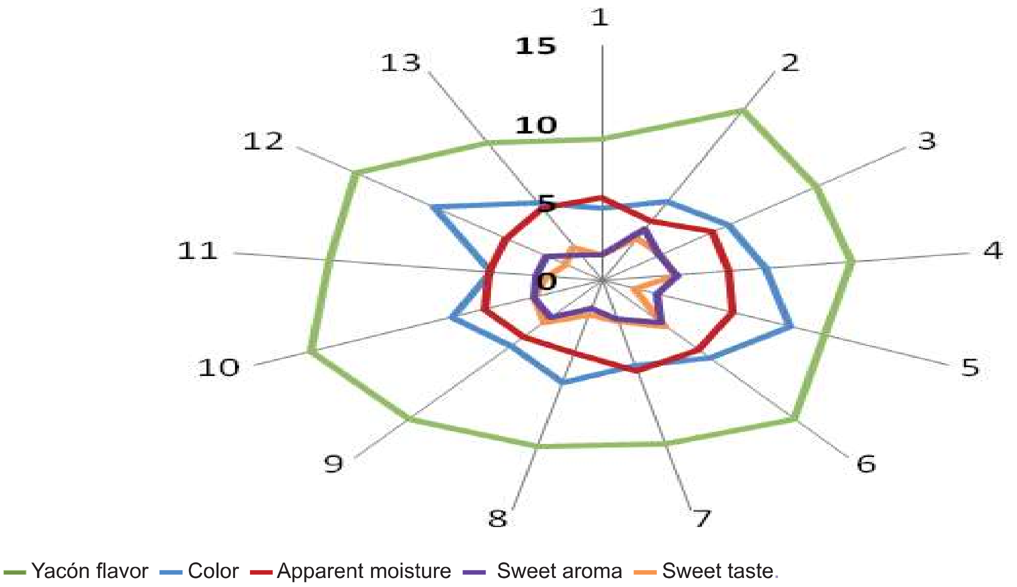
3. RESULTS AND DISCUSSION

3.1 DESCRIPTIVE SENSORY ANALYSIS OF MINIMALLY PROCESSED YACÓN

Six assessors evaluated the minimally processed yacón. From the ten items examined only one of them, astringency, showed a significant effect ($p \leq 0.05$) for the interaction assay*assessor. A graph of an average score by assay per assessor showed what caused this interaction and the type of interaction. Some data were excluded from the analysis, as significant interaction occurred due to the presence of discrepant scores (6.5 higher relative to the average) of some assessors. Thus, the scores of an assessor for five assays and another for one assay only were eliminated. Discrepant scores for this sensory attribute were removed, ANOVA was recalculated and the interaction assay*assessor was not significant ($p>0.05$) for all attributes.

The analysis of results found that regardless of the assay used in the treatment of minimally processed yacón, there was no significant difference ($p> 0.05$) for the sensory attributes color, sweet taste, yacón flavor, sweet aroma and apparent moisture. To illustrate the results, the profile of these sensory attributes was expressed graphically according to the mean of each attribute for each mixture of acids tested (assays) and it can be observed in Figure 1. The central point of the scale refers to 0 and the intensity increases from the center to its periphery.

FIGURE 1. SENSORY PROFILE OF MINIMALLY PROCESSED YACÓN TREATED WITH ANTI-BROWNING AGENT'S MIXTURE FOR ATTRIBUTES WITHOUT SIGNIFICANT DIFFERENCE (P>0.05).



The means for the sensory attribute color were next to 5 cm in the scale of 15 cm, suggesting that the presence of anti-browning agents favored the maintenance of yacón light color. The taste of yacón close to 10 cm alludes that possibly the presence of these acids changed its distinctive flavor. Sweet taste and sweet aroma exhibited similar means, around 3 cm. The sweet taste in yacón root is variable depending on the storage time, and the freshly harvested form has a higher content of fructooligosaccharides (FOS) and inulin, which in time are hydrolyzed to form simple sugars (glucose, fructose and sucrose) and consequently accentuate the sweet taste of the root (Seminario et al. 2003). Another factor that may have contributed to the low scores of these attributes is related to the presence of the acid taste, masking the perception of the first ones. The apparent moisture also close to 5 cm indicates that all samples show this effect comparably between the carrot and apple, used as references, confirming certain crispness to the root. For the attributes that varied significantly ($p \leq 0.05$), regression equations were adjusted as shown in Table 3.

