

Physiological and Physico-Chemical Characterization of the Soursop Fruit (*Annona muricata* L. cv. Elita)

Caracterización Fisiológica y Físico-Química del Fruto de la Guanábana (*Annona muricata* L. cv. Elita)

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Resumen. En el mundo, el consumo de frutas es creciente, al igual que la población. La Organización Mundial de la Salud recomienda un consumo mínimo de 120 kg/persona al año. Frutas como la guanábana aportan nutrientes, fitoquímicos y antioxidantes de vital importancia para la salud humana, además de sustancias bioactivas como vitamina C, flavonoides, antocianinas y carotenoides, entre otros. En la presente investigación, frutos de guanábana (*Annona muricata* L. cv. Elita), fueron recolectados en estado de madurez fisiológica o de cosecha, en dos épocas de producción, para determinar sus características fisiológicas (tasa de respiración, producción de etileno y pérdida fisiológica de peso), y físico-químicas (rendimiento en pulpa, semillas y piel, sólidos solubles totales (SST), acidez total, pH y firmeza). Se pudo constatar que la producción de etileno alcanzó un máximo en el día 6 de la poscosecha, con un valor de $133,2 \mu\text{L kg}^{-1} \text{h}^{-1}$. Este parámetro presentó un comportamiento creciente durante la poscosecha, con picos en los días 4 y 6, coincidentes con los picos climatericos bifásicos de la respiración, el mayor de los cuales alcanzó un valor de $186,9 \text{ mg de CO}_2 \text{ kg}^{-1} \text{h}^{-1}$. Esto, probablemente es el activador de la cascada de eventos propios de la maduración, donde sobresalen los cambios los niveles de sólidos solubles totales, acidez y la firmeza de los frutos.

Palabras clave: Respiración, etileno, transpiración, fisiología poscosecha.

Abstract. Fruit consumption is increasing around the world, just as its population. The World Health Organization recommends a minimum consumption of fruit 120 kg/person - year. Fruits such as soursop provide nutrients, phytochemicals and antioxidants which are vital to human health, as well as bioactive substances such as vitamin C, flavonoids, anthocyanins and carotenoids, among others. In this research, soursop (*Annona muricata* L. cv. Elita) fruits were collected at physiological maturity in two production seasons for their physiological (respiration rate, ethylene production and physiological loss of weight) and physico-chemical characterization (pulp, seeds and skin yield, total soluble solids (TSS), total acidity, pH and firmness). We found that ethylene production peaked at day 6 after-harvest, scoring $133.2 \mu\text{L kg}^{-1} \text{h}^{-1}$. This parameter was found to increase during postharvest, with peaks on days 4 and 6, coinciding with the climacteric peaks of biphasic respiration, the largest of which reached a value of $186.9 \text{ mg CO}_2 \text{ kg}^{-1} \text{h}^{-1}$. This is probably the starter for the cascade of events that feature the ripening process, among which changes in TSS, acidity and fruit firmness were outstandingly visible.

Key words: Respiration, ethylene, transpiration, postharvest physiology.

Soursop (*Annona muricata* L.) is grown in the Caribbean and the equatorial belt of the Americas, primarily in Bermuda, Bahamas, Cuba, Dominican Republic, St. Vincent, Grenada, southern Mexico, Costa Rica, Puerto Rico, Colombia, Brazil and Ecuador. In addition, there are some commercial crops in Vietnam, Australia, New Zealand, Africa, Southeast China and Southern United States (Ramí *et al.*, 1995; Orellana and Martínez, 2002; Cuadros, 2008).

Colombia has 114,174,800 million hectares of total area, out of which 54% corresponds to sparsely studied, mostly forested lands (Arango and Bacanumenth, 1999). Of the remaining area, only 0.23% (equivalent to 259,409 ha) is devoted to fruit crops, out of which about 1,300 ha correspond to soursop plantations, with a production of $10,010 \text{ t year}^{-1}$, and $7.7 \text{ t ha}^{-1} \text{ year}^{-1}$ average yield. The departments of Valle, Tolima, Cauca and

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Cundinamarca are the most productive ones (Ramírez *et al.*, 1998).

