

PHYSICOCHEMICAL CHARACTERIZATION OF THE LULO (*Solanum quitoense* Lam.) CASTILLA VARIETY IN SIX RIPENING STAGES

CARACTERIZACIÓN FÍSICOQUÍMICA DE LA VARIEDAD CASTILLA DEL LULO (*Solanum quitoense* Lam.) EN SEIS ESTADIOS DE MADURACIÓN

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ABSTRACT

Background: Lulo (*Solanum quitoense* Lam) is considered a fruit with a high potential for exportation due to its exotic aroma, its bittersweet taste and the bright green color of its pulp. As a climacteric fruit is harvested when the fruit has reached full development, and 75% of the ripening is apparent by its characteristic yellow color with some small green points. However, this parameter is not enough to decide the right time for the crop, due to the irregularity of the fruits, especially for selling purposes in international markets, hence studies have been conducted to establish the changes during ripening and postharvest. **Objectives:** The aim of this work was to determine the relationship between color measurement and the other physicochemical parameters in the six ripening stages of lulo Castilla variety, in order to define the optimal harvesting time. **Methods:** Three fruits were studied for each stage, and were analyzed in the fresh state: ° Brix, pH, titratable acidity and color changes in the cortex, according to CIELab system. **Results:** The ranges obtained for the six evaluated stages were: ° Brix: 4.2 - 10.3, pH: 3.67 - 3.90, acidity: 2.63 - 3.00 and color (ΔE): 0 - 53. We found that the epicarp of the fruit varies from green to yellow intense, indicating the maturity for consumption. Total soluble solids increased with fruit maturation and the tritatable acidity decreased reaching a minimum values at stage 3, which was considered optimum for harvesting. The pH increased in stage 5 due to the drop of vacuolar H⁺ ion concentration. **Conclusion:** By measuring color parameters, 3 ripening stages were defined: green (0, 1, 2); semi ripe (3, 4) and ripe (5).

Keywords: Naranjilla, tritatable acidity, pH, soluble solids, color.

RESUMEN

Antecedentes: El lulo (*Solanum quitoense* Lam) es una fruta de alto potencial de exportación por su aroma suave, su sabor agrídulce y el color verde brillante de su pulpa. Por ser un fruto climatérico es recolectado cuando el fruto ha alcanzado su completo desarrollo y presenta un 75% de madurez, manifestado por el color amarillo característico con ligeros puntos verde. Este parámetro no

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es suficiente para definir el momento propicio de la cosecha, debido a la irregularidad de los frutos, especialmente para la venta de este producto en mercados internacionales; por lo que se ha realizado estudios para establecer los cambios producidos durante su maduración y poscosecha. **Objetivos:** El objetivo de este trabajo fue determinar la relación de las medidas del color y otros parámetros fisicoquímicos en seis estadios de maduración de lulo variedad Castilla, para definir el tiempo óptimo de cosecha. **Métodos:** Se tomó 3 frutos por cada estadio y por cada medición, analizando en estado fresco: grados brix, pH, acidez titulable, y los cambios de color de la corteza, de acuerdo al sistema CIELab, durante la maduración de éstos. **Resultados:** Los rangos de estos parámetros para los 6 estadios evaluados fueron: °Brix: 4,2 – 10,3, pH: 3,67 – 3,90, % acidez: 2,63 – 3,00 y color (ΔE): 0 – 53. Se encontró que la corteza del fruto varía de verde a naranja intenso, indicando la madurez de consumo. Los sólidos solubles totales se incrementaron a medida que el fruto maduraba. La acidez titulable disminuyó durante la maduración, observándose una caída en el estadio 3, estimado el momento óptimo para su cosecha. El pH aumentó en el estadio 5, por la disminución de la concentración de iones H^+ vacuolar y el aumento de aminoácidos libres. **Conclusión:** Mediante la medición de los parámetros de color se definió 3 estadios de maduración: verdes (0, 1 y 2), pintones (3 y 4) y maduros (5).

Palabras clave: Naranjilla, acidez titulable, pH, sólidos solubles, color.