TABLE 3. REGRESSION EQUATION MODEL OF MIXTURE FOR VARIATION OF SENSORY ATTRIBUTES BRIGHTNESS, ACID TASTE, ASTRINGENCY, ACID AROMA AND YACÓN FLAVOR AS A FUNCTION OF THE CONCENTRATION OF ANTI-BROWNING AGENT (C, A OR T), IT'S COEFFICIENT OF DETERMINATION (R^2) AND PROBABILITY LEVEL (P).

Sensory attribute	Regression model	R^2	p(F)
Brightness	11.8069C + 11.6819A + 14.6164T	0.9957	<0.0001
Acid taste	7.4430C + 4.9032A + 3.3224T	0.9334	<0.0001
Astringency	4.4104C + 2.3960A + 3.1487T	0.9181	<0.0001
Acid aroma	7.8372C + 4.8259A	0.9226	0.0004
Yacón aroma	8.8030C + 11.1980A + 11.7218T	0.9226	<0.0001

C = citric acid, A = ascorbic acid, T = tartaric acid.

According to the adjusted equations, it is observed that there was a predominance of some acids in the sensory perception of the attributes. Sour taste, astringency and acid flavor were more significant at higher concentrations of citric acid and the acid flavor did not vary as a function of tartaric acid. Conversely, yacón aroma that was less noticeable at higher concentrations of tartaric acid. The sensory brightness was mainly expressed in larger amounts of this acid.

The score variation in function of the concentration of anti-browning agents for thesensory attributes brightness, acid taste, astringency, acid flavor and yacón aroma, based on adjusted equations can be seen through the response surface (Figures 2, 3 and 4). The attribute acid aroma varied only in the function of ascorbic and citric acid concentrations.

FIGURE 2. RESPONSE SURFACE OF THE SENSORY ATTRIBUTES BRIGHTNESS (LEFT) AND ACID TASTE (RIGHT) OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF (C) CITRIC, (A) ASCORBIC AND (T) TARTARIC ACID CONCENTRATIONS.

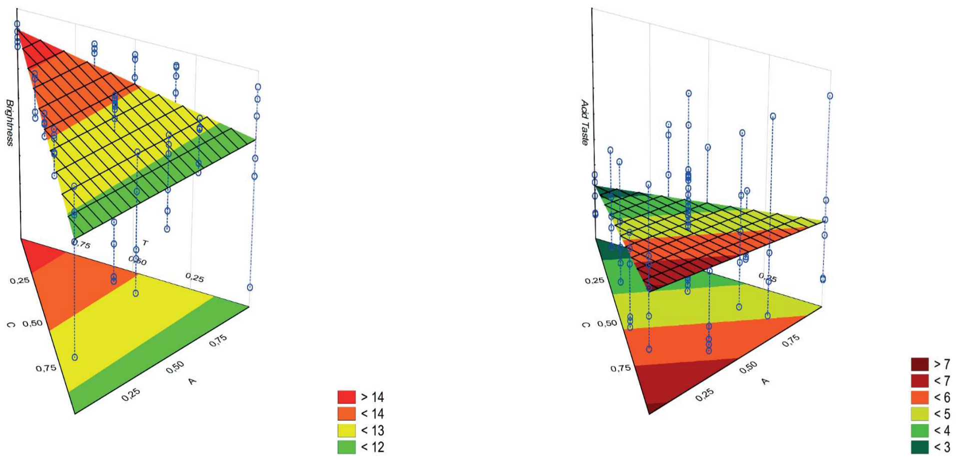


FIGURE 3. RESPONSE SURFACE OF THE SENSORY ATTRIBUTES ASTRINGENCY (LEFT) AND YACÓN AROMA (RIGHT) OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF (C) CITRIC, (A) ASCORBIC AND (T) TARTARIC ACID CONCENTRATIONS