In climacteric fruits such as soursop, ripening is a complex, genetically controlled process marked by the synthesis and degradation of new compounds (Salisbury and Ross, 1994; Taiz and Zeiger, 2006; Azcón, 2008). Some of the changes that occur during ripening and postharvest directly affect the shelf life and sensorial and nutritional quality of fruits (Hosakote *et al.*, 2005; Wu *et al.*, 2005; Ornelas *et al.*, 2008).

The annonaceae exhibit important metabolic changes, manifested as color and brightness variations, as well as decrease of green hue; all of them resulting from the degradation of chlorophyll to pheophorbide and clorofilida (Castro *et al.*, 2008). Another aspect that reveals intense metabolic activity is related to the hydrolysis of high molecular weight carbohydrates (starch type) to simpler compounds such as disaccharides and monosaccharides (mainly sucrose, glucose and fructose), usually due to high enzymatic activity of α and β amylases (DeMan, 1999; Kader, 2002).

Postharvest handling and processing of these fruits is clearly deficient. This can be largely attributed to lack of knowledge on many of the features that typify their ripening, among which physiological (Trincherro *et al.*, 1999) and physicochemical (Mercado *et al.*, 1998) ones certainly stand out.

In this context, the purpose of the present study was the assessment of the physiological and physicochemical features that express the most significant metabolic changes taking place in the soursop fruit (*A. muricata* L. cv. Elita) during postharvest, by means of a study conducted on fruits from the Cauca valley, in Colombia.

MATERIALS AND METHODS

The present research was carried out on soursop fruits obtained from the orchards of "La Española" farm (1,070 m.a.s.l., 23 °C average temperature, 1,225 mm annual precipitation, mean solar radiation of 4.8 W m² - d, and relative humidity of 83%), which belongs to the firm "Agrícola Varahonda", and is located in the municipality of Pradera, in the agroindustrial area of the Cauca Valley.

The fruits were collected at physiological maturity, which corresponds to 16 weeks after fruit set,

featured by the proper harvest color and firmness of this cultivar. On the same day, they were transported in styrofoam containers to the Fruit and Vegetable Laboratory of the Faculty of Agricultural Science at Universidad Nacional de Colombia, Medellín campus, where all the analyses were conducted.

For the analysis of the physiological traits, we used 12 experimental units (EU) on which, for a period of 10 d, we recorded the following parameters. In the first place, the respiration rate was determined by adapting the chemical method of Petenkoffer, which consisted in weighing and placing the sample in an airtight container. CO₂ resulting from respiration was driven through a basic solution (NaOH 0.1 N), which it partially neutralized. The amount of carbonic acid (H₂CO₃) produced by this reaction was calculated as the difference between the initial and final concentrations. The amount of CO₂ released by the fruit in 1 h of experiment at 23 °C was calculated by stoichiometry and expressed in milligrams of CO₂ kg⁻¹ h⁻¹ (Angueira *et al.*, 2003). In the second place, the production of ethylene was analyzed in a Varian™ 3800 gas chromatographer (GC) equipped with a 60 m long DB capillary column of internal diameter 53 mm (J & W Scientific™). On a Flame Ionization Detector (FID), we used Helium (He) carrier gas at a flow rate of 3 mL min⁻¹. The temperature conditions were 150 °C for the injection port, 280 °C for the detector, and 40 °C for the oven, which was then increased at a rate of 40 °C min⁻¹, to finally reach 300 °C. Each run took a total of 20 min. The areas under the curve obtained from the analysis were compared to a calibration curve based on the performance of a standard gas (Oxígeno de Colombia™, 20 ppm). Ethylene production was expressed in L of ethylene/kilogram/hour (adapted from Cruz *et al.*, 2007). Assessed on a daily basis, the physiological loss of weight was calculated by gravimetry as the difference with the initial record using an Ohaus™ Navigator 1121 analytical balance (Bernal, 1993).