INTRODUCTION

Lulo or naranjilla (*Solanum quitoense* Lam.) is a fruit original from the highlands of the Andes mountains in South American, more specifically from Colombia, Ecuador and Peru, in regions with mild weather and low luminance, between 1.500 m and 2.800 m of altitude (1, 2). Lulo belongs to the family Solanaceae which has hortofruicultural value in tropical regions in America, Africa and Oceania (3). Estimation of the area cultivated with lulo in Colombia is roughly 6.637 hectares, with an annual production of 64.432 tons (4, 5). In order to supply the Colombian demand it is necessary to import from Ecuador roughly 21% of the volume consumed per year, reaching a volume of 2.746 tons (5). Based on studies from European Economic Community, lulo is one of the Andean fruits with more potential for exportation (6).

The yield and quality of lulo production are defined by seed selection and the site of the plantation. The effort, care and resources invested in the plantation, can be lost due to an untimely and inadequate crop. The time for the crop is determined by identifying the fruit ripening degree (color of the fruit epidermis). Ripening is a complex process involving the coordinated regulation of several metabolic pathways related to color, flavor, aroma and texture, and the fruit nutritional value (minerals, vitamins, fibers and antioxidants) is also affected

(7). During ripening there is an initial increase in respiration that slowly decreases up to the stage of physiological maturity. However, in some fruits, after reaching a minimum value there is a new increase in respiration with a maximum called climacteric peak and a new decrease afterwards. These fruits are called climacteric and lulo (*Solanum quitoense* L) is one of them (8). It is optimally harvested when the fruit has reached full size development and 75% of the ripening is apparent by its characteristic yellow color with some small green points, soluble solids at 10° Brix, and total average acidity of 3.84% of citric acid. The color is not enough to decide the right time for harvesting, due to the different ripening of the fruits even in the same tree, thus making this time of especial importance in the selling of this product in the international markets.

The evaluation of quality of a fruit is based on the identification of specific values in physicochemical parameters such as pH, acidity, ° Brix and color (9-11). Among the different changes that fruits experiment during ripening, color is the most noticeable and it is frequently the most important criterion that consumers utilize to decide if a fruit is ripe or not. Color changes are due to chlorophyll degradation, by enzymatic systems that lead to unmasking the carotenoid pigments (orange and yellows) and the anthocyanins (blue, red) (12, 13). These substances play an important role in the quality of fruits and vegetables, in as much as the

quality and quantity composition of these pigments is related to the maturity stage (14), showing differential contents during the ripening process.

Taking into account the fact that human identification of color is complex, because sensations such as brightness, luminance, intensity among others, influence the perception of primary colors (red, blue, yellow) and their combinations (orange, green, purple, etc), the *Commission Internationale del'Eclairage* (CIE) made possible to express color in quantitative and numerical terms. This system conceives all colors in a color sphere, defined by three perpendicular axes, L* (from white to black), a* (green to red) and b* (blue to yellow). Coordinate L* receives the name of **Luminance** (measurement) (0% black to 100% white), coordinate a* corresponds to **Clarity** (ranging from -60% green to +60% red) and the coordinate b* corresponds to **Chromaticity** (ranging from -60% blue to +60% yellow) (15, 16). Color measurements in the food industry are made by sensory evaluation (visual) or by instruments, the last one can be done by colorimeters, spectrophotometers or spectroradiometers (17-20).

This work was carried out to determine the relationship between color measurement and the other physicochemical parameters in different stage of lulo maturation in order to define the optimal harvesting time to achieve the best fruit quality for future consumption.