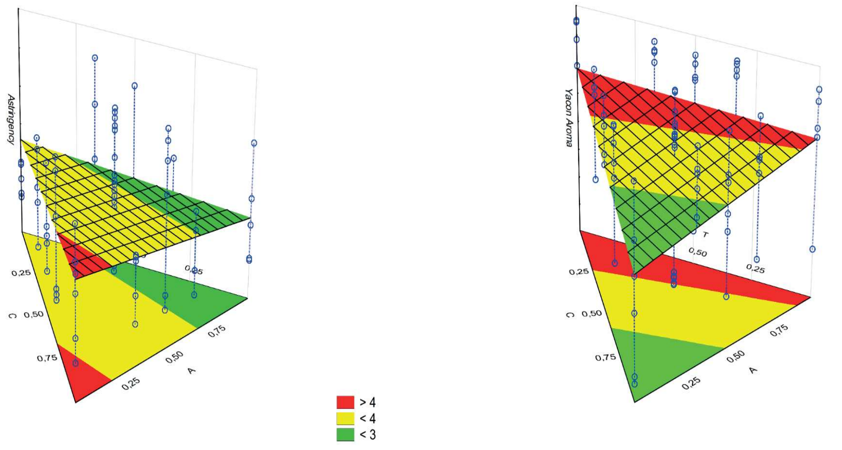
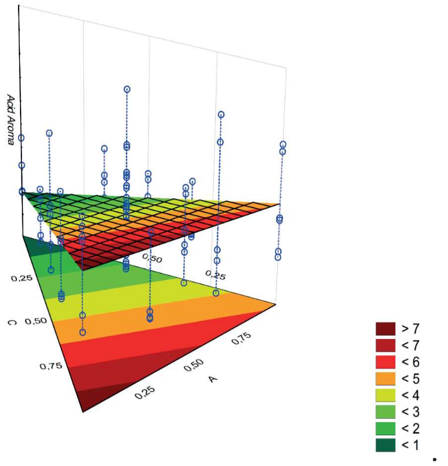


FIGURE 4. RESPONSE SURFACE OF THE SENSORY ATTRIBUTES ACID AROMA OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF (C) CITRIC, (A) ASCORBIC AND (T) TARTARIC ACID CONCENTRATIONS.



Regarding tartaric acid as anti-browning agents, the references addressing the mechanism of action and effect of this agent in the oxidative reactions are insufficient. As an acidulant, tartaric acid has several distinct advantages, such as its fresh crisp taste, high microbial stability, and dissociation constant, which allows reducing the pH (Zoecklein 2012), but there are no studies relating tartaric acid with brightness. Studies with tartaric acid are more accomplished in the acidification of grapes and wines.

According to the correlation coefficients obtained (Table 4), it can be noticed that the attributes yacón flavor and aroma negatively correlate ($p \leq 0.05$) with acid taste and aroma, both from the presence of the evaluated anti-browning agents, while these acid attributes are positively correlated, as well as acid taste and astringency. Thus, it is possible to conclude that the concentration of anti-browning agents in the minimally processed yacón reduces its peculiar characteristics.

TABLE 4. CORRELATION COEFFICIENT BETWEEN THE AVERAGE SCORES OF THE SENSORY ATTRIBUTES OF MINIMALLY PROCESSED YACÓN EVALUATED IN DESCRIPTIVE SENSORY ANALYSIS.

	Brightness	***Sweet taste***	***Acid taste***	***Astringency***	***Yacón Flavor***
Color	0.6434*	-0.2336	-0.1073	0.0627	0.1630
Brightness		0.0951	-0.2900	-0.2379	0.1369
Sweet taste			-0.3304	-0.2401	0.5195
Acid taste				0.7159*	-0.6953*
Astringency					-0.3968

	Yacón aroma	***Sweet aroma***	***Acid aroma***	***Apparent humidity***
Color	0.4256	0.1227	-0.1440	0.1858
Brightness	0.4686	-0.0268	-0.3582	0.4804
Sweet taste	0.3877	0.7214*	-0.4667	0.0387
Acid taste	-0.7738*	-0.4436	0.8045*	0.1109
Astringency	-0.5296	-0.3116	0.4569	-0.2101
Yacón Flavor	0.8131*	0.7050*	-0.6846*	-0.4564
Yacón aroma		0.5803*	-0.7668*	-0.1539
Sweet aroma			-0.4134	-0.1180
Acid aroma				0.3144

* significant at 5 % probability.