Studied at a rate of 12 per day, 120 fruits were available for the physico-chemical assessment, covering a period of 10 days, during which they started senescence. The parameters in question were external and internal color (i.e., epidermal and endocarpic, respectively), assessed with an X-Rite™ SP-60 sphere spectrophotometer, measurement aperture size 4 mm, and illuminant D-65 with a 10° observer angle. From the reflection spectra we obtained the color coordinates CIE L*, a* and b*, where L* is a measure of brightness, a* represents

green (-) to red (+) chromaticity, and b^* stands for blue (-) to yellow (+) chromaticity. Three equatorial measures expressed as arithmetic average were taken on the epidermis of each EU. The same was done for the endocarp or pulp (Almela *et al.*, 2000). Pulp, seeds and skin yield values were obtained by gravimetry, the results expressed as percentage (Camacho and Romero, 1996). The concentration of total soluble solids (TSS) was determined at 23 °C using a Leica auto ABBE™ refractometer (0-32% measuring range), and the results were expressed in Brix degrees. Total acidity was obtained by potentiometric acid-base titration and expressed as percentage of malic acid using a CG-840B Schott™ potentiometer, which was also used to determine pH (Bernal, 1993). Unidirectional compression tests were applied to measure fruit firmness using a cylindrical stainless probe, diameter 8 mm, used at a penetration rate of 2 mm s⁻¹, to reach a deformation depth of 40 mm. The experiments were conducted under average laboratory conditions of 23 °C and 65% RH. The equipment was a TA-XT2™ texture analyzer operated through Texture Expert Excced 2.64 (Stable Micro Systems, London, UK 2002™) (Ciro *et al.*, 2005).

The results were statistically processed through standard deviation and arithmetic average (on STATGRAPHIC plus 5.1.), which were then plotted against time after harvest.

RESULTS AND DISCUSSION

Figure 1 shows the first respiratory peak on day 4 after harvest, with a production rate of 148.1 mg CO₂ kg⁻¹ h⁻¹ ± 9.43. On day 5, there was a slight decrease, followed by a sharp increase, reaching its maximum intensity (highest climacteric peak) on day 6, with a value of 186.1 mg CO₂ kg⁻¹ h⁻¹ ± 9.56 SD. This allows classifying this fruit as climacteric of the biphasic type, as reported by Worrel *et al.* (1994). This feature contrasts partially with Cherimoya (*A. cherimola* Mill), whose respiratory behavior has shown only one climacteric peak (Paull, 1996). These respiratory peaks are probably reflecting an shift in mitochondrial activity, which in turn results from increased availability of carboxylate substrates. The later are likely to be induced by starch enzymatic degradation resulting in the formation of low molecular weight carbohydrates due to very intense activity of specific tissues and structures (Bruinsma and Paull, 1984). Increase in CO₂ production during climacteric respiration is associated to the availability of large amounts of ATP, which is required for the biochemical and physiological processes that are in turn associated to ripening, and allow the fruit to reach both the desirable organoleptic features required for consumption and its later senescence. This feature is confirmed by the short shelf life and high perishability of this product (Kader, 2002).

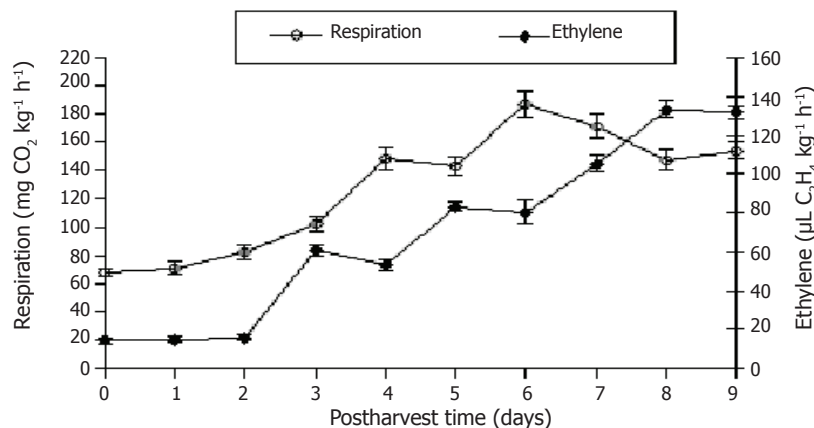


Figure 1. Respiratory behavior and production of ethylene in fruits of soursop (*Annona muricata* L. cv. Elita) stored at average conditions of 23 °C and 65% RH. The symbols show the mean, and the vertical bars the SD values for n = 12.