MATERIALS AND METHODS

Plant material

Lulo fruits were collected from different trees at morning time in the "Porvenir" farm, "La Pola" county, in Circasia (Quindio). Healthy fruits without bruises or signs of insects or other plague attack were chosen. The sampled trees were selected in a blueprint of plantation, assigning random numbers to the trees. Then, our collection can be assimilated to a simple random sample. Five fruits were collected randomly for each maturation stages according to the color scale of the Colombian Technical Norm 5093 (21). The fruits were collected manually using gloves and the peduncle was cut with scissors. These fruits were placed in Styrofoam containers, adequately ventilated to allow gas exchange. Since no previous studies of this type were found, to allow a variance estimation, no sample size

calculation was carried out and a 30 fruits sample was used. Once harvested, the fruits were grouped according to their degree of ripening, washing and disinfected according to the minimal requirement of the Codex Alimentarius for Fresh fruits and Vegetables (22). They were stored at 4°C afterwards.

Physicochemical Characterization

Physicochemical analyses were performed by only one measurement with the following indicators: Brix degrees (% of soluble solids), pH, titratable acidity and color (accordingly to CIELabs system). The lulo fruits selected were free of bruises, cuts, insect attacks or any other plague. They were washed and disinfected according to minimal requirements of Codex Alimentarius for fresh fruit and vegetables (22) and were stored at 4°C until the time of analysis.

Brix degree measurement (°brix)

Measurements of the °Brix were done with a table refractometer (Thermo Electron Corporation model 334610), which was calibrated with distilled water and was kept in a constant temperature of 25°C (NTC 4624) (23).

Color measurement

The color measurement was carried out by instrumental techniques. It was used a colorimeter Minolta CR-10, color measurement were taken under the conditions of standard illuminant D65 and 10° observer in the CIELab system. The tree values of stimuli L* (vertical axes that show color luminance, having black in the lower region with a value of zero, and white in the upper region with a value of 100), a* (tendency from green (-) to red (+) or x axis) and b* (tendency from blue (-) to yellow (+) or y axis) were obtained directly from the colorimeter and were used to calculate the saturation value (C*, indicates the distance from the center to a given point) and the hue angle (h°, measurement of hue angle, ranges between 0 and 360°), using the following equations (24):

$$C^* = \sqrt{(a^*{}^2 + b^*{}^2)} \quad \text{Equation 1.}$$

$$h^{\circ}_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \quad \text{Equation 2.}$$

To determine the color variation (ΔE) in each ripening stage, an equation proposed by Chen and Ramaswamy, 2002, was used (25):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{Equation 3.}$$

Color measurements were carried out on each fruit surface by triplicate using 3 fruits for each maturation stage, 4 measurements were made around the fruit equator and 2 in the upper and lower parts each.

Titrateable acidity measurement

Acidity was measured by doing an acid-base titration with NaOH 0.1 N (Colombian Technical Norm, NTC 4623) (26) and was labeled as % of citric acid (acid prevailing in lulo) in 100 g of a sample as shown by the equation:

$$\% \text{Titrateable acidity} = \frac{V_{NaOH} \times N_{NaOH} \times mEq_Weight_{AcCitrico}}{V_{Muestra}}$$

Equation 4.

pH measurement

The pH was measured by using a pH-meter (IQ Scientific model IQ 240), with an electrode for liquids previously calibrated. The fruits were washed, peeled, liquefied and filtered to obtain juice. 10 ml of lulo juice were taken for each maturation stages and 3 drops of phenolphthalein (1%) were added. The pH and titrable acidity measurements were made using three different fruits in each maturation stages.

Statistical Analysis

The statistical significance study of the different effects and their possible interactions was conducted

through One way Analysis of Variance (ANOVA) using the LSD (least significant differences) method as the method for multiple comparisons, with a confidence level of 95% ($\alpha = 0.05$). The variance analysis was performed with the Statistical Package Statgraphics Centurion XV (Version 15.2.06).

RESULTS

Characterization of fresh lulo

The characterization of the maturation states of fresh lulo was performed based on the color changes according to Colombian Technical Standard NTC 5093 (21) as showed in the Figure 1 and Table 1.



Figure 1. Table of color of lulo Castilla variety (21).

Table 1. Color of lulo according to its ripening stage (21).

Color 0	Physiologically ripe fruit dark green
Color 1	Dark green fruit with light green hues
Color 2	Light green fruit with some orange hues
Color 3	Green orange fruit with hints of fruit to the center
Color 4	Orange fruit with few green overtones
Color 5	Orange fruit

In Table 2 are summarized the results of descriptive statistics for physicochemical parameters evaluated for the 6 ripening stages (0-5) of lulo Castilla variety.