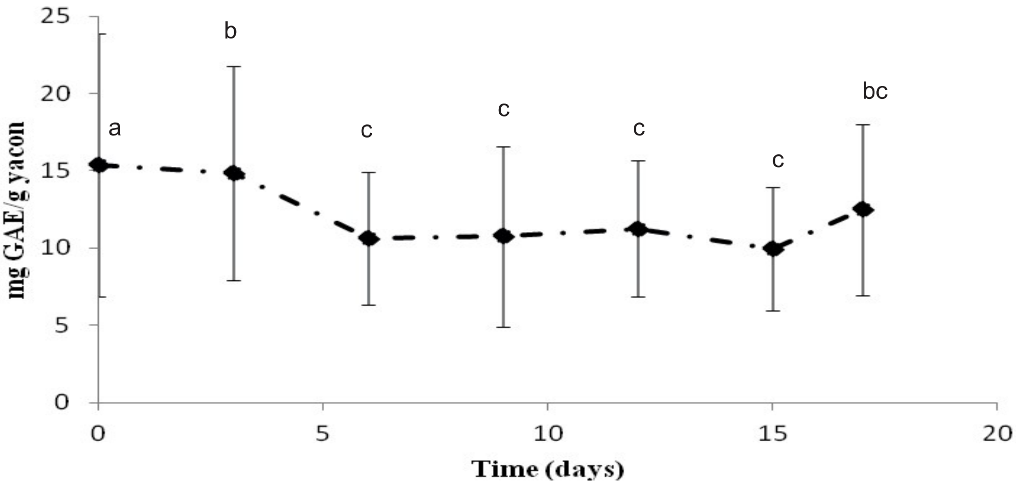
Yacón taste and aroma are also positively correlated ($p \leq 0.05$) with sweet taste and aroma, suggesting that the greater are the yacón sweet attributes the most characteristic is its flavor.

3.2 PHYSICOCHEMICAL ANALYSIS OF MINIMALLY PROCESSED YACÓN

3.2.1 Total Phenolic Compounds in Minimally Processed Yacón

The analytical curve prepared to express the results in milligrams of Gallic Acid Equivalents (GAE) allowed the adjustment of the linear equation $Abs = 2.5601F - 0.0008$ with $R^2 = 0.9646$; where 'F' is the amount of total phenolic content represented by gallic acid in 1 g of a sample of minimally processed yacón and 'abs' refers to the absorbance of the solution in the equation of the calibration curve of gallic acid. From the data obtained with this curve, was performed ANOVA. The phenolic compounds content did not vary significantly concerning the interaction Assay*Time ($p>0.05$), indicating that the acids and their concentrations acted similarly during storage. However, the number of phenolic compounds varied with the time of storage as a single factor ($p<0.001$). It was not possible to set an equation model to explain this behavior, so the average values in mg GAE/g of minimally processed yacón and their standard deviations for the three acids evaluated were plotted on a graph, being these average values submitted to the Duncan test at 5% probability (Figure 5).

FIGURE 5. AVERAGE VARIATION AND STANDARD DEVIATION OF PHENOLIC COMPOUNDS CONTENT IN MG GAE / G OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF STORAGE TIME.



Different letters differ at 5% probability, by Duncan Test.

The number of phenolic compounds showed an oscillatory variation with storage time. A justification for this behavior would be related to the oxidative enzymes that, besides the active form present in the cytosol, present latent forms found both in the cytosol and in plastids isolated from the rest of the cell (Sellés-Marchart et al. 2006). Due to cold storage, the enzymes found in latent form, and plastids still intact may undergo modification with time, leading to the occurrence of these oscillations in the oxidation of phenolic compounds. The stress caused by cold storage of sliced yacón may also have activated the secondary metabolism of the cells, which is one of the routes for the formation of phenolic compounds (Ding et al. 2001). However, all these changes are more pronounced at the beginning of the post-harvest period.