As for the production of C₂H₄, the lowest concentration records remained steady for the first 3 days after harvest. On day 4, there was an increase that reached a value of 44.9 µL of C₂H₄ kg⁻¹ h⁻¹, followed

by a slight decrease on day 5. Again on day 6, there was a raise, and then a new decrease on day 7, after which we observed a steady increase until day 9, when ethylene production reached a maximum of

133.2 $\mu\text{L kg}^{-1} \text{h}^{-1}$. This reveals an intense synthesis of this product during the studied period, accompanied by rapid respiratory activity.

As for the production of C_2H_4 , the lowest concentration records remained steady for the first 3 days after harvest. On day 4, there was an increase that reached a value of 44.9 $\mu\text{L of C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$, followed by a slight decrease on day 5. Again on day 6, there was a raise, and then a new decrease on day 7, after which we observed a steady increase until day 9, when ethylene production reached a maximum of 133.2 $\mu\text{L kg}^{-1} \text{ h}^{-1}$. This reveals an intense synthesis of this product during the studied period, accompanied by rapid respiratory activity.

Marking the end of the ripening stage and the onset of senescence, this process occurs without any external influence. It is characterized by a series of changes in the chemical composition of the pulp, involving the action of several enzymes such as polygalacturonase (PG), pectin methyl esterase (PME), endo-1-4 β -glucanase, α -arabinosidase and β galactosidase (β -GAL), all of which exhibit activity changes as part of the metabolic dynamics that induce ripening (Bruinsma and Paul, 1984; Brovelli *et al.*, 1999; Armida *et al.* 2011).

Physiological loss of weight. According to Figure 2, the physiological loss of weight showed a linear increase throughout the postharvest stage (represented as $Y = 2.15X + 1.19$; $R^2 = 0.98$), although a slight slow down could be observed since day 6, marked by a weight loss of 14.71%. Throughout the post-harvest stage (including ripening and senescence), the studied soursop fruits lost a total of 21.72% of their weight. In fruits of custard apple (*Annona cherimola* Mill) stored at 22 °C and 60% RH, this parameter has been observed to reach 14.2% on day 8 after harvest (Paull, 1996); while fruits of sugar apple (*Annona squamosa* L.) stored for 7 d at 4 °C and 95% RH have shown records of 6.7% (Poot, 2003). Remarkable physiological losses in fruit weight in the Annonaceae lead to their rapid physical deterioration as compared to other climacteric fruits such as sapote (*Pouteria sapota* Jacq.), which have been found to lose 10.8% of their weight in 6 d of storage at 25 °C (Díaz *et al.*, 2000); or mango (*Mangifera indica* L. cv. Namdok Mai), registering losses up to 16% after 17 d of storage at 30 °C. Physiological loss of weight is defined as the result of transpiration and respiration, which deteriorate the fruit by reducing tissue turgor (Sothornvit and Rodsamran, 2008).

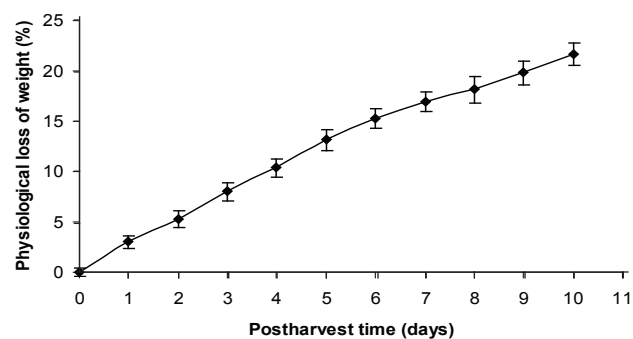


Figure 2. Behavior of physiological loss of weight in soursop fruits (*Annona muricata* L. cv. Elita) stored at average conditions of 23 °C and 65% RH. The symbols show the mean, and the vertical bars the SD values for $n = 12$.