Table 2. Physicochemical properties in six ripening stages of lulo according to the table of color. It is shown the mean (\bar{x}), standard deviation (s) and coefficient of variation (CV) values for each parameter.

Ripening stage (Table of color)	Parameters											
	Brix degree			pH			Acidity (% citric acid)			Color ΔE (CIELab system)		
	\bar{x}	σ	CV (%)	\bar{x}	σ	CV (%)	\bar{x}	σ	CV (%)	\bar{x}	σ	CV (%)
0	4.2	0.10	2.38	3.67	0.006	0.16	2.63	0.04	1.53	0.00	1.77	40.52
1	5.4	0.20	3.70	3.62	0.006	0.16	3.05	0.08	2.64	5.83	3.14	53.00
2	6.4	0.20	3.13	3.66	0.006	0.16	3.14	0.06	2.07	7.71	2.39	30.94
3	8.6	0.20	2.33	3.63	0.010	0.27	2.21	0.03	1.35	40.32	3.93	9.74
4	9.3	0.35	3.61	3.60	0.015	0.42	2.70	0.05	1.70	52.05	0.66	1.27
5	10.3	0.20	1.92	3.90	0.006	0.15	3.00	0.07	2.50	40.00	2.35	5.88

According to the Colombian Technical Norm NTC 5093 (21), the maximum content of citric acid, independent of the ripening stage, is 3.23%. Therefore, the selected lulo fruits used in these experiments present values within the allowed parameters (%) of acidity.

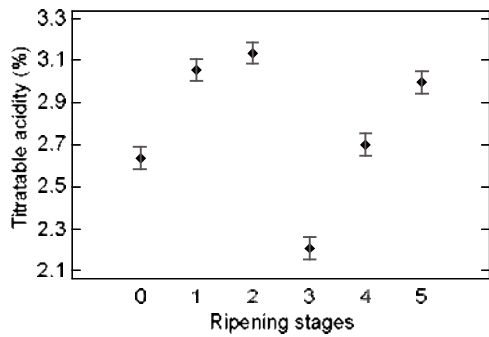


Figure 2. Percentage of acidity expressed as % of citric acid in the six ripening stages of lulo Castilla variety.

In figure 2 is shown the percentage of acidity expressed as citric acid, there is not a growing tendency of this acid, given that some significant differences appear in the different ripening stages, showing an increment between the green stages (0-2) and a remarkable decrease in stage 3, considered as the optimal time for the harvest, rising again in stages 4 and 5.

The pH values of the harvested lulos in the different ripening stages present a significant statistical difference, showing an increase in maturation stage 5 (orange fruit), which is shown in Figure 3.

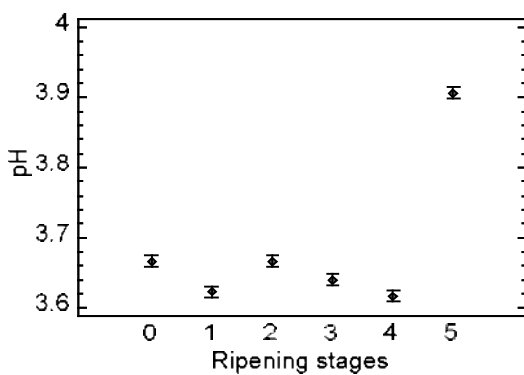


Figure 3. pH in the six ripening stages of lulo Castilla variety.

In Figure 4 is shown that the values of total soluble solids (°brix) increase during the ripening time, ranging from 4.2 (stage 0) to 10.3 (stage 5), due to the hydrolysis of stored starch in vacuoles and intercellular spaces during the fruit growth.