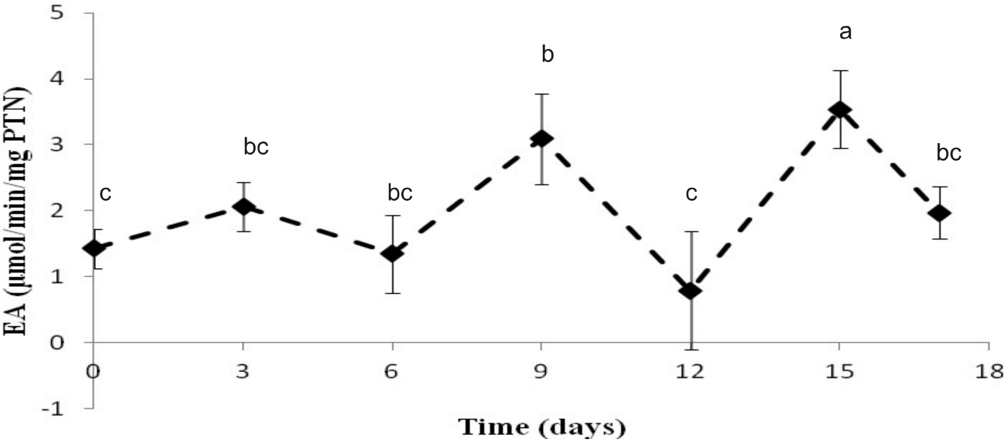
The phenolic compounds content found in this study (mean = 12.2 ± 6.3 mg GAE /g yacón) is superior to the findings by Valentová and Ulrichová (2003) and Lachman et al. (2003) who obtained approximately 2 mg/g of fresh yacón. This probably occurred because the results were overestimated by the presence of interfering causes. The spectrophotometric method of Folin-Ciocalteu is the most commonly used, however, is not specific, because the reaction involves all phenolic groups present in the medium in addition to reducing substances added to food or naturally present therein, such as ascorbic acid, sugar and some amino acids (Agbor et al. 2014). These substances are present in yacón and significant concentrations concerning fresh mass and others were still used as anti-browning agents, which contributed to these relatively high results.

3.2.2 Peroxidase (POD) Activity in Minimally Processed Yacón

The calibration curve for determining the concentration of total proteins allowed the adjustment of the linear equation Abs = 34.44P - 0.031 with $R^2 = 0.9718$, where “P” is the amount of protein (mg /ml) in the solution prepared from 1g of minimally processed yacón sample and ‘Abs’ refers to the absorbance of the solution in the equation of the calibration curve at 420 nm. From the data obtained with this curve, the enzymatic activity of peroxidase was calculated and the results were evaluated by ANOVA. The peroxidase activity did not change during all the storage time for each mixture of anti-browning agent, as also regarding the mixture composition ($p>0.05$). Only the storage time, as an isolated factor significantly affected ($p<0.0001$) the enzymatic activity of peroxidase;

however, it was not possible to set a regression model. Thus, the mean values and their standard deviations were plotted on a graph, being these average values submitted to the Duncan test at 5 % probability (Figure 6). Acids used to treat yacón, also known as acidulant agents are generally used to maintain the pH of the medium below the optimum for the catalytic action of enzymes. According to Richardson and Hyslop (2000), the ionizable groups of the enzyme protein structure is affected by the pH. These groups should remain in the appropriate ionic form to maintain the conformation of the enzyme active site, to recognize substrates and catalyze the reaction. Changes in the ionization degree of the enzymes are usually reversible, except in extreme conditions of pH change. Depending on how the enzyme is affected, reversibility can be more or less slow, leading to oscillations observed in Figure 6, during the storage time of the minimally processed root.

FIGURE 6. AVERAGE VARIATION AND STANDARD DEVIATION OF ENZYMATIC ACTIVITY VALUES (EA) OF PEROXIDASE OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF STORAGE TIME.