Skin and endocarp color. Figure 3 presents the results of epidermis and endocarp tristimulus color assessment regarding coordinates L^* , a^* and b^* . Epidermal brightness (L^*) showed a slightly decreasing behavior until day 5 of postharvest, after which the process sped up, to be further intensified on days 7, 8 and 9, which correspond to overripening and the onset of senescence. In the pulp and endocarp, brightness decreased steadily throughout postharvest. For both

epidermis and endocarp, this parameter showed less variation in the stage corresponding to unripe fruit (days 0, 1 and 2 after harvest). The results obtained here for fruit brightness are consistent with those reported by Islam *et al.* (1996) and Umme *et al.* (1997).

As for the epidermis, coordinate a^* scored the most negative values in the immature fruit (day 0 after

harvest). Then it started to increase, a tendency that was later intensified during ripening and overripening, to finally show positive values in the last stage of post-harvest. Indicating a loss of green hue, this process is associated to decreased levels of chlorophyll b through chlorophyllase mediated hydrolysis to chlorophyllid and phytol (Yang *et al.*, 2009). For the endocarp this coordinate is not considered as important as for the the epidermis. However, it shows a decreasing behavior throughout post-harvest, coinciding with reports by Umme *et al.* (1997).

As for color coordinate b^* , the epidermis presents a slight and continuous decrease until day 4 of post-harvest, after which it drops quickly, and even quicker during ripeness. This results in a color intensity decrease, probably due to reduced levels of chlorophyll a, whose structure is similar to that of Chlorophyll b; the difference consisting in the replacement of group 3-formyl of the latter by group 3-methyl of the former. This small difference leads to changes in their visible absorption spectra, thus conferring to chlorophyll b solutions their characteristic green