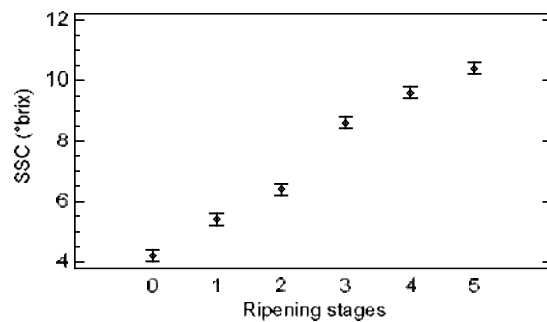


Figure 4. Total soluble solids (SST), expressed as °brix, in the six ripening stages of lulo Castilla variety.

Figure 5 shows that component a* presents a typical change, followed by a sigmoid tendency. There were no important changes in coordinates a*, b* and L* when fruits were green (maturation stages from 0 to 2) or yellow (maturation stages from 3 to 4), but there appeared a marked increase between maturation stage 2 and 3 and a slight fall in the maturation stage 5. There is a change from negative to positive in the a* value, as a consequence of chlorophyll degradation and the increase in concentration of carotenoid pigments due to an increase in the ethylene concentration (27).

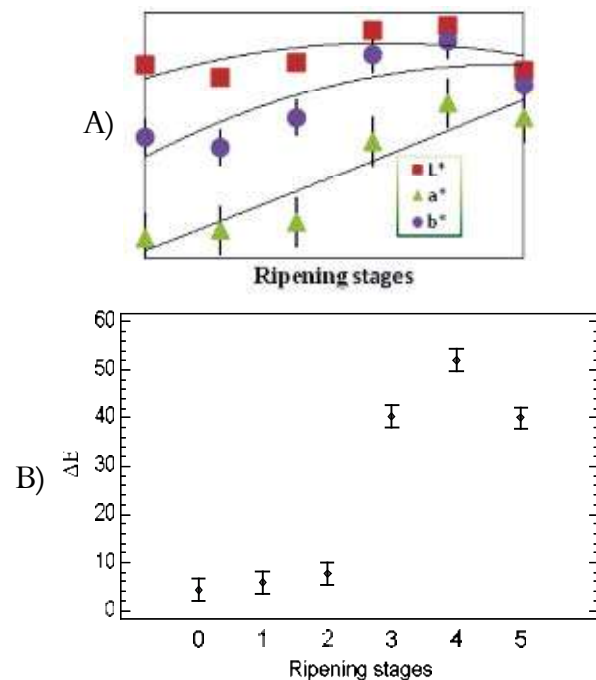


Figure 5. A). Average values of L*, a*, b* in the different ripening stages of lulo Castilla variety. B). Color variation (ΔE) in the 6 ripening stages.

Lightness intensity analysis (L*) on the lulo epidermis in the six ripening stages showed significant

statistical differences between 0, 1, 2 and 5 and 3, 4 ($p = 0.0000$).

Figure 6 shows a luminosity increasing tendency in fruits of stages 1-4, which indicates an increase in reflectance, making the color saturation less pure and therefore more luminous (28).

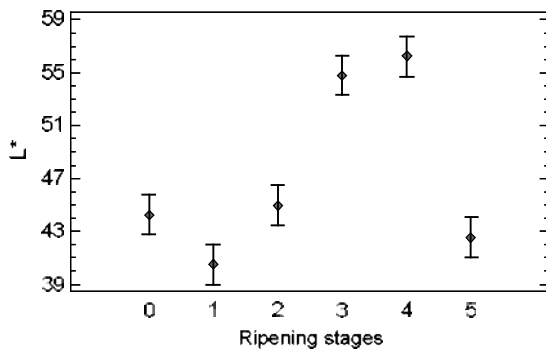


Figure 6. Lightness (L^*), on the epidermis of the six ripening stages of lulo Castilla variety.

The chromaticity parameters a^* ($-a^*$ green, $+a^*$ red) and b^* ($-b^*$ blue, $+b^*$ yellow) presented highly significant differences ($p = 0.0000$), with an increasing tendency to green and yellow hues respectively, while diminishing during ripening (stage 5) and senescence. These results can be attributed to carotenoid pigment concentration that together with anthocyanin presence generates the fruit epidermis natural color (24, 26). The a^* negative values shown in figure 7, reveals a change in the epidermis toward less intense green hues, with an increase until maturation stage 4 and a further decrease in stage 5, indicating the loss of green color and tendency to yellow hues at the end of the organoleptic maturation.