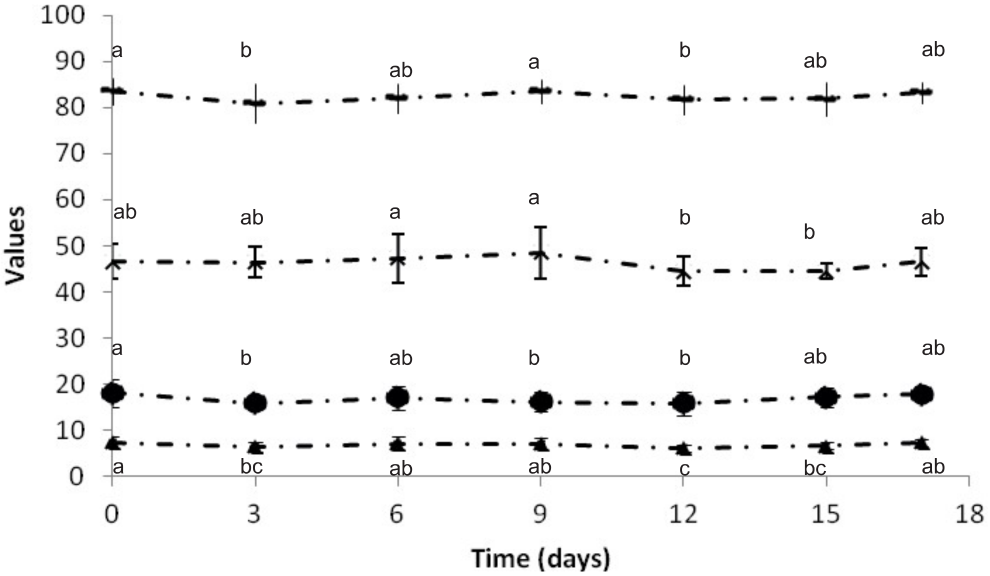


Different letters differ at 5% probability, by Duncan Test.

3.2.3 Color Evaluation of Minimally Processed Yacón

ANOVA results for all color measurements of minimally processed yacón showed that there was no significant interaction (p> 0.05), suggesting similar behavior of the acid mixtures in maintaining color during storage. In the same way, no significant variation (p> 0.05) for the effect of the mixture indicates that there were no concentrations more strongly pronounced; thereby allowing inferring the effectiveness of the acids assessed independently of their concentration, once totaling 0.2 mol. L^{-1}. The L coordinate values (p = 0.0092), Chroma (p = 0.0046), Hue (p = 0.0043) and Browning Index (p = 0.0122) presented significant differences relative to storage time, however, it was not possible to adjust a regression model. Thus, the mean values and their standard deviations were only plotted on a graph, being these mean values submitted to the Duncan test at 5 % probability (Figure 7).

FIGURE 7. AVERAGE VARIATION AND STANDARD DEVIATION OF THE L* (X), CHROMA (▲), HUE (-), AND BROWNING INDEX (●) VALUES OF MINIMALLY PROCESSED YACÓN AS A FUNCTION OF STORAGE TIME.



Different letters in the same line differ at 5% probability, by Duncan Test.

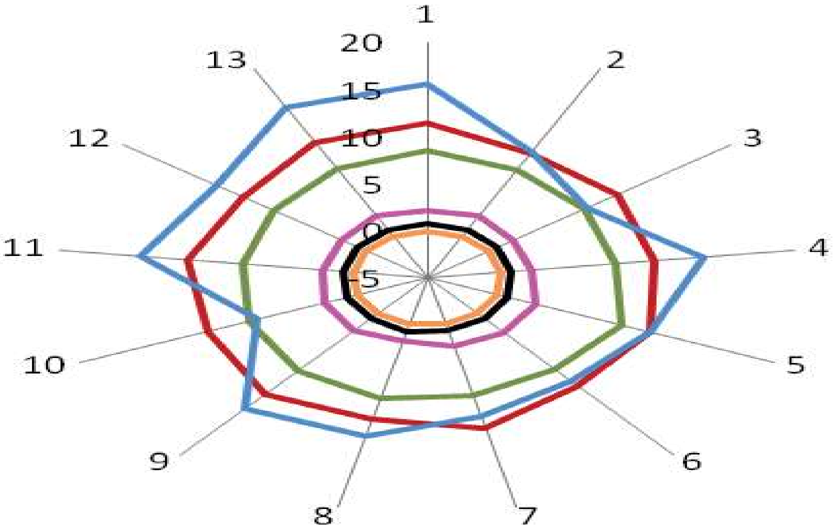
The graph shows that all color measurements presented minor fluctuations over time, corroborating the results for total phenolic compounds content and peroxidase enzymatic activity. The higher L* value, the brighter the sample and, therefore, the lower the browning. In this study, once there was no change in this color measurement ($p>0.05$) for the evaluated assays, it is possible to say that the brightness of minimally processed yacón at the end of 17 days of storage remained close to that after processed. Chroma Index (C*) refers to the intensity, or purity of the color, to gray, so the higher value means more intense and pure color (Rodrigues et al 2003). Once fresh yacón does not present high color intensity, low values were expected for this measurement (means between 4.31 and 11.82). The hue angle (h*) is the color tone of the sample and it may vary from 0 to 360 °, the angle 0° or 360° represents the red color, 90° the yellow, 180° the shades of green and 270° the blue (Barreiro et al 1997; Lopez et al 1997.). All readings were around 90° (between 70.87 and 87.87º), showing a close hue to yellow for all the samples, which was expected, because it represents the natural color of the root. The Browning Index (BI) presented reduced values between 10.85 and 27.38; and as there was no change in relation to the mixture of anti-browning agents ($p> 0.05$), it can be assumed that all concentrations up to 0.2 mol L^{-1} of these agents were effective in controlling enzymatic browning.