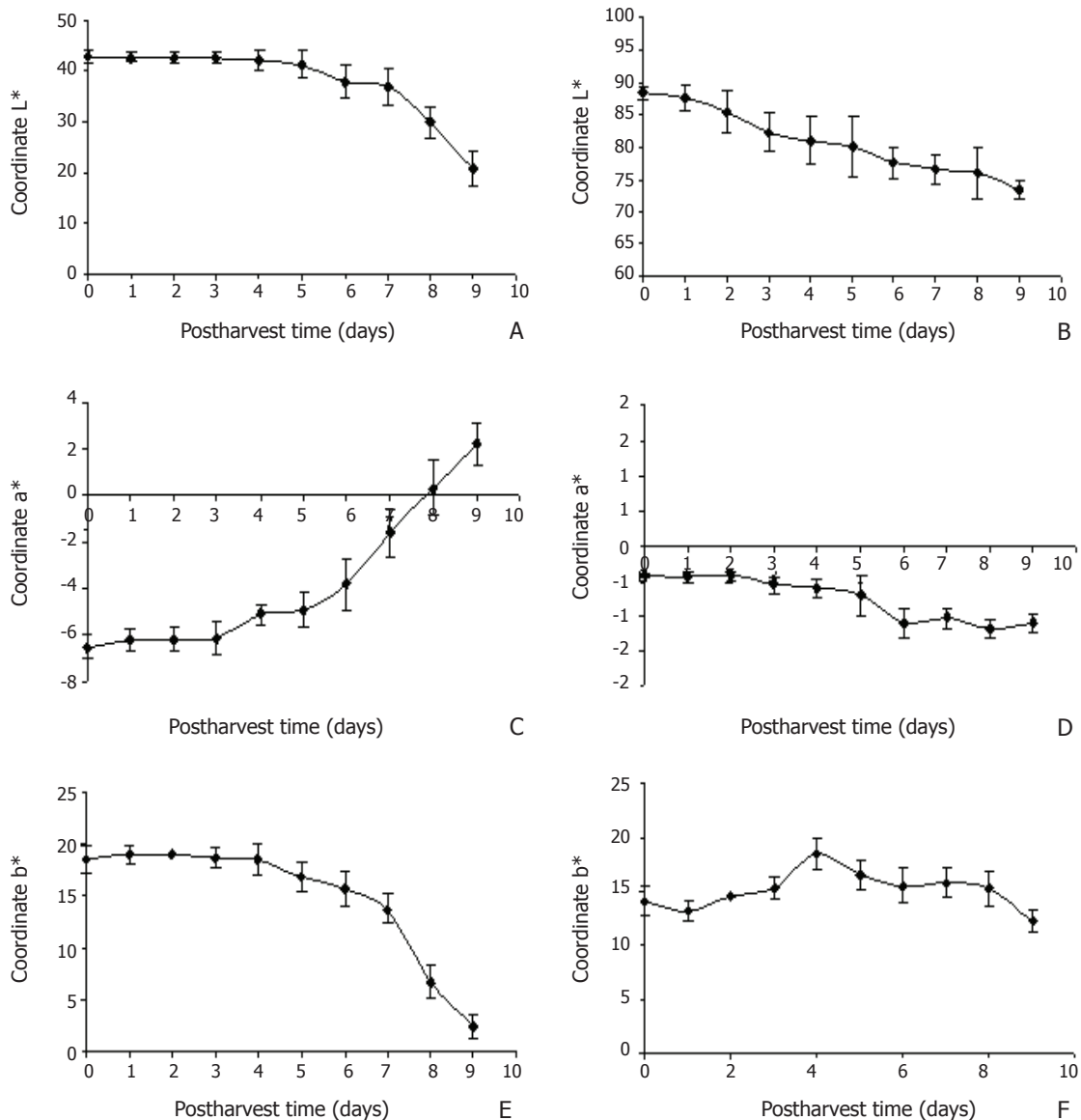


Figure 3. Scores of color coordinates L^* , a^* and b^* for the epidermis (charts A, C and E, respectively), and endocarp (charts B, D and F, respectively) of soursop fruits (*Annona muricata* L. cv. Elita) stored at 23 °C and 65% RH. Vertical bars show SD values for n = 12.

color, and blue to those of chlorophyll a. Thus, color coordinate b^* is likely to be associated to chlorophyll a, whose concentration reduces with maturation, bringing about a concomitant decrease of coordinate b^* . Regarding this same coordinate, the endocarp showed a slightly increasing behavior until day 4, and then a continuous decrement until ripening, when said color drop was more accentuated, as also reported by Islam *et al.* (1996).

Color coordinates L^* , a^* and b^* are indicators of soursop ripeness. Therefore, according to the reproducibility of the procedure used for their determination, its ease of application and generally low result variability, they could be used to determine harvest maturity in the field in a rapid, non destructive and especially useful manner.

Fruit yield. The main product obtained from a fruit is its pulp, a basic raw material for different agro-

industrial products or for direct consumption. In the case of soursop, the fractions corresponding to skin and seeds are of great importance as well (Camacho, 1995).

Table 1 presents the different fractions obtained from the studied soursop fruits, assessed on a daily basis during post-harvest. Pulp yield for day 6 (ripe fruit) was 60%, which is higher than a previous report by Camacho (1995), who reported a score of 55%, but lower than the Colombian Technical Standard (NTC, 5208), set at 74%. The average number of seeds was 129, ranging from 58 to 250. Average seed weight was 0.87 ± 0.11 g. The remaining fraction corresponds to the central axis of the fruit, and to losses due to processing. A previous study on other soursop cultivars reports values ranging from 90 to 266 seeds, with an average of 175. Endocarp reduction is probably due to the biochemical demand that is proper of the metabolic processes of ripening and overripening (Laboren, 1994).

Table 1. Participation of the main fractions of soursop fruits (*Annona muricata* L. cv. Elita) stored at average conditions of 23°C and 65% RH. The values correspond to the mean \pm SD for $n = 12$.

Days after harvest	Endocarp (%)	Epidermis (%)	Seeds (%)
0	70.9 \pm 2.7	18.9 \pm 1.37	4.8 \pm 0.90
1	69.8 \pm 3.3	20.5 \pm 1.22	4.1 \pm 1.15
2	66.8 \pm 6.6	20.6 \pm 1.31	4.8 \pm 0.86
3	68.9 \pm 3.8	18.6 \pm 1.94	4.3 \pm 0.83
4	65.5 \pm 1.4	17.2 \pm 1.59	4.9 \pm 1.22
5	63.3 \pm 2.5	21.5 \pm 1.87	5.1 \pm 0.57
6	60.0 \pm 2.2	19.7 \pm 1.63	4.9 \pm 0.85
7	61.5 \pm 1.2	22.8 \pm 1.60	4.6 \pm 1.04
8	60.1 \pm 3.1	21.8 \pm 1.77	4.2 \pm 0.98
9	59.8 \pm 3.4	22.0 \pm 2.71	5.2 \pm 1.13