The value of b^* , according to Figure 7, increase in the most advanced maturation stages, as shown by the color change in the epidermis from yellow to orange, starting in stage 3. For this reason, b^* is considered the main responsible of the external appearance in fruit ripening. In effect, our statistical results for b^* are more significant than those for a^* .

To better appreciate the changes in a^* and b^* they were located in the chromatic plane a^*-b^* as shown in Figure 7. It is observed that the maturation stages 0-1 are found in the green zone and for stages

3-5 the color begins to change the yellower hues, which can be attributed to chlorophyll degradation.

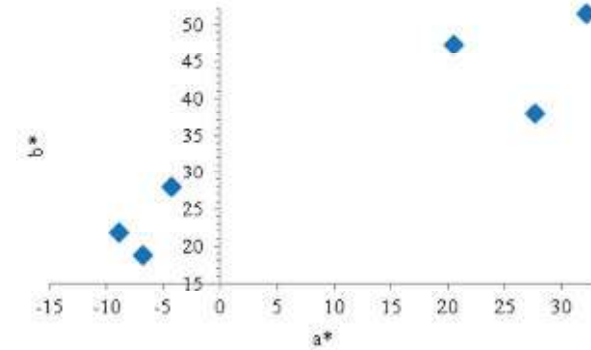


Figure 7. Variation in the chromatic plane a^*b^* in the epidermis of lulo fruits through six maturation stages.

Statistically, the chroma variation value ($p = 0.0000$) indicates that this parameter has a high significance level. In Figure 8 is illustrated the color saturation, C^*_{ab} of lulo fruits, which showed a similar behavior to luminance L^* and to chromaticity b^* , suggesting that color in ripening stages 3, 4 and 5 indicates greater purity and saturation of yellow and orange tonalities, and less in gray tonality, given that gets away from the center of the sphere color and gets closer to the central axis.

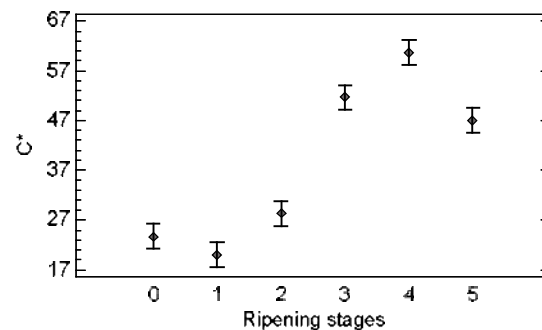


Figure 8. Saturation values (C^*_{ab}), on the epidermis of the six ripening stages of lulo Castilla variety.

The color of lulo skin turned less green, showing a tendency toward a more intense and bright yellow with the age of harvest, through the increase in L^* and C^*_{ab} values and a decrease in h°_{ab} angle as shown in figure 9, thus suggesting a decrease in tone or hue of color at stages 0, 1 and 2.

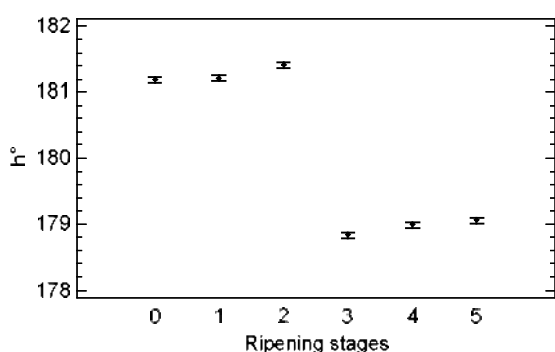


Figure 9. Hue angle values (h°_{ab}), on the epidermis of lulo Castilla variety in the six ripening stages.

DISCUSSIONS

The results and the characterization of lulo Castilla variety evaluated in this investigation are in agreement with the report by Casierra-Posada *et al.*, 2004 (29), who worked with the same variety of lulo.