3.2.4 Instrumental Texture Profile Analyses in Minimally Processed Yacón

Texture properties - hardness, fracturability, adhesiveness, chewiness, cohesiveness and elasticity – of minimally processed yacón were analyzed by the difference between the value obtained after processing and the last day of storage. All instrumental measurements of texture presented $p>0.05$ indicating that storage time and/or a mixture of anti-browning agents did not cause changes in this attribute. Thus, the presence of anti-browning agents allowed the maintenance of the texture characteristics during 17 days of storage.

To visualize the results, the instrumental texture profile was expressed graphically according to the averages for each attribute for each of the tested anti-browning agent mixture (assays) and can be observed in Figure 8. In the graph, the scale central point refers to -5, once there are negative values from the instrumental attributes evaluated, and the intensity increases from the center to the periphery. The average observed of each instrumental texture attribute for each assay applied in the minimally processed yacón is marked on the corresponding axis.

FIGURE 8. INSTRUMENTAL TEXTURE PROFILE OF MINIMALLY PROCESSED YACÓN TREATED WITH ANTI-BROWNING AGENTS MIXTURE.



▬ Chewiness (J); ▬ Hardness (N); ▬ Fracturability (N); ▬ Elasticity (mm); ▬ Cohesiviness (adimensional); ▬ Adhesiviness (Ns).

Analysis of the texture profile can be considered as an operation of imitation of the chewing process. The instrument used for the analysis operates with two compression cycles (Besbes et al. 2009). Force-time curves are recorded and from these the mechanical indicators can be calculated (Szczesniak 2002; Caine et al., 2003). The instrumental measurements adhesion, cohesiveness and elasticity, presented very similar values with each other and close to zero, which was expected since these indicators are not characteristic of this type of food material.

The hardness (maximum force required for sample compression) is one of the factors that determine the acceptability of the food by the consumer and as well as the fracturability (force by which a sample jumps from the teeth when crumbles or breaks into pieces) (Bourne 2002), their values should be low.

The chewiness as well as gumminess (energy required to disintegrate one semi-solid food) are instrumental texture measurements obtained by calculation. The chewiness refers to the force required to chew up to swallowing solid foods, e.g. the number of chews required to make the food capable of being swallowed. This measure is a secondary property of instrumental texture profile, and the result is the product of the indicator's hardness, cohesiveness and elasticity, and therefore has a high positive correlation with hardness (Bourne 2002). These results showed that independently of the mixture of acids, minimally processed yacón maintained its characteristic crispness without changes that could compromise its storage.

4. CONCLUSION

The descriptive sensory analysis indicated that the minimally processed yacón treated with ascorbic, citric and tartaric acids isolated or in combination, totaling 0.2 mol L^{-1}, did not change the sensory attributes color, yacón flavor, sweet taste, sweet aroma and apparent moisture. However, the attributes brightness, acid taste and aroma, astringency and yacón aroma varied with the concentrations of these anti-browning agents. In general, the higher the concentration of acids, especially citric acid, the greater the perception of acid taste and aroma and as well as astringency. The brightness and yacón aroma presented higher scores in the presence of tartaric acid.

For the physicochemical characteristics performed, the presence of the acids, independently of concentration, allowed to preserve phenolic compounds, control the enzymatic activity of peroxidase and therefore to maintain the light color of the root. There was variation ($p \leq 0.05$) only for physicochemical characteristics concerning storage time, with small oscillations observed in the results; however, anything that would compromise the quality of minimally processed yacón.

Regarding the instrumental texture properties, no significant change ($p > 0.05$) was observed in the mixture of anti-browning agents and/or storage time.

Therefore, it is recommended the use of ascorbic, citric and tartaric acids isolated or in mixture at a concentration of 0.2 mol. L^{-1}, which is safe for human consumption, cheap and may also extend the storage life of minimally processed yacón. Furthermore, to obtain minimally processed yacón with more attributes similar to fresh yacón, a lower concentration of citric acid can be used.

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