Total soluble solids (TSS). Figure 4 shows a rapid increase in the level of TSS from day 1 to day 6 after harvest, when it reached a concentration of 12.8 °Brix. During this period, a typical climacteric fruit reaches peak production of CO₂, probably revealing the great metabolic dynamics involved in the activity of α and β amylases. During ripening, these enzymes hydrolyze starch to simpler carbohydrates such as mono and disaccharides (mainly sucrose, glucose and fructose). According to our results, TSS decrease as observed since day 6, may be the result of the consumption of these sugars as respiratory

substrates (DeMan, 1999; Kader, 2002). The concentration found in the present research coincides with that reported by Chaparro *et al.* (1993), who found concentrations of 13°Brix for ripe soursop fruits. The Colombian Technical Standard (NTC², 5208) establishes minimum values of 13.5 °Brix for this parameter at consumption ripeness; which is slightly higher than our findings. Just as well, our results were lower than those reported by Ojeda de Rodríguez *et al.* (2007) for different soursop cultivars

² Colombian Technical Standard

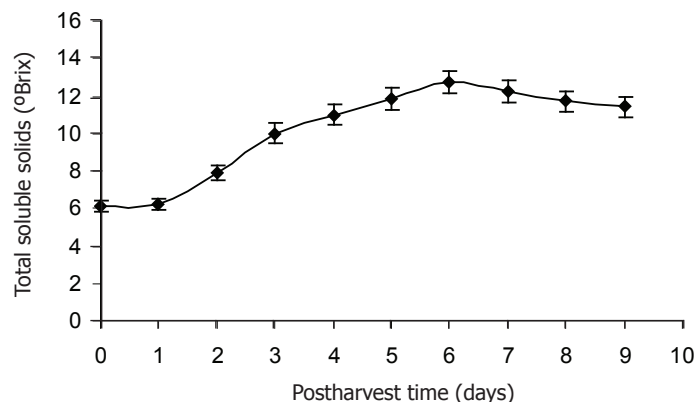


Figure 4. Behavior of total soluble solids (°Brix) in soursop fruits (*Annona muricata* L. cv. Elita) stored under average conditions of 23 °C and 65% RH. Symbols represent the mean, and vertical bars the SD values for n = 12.

at consumption ripeness, which ranged between 14 and 17 °Brix. Total acidity and pH.

Figure 5 shows total acidity behavior in the studied soursop fruits. This parameter increased throughout the climacteric stage to reach a maximum of 0.74% on day 6 after harvest, coinciding with ripeness. pH, whose minimum score was obtained on day 6 after harvest, naturally showed an inverse behavior to that of acidity. According to the results, soursop can be classified as a fruit of intermediate acidity (Camacho,

1995). During the climacteric period, the metabolic activity of this fruit coincided with the production of organic acids, which confer it its characteristic sensory contrast, probably due to the specific genetic load of this cultivar. In the post-climacteric stage, lower acidity may be due to the metabolic consumption of these organic molecules to provide the energy required by the fruit. In addition, many organic acids are involved as precursors of volatile substances, which are more abundant during this period (Park *et al.*, 2006).

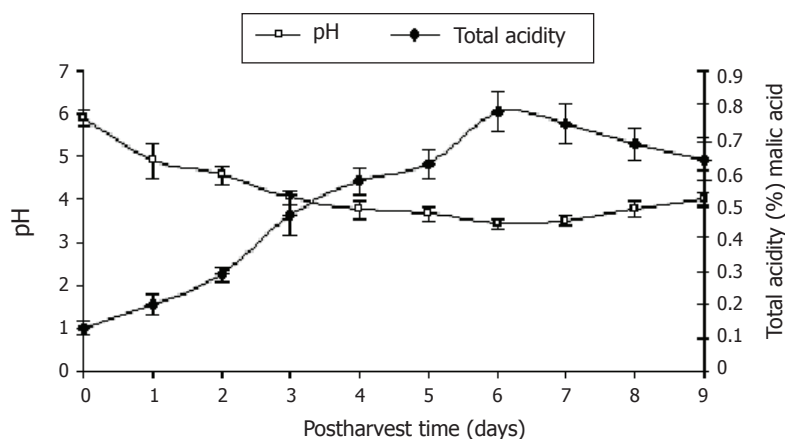


Figure 5. Changes in total acidity and pH during the postharvest of soursop fruits (*Annona muricata* L. cv. Elita) stored at 23°C and 65% RH (average conditions). Symbols represent the mean, and vertical bars the SD values for n = 12.