The decrease in the acidity percentage is due to the activity of dehydrogenases and to the organic acids that are used as substrates for respiration in the synthesis of new components during maturation. This decrease coincides with the start of the ripening and the accumulation of sugars (26). This observation has been carried out in other fruits and vegetables during ripening (30). Organic acids have been identified as an energy source during the very active respiration that occurs in ripening, with their consequent decrease (31, 32). The organic acid content makes a large contribution to color, aroma and flavor in both fruits and vegetables, with parameters that vary during ripening, depending on the maturation index or the relationship between total soluble solids and the acidity percentage (33).

The decrease of pH in the first stages could be related to the increase of the concentration of organic acids (citric, malic and oxalic acid). The increase of pH in stage 5 appears because during the filling of fruits, a great part of the accumulation activity is performed by symport, where H^+ ions play an important role; they are part of the formation of substrates such as sucrose and glucose, and lower the concentration in the vacuole, during the final ripening stages, which slightly increases the pH (26). These results differ from those reported by Galvis *et al.*, 1999 (27), and Casierra-Posada *et al.*, 2004 (29), who observed that during the ripening period of lulo, the pH value is very similar and

ranges between 2.9 and 3.2, between the first and seventh day after the harvest. It appears that the results obtained, also differ from those obtained by Ospina-Monsalve *et al.*, 2007 (28), for lulo fruits in two ripening stages: semi ripe and ripe, where the pH does not change and has an average value of 3.30. According to the literature, fruits present a small increment in pH in the period between the organoleptic maturation and senescence. This observation is probably due to an increment in the free amino acids content due to enzyme degradation of proteins and other polymeric structures in the fruit (34-36).

Starch hydrolysis occurs by the great increase in the activity of enzymes α -amylase, β -amylase and starch phosphorylase (26) which transform starch into reducing sugars. Besides, there is a direct increase with the maturation degree in which the fruits were harvested. So, there will be a higher concentration of total soluble solids in the samples harvested in later maturation stages than in those fruits that were collected in an early maturation stage (25, 28). The data obtained in this investigation differ from those reported by Ospina-Monsalve *et al.*, 2007 (28), where $^{\circ}$ brix values were between a small range of variation, between the maturation stages semi ripe and ripe, having an average of 8.25 $^{\circ}$ brix.

The luminosity parameter behavior can be explained by the increase in the concentration of carotenoids that appears in these stages, where a physiologically mature tissue changes to one more visually attractive (37, 38). Furthermore, a decrease in luminosity is observed in stage 5, possibly due to the beginning of degradation of carotenoid pigments by oxidation, which leads to color darkening (stages 4 and 5) and is confirmed by the a^* and b^* values (39).

These results are similar to the reported for tomato fruit by López *et al.*, 2004 (40), where a decrease in L^* values is observed, due to the darkening of red color (from pink to dark red) by the start of synthesis of pigments (lycopene).

The results obtained in the color and shade parameters are similar to those reported by Poca-sangre *et al.*, 1995 (41), who attributed this change to the increase in chlorophyll degradation, having a rapid transition from a greenish yellow to yellow. In the same way, Abbott *et al.*, 1999 (42), reported how L^* and the angle h°_{ab} are enough to quantify the color of a product.

CONCLUSIONS

The physicochemical changes evaluated to Lulo on its 6 ripening stages showed an increase in the content of sugars, from 4.2 to 10.3°brix, in pH from 3.67 to 3.9, and a decrease of the percentage (%) of acidity in stage 3 of 2.21. It was observed that these parameters are highly correlated and each one depends on the other.

Based on the results obtained in concern to color variation (ΔE), Lulo fruits of Castilla variety can be grouped in three groups according to the ripening stage, green (stages 0 to 2), semi ripe (stages 3 and 4) and ripe (stage 5), corresponding to the physiological maturity, harvest maturity and consumption maturity, respectively. It was observed that b^* is a key variable in the color change of the epidermis of lulo fruits during ripening.

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