Total acidity as assessed in the present work was slightly lower than a previous report by De Lima *et al.* (2003), and is consistent (in terms of acidity and pH) with values

reported by Hernández *et al.* (1988). Our acidity and pH values are consistent with those recommended by the Colombian Technical Standard (NTC, 5208).

Firmness. This parameter showed great variability in the present study. Figure 6 indicates how immature fruits (day 0) were found to have an average strength of 80 N, a value that is considered to be very high, which persisted until day 2 after harvest. The maximum acceleration in the decline of firmness was observed between days 2 and 4. Since day 4, the curve decreases with a gentle slope, exhibiting respective values of 7.48 and 4.72 N on days 5 and 7 (ripe fruits). Overripe fruits (day 9) showed an average firmness of 3.62 N, which characterizes the tissue as extremely soft. The behavior of this parameter was consistent with that reported by Ciro *et al.* (2007). Other soursop cultivars have shown values of 60 N on day 0 after harvest, and a

similar behavior to that found in the current research during the remaining period (De Lima *et al.*, 2003). In this regard, changes in firmness are the result of the gradual degradation of cell wall polysaccharides, which are solubilized and depolymerized by the enzymatic action of PG, PME, β -GAL, xiloglucanasa and xylanase (Silveira, 2007; Sañudo *et al.*, 2008).

Furthermore, the decrease in firmness also affects the turgor loss underwent by the fruit due to transpiration as ripening progresses. It is important to note that – due to hydraulic pressure on the cell wall – swelling is responsible for cell firmness and rigidity (Muy *et al.*, 2004).

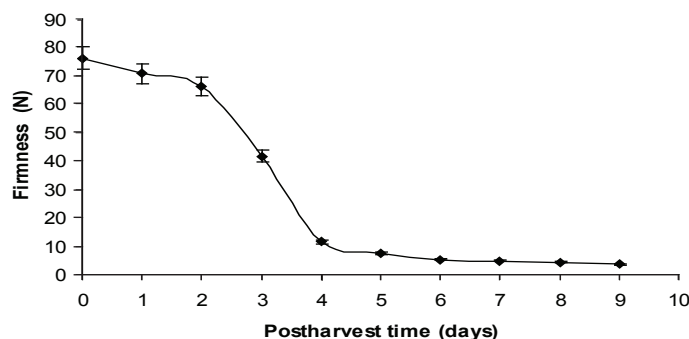


Figure 6. Effect of post-harvest time on the strength of soursop fruits (*Annona muricata* L. cv. Elita) stored at average conditions of 23°C and 65% RH. Symbols represent the mean, and vertical bars the values of SD for n = 12.

CONCLUSIONS

Ethylene production in the studied soursop fruits was observed to grow during post-harvest, with outstanding peaks on days 4 and 6, the latter being the largest one, with a production of 186.9 mg CO₂ kg⁻¹ h⁻¹.

The fruits reached their maximum ethylene production on day 8 after harvest, by scoring 133.2 μ L kg⁻¹ h⁻¹, when they were already ripening and close to senescence. In turn, the physiological loss of weight reached 14.7% on day 6, and 21.7% at ripeness.

During ripening and until day 6, the concentration of total soluble solids was found to increase; whereas during the overripening period it underwent a slight decline. However, the values found for the physico-chemical parameters, namely TSS, pH and total acidity expressed as malic acid are among those recommended by the Colombian Technical Standard for fruit soursop (NTC, 5208).

Attending color, coordinates L*, a* and b* allowed classifying fruit ripeness. Consumption ripeness was reached on day 6 after harvest, coinciding with the major climacteric peak, with a pulp yield of 60%.

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