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## Growth and maturation of pequi fruit of the Brazilian cerrado

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### Abstract

The objective of this study was to characterize the development of pequi fruit (*Caryocar brasiliense*) of the Brazilian *cerrado*. It takes 84 days (12 weeks) for pequi to develop with the onset of flowering in September and early fruit set in January. Pequi fruit showed a simple sigmoid growth curve, and its growth was characterized based on fresh mass and longitudinal and transverse diameters. The contents of titratable acidity, soluble solids,  $\beta$ -carotene, and vitamin C increased during fruit growth, reaching their maximum values at the 12th week (84 days) after anthesis. Pequi is a fruit with an extremely high respiratory activity; its respiratory rate decreased during its development. Pequi fruit has been classified as a non-climacteric fruit due to the decrease of both respiration and ethylene production rates during maturation and ripening.

**Keywords:** *Caryocar brasiliense*; development; ripening; respiratory activity; ethylene.

### 1 Introduction

*Cerrado* is the second largest Brazilian biome; it is overcome only by the Amazonian biome. It occupies 21% of the national territory and is considered the planet's last agricultural frontier. The Brazilian ecosystem has a high biodiversity, and it has the richest savanna in the world, with over 7,000 species and high levels of endemism. However, this amazing biome is not well appreciated (Ratter et al., 2003).

The consumption of native species by the local population, mainly the fruit plants, which are economically promising for the *cerrado*, is still low in due to extractivism. Among the fruit-bearing plants, it is worth highlighting the importance of the species *Caryocar brasiliense* Camb., popularly known as pequi fruit. The pequi fruit is known for its agreeable color, aroma, and flavor, and it has medicinal and aphrodisiac properties, according to popular belief. Pequi is a rich source of energy, proteins, fibers, minerals, and vitamins, especially vitamin C (105 mg of ascorbic acid per 100 g of pulp) and  $\beta$ -carotene (2,000 I.U.) (Rodrigues et al., 2009). However, the lack of information and research on it has contributed to huge levels of post-harvest losses of pequi fruit.

The pequi tree is an arboreal plant belonging to the family Caryocaraceae and to the genus *Caryocar* L., which includes about 20 species. In Brazil, there are at least eight of these species, and most of them are large-scale species and are found in the Amazonian forest vegetation. The species *C. brasiliense* is shorter, reaching a maximum height of 15 m (Rizzini, 1971). Its fruits are globose drupe consisting of a green pericarp enveloping

from one to four pyrenes, known as stones. The mesocarp is subdivided into external (leathery fleshy) and internal (yellow, fleshy edible part) and involves the woody, thorny endocarp and a white kernel or seed (Almeida et al., 1998).

The species *C. brasiliense* is widely spread in the Brazilian *cerrado*. It is found in a vast area that includes the states of São Paulo, Minas Gerais, Rio de Janeiro, Mato Grosso, Mato Grosso do Sul, Goiás, Tocantins, Bahia, Pará, Piauí, and Ceará (Almeida & Silva, 1994). It plays an important role in the life of the people of that region, both economically, with the sale of fresh fruits, and for local consumption.

This fruit is very popular and widely consumed, and it is known as "Cerrado Gold" due to its high nutritional, medicinal, melliferous, and ornamental value and high timber and oleaginous quality (Ribeiro, 2000). A number of studies have demonstrated the potential of use of fresh and low processed pequi fruit (Damiani et al., 2008; Souza et al., 2007; Rodrigues et al., 2007). It has been exported to Australia, which shows its great export potential to other countries.

There is no standardization and consensus about the ideal pequi fruit harvest maturity stage. They are normally collected from the ground after their abscission from the mother plant. The maturity stage at harvest determines their ultimate quality; when collected in an immature state, they show a poor quality, both sensorial and nutritional. On the other hand, if harvested overripe, they will reach senescence rapidly, hindering the use of technologies aimed at their conservation.

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The objective of this study was to characterize the development and maturation of pequi fruit (*C. brasiliense*) of the Brazilian *cerrado* by evaluating its physical and chemical parameters, respiratory activity, and ethylene production. These data will contribute to the understanding of the physiological processes during the development of the pequi fruit and will support the optimization of technological methods to minimize the post-harvest losses and increase its production.

## 2 Materials and methods

### 2.1 Plant material and experiment

The experiment was conducted from October 2004 to February 2005 in a native grassland area with a typical *cerrado* formation, located in the town of Itumirim (Altitude: 871m; Latitude: 21° 19' 01" South; Longitude: 44° 52' 16" West), in the southern part of the state of Minas Gerais, Brazil.

Around 60 specimens of the *C. brasiliense* trees of similar size were selected at random, and those with wide-open flowers during anthesis in different positions were identified. One hundred and fifty fruits were harvested immediately after fruit set and were divided into three equal batches, representing the replicates. After fruit development, the number of fruit available to be collected was low; therefore, each replicate consisted of 25 units.

The fruits were collected in weekly intervals (7 days apart) from the time they were set until the time when, still in the tree, they showed cracked outer skin (green epicarp) revealing the mesocarp (yellowish pulp, edible part).

The fruits were collected in the morning and packed in high-density polyethylene bags which were placed in cleaned foam boxes and transported (20-25 °C, approximately 1 h) to the Fruit and Vegetable Postharvest Laboratory of the Food Science Department at the Federal University of Lavras under environmental conditions.

After arriving at the laboratory, the fruits were immediately selected based on the absence of defects or pests and were washed with a neutral detergent and running water for the removal of surface dusts coming from the field, and were subjected to specific analyses.

### 2.2 Fruit analysis

The fruits were weighed (fresh mass), and the longitudinal and transverse diameters of the whole fruit and the stone (internal mesocarp including the thorny endocarp and seed) were measured. The color was determined at five different spots on the peel (epicarp) and pulp (internal mesocarp) in the CIE L\*a\*b\* color space using a Minolta CR-400 colorimeter (Lavras, Brazil).

The pulp  $\beta$ -carotene was extracted with acetone:hexane (4:6) and determined according to Nagata & Yamashita (1992); the results were expressed in international units (I.U.) and calculated by the equation  $\beta\text{-carotene} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$ ; where  $A_{663}$ ,  $A_{645}$ ,  $A_{505}$  and  $A_{453}$  are absorbance readings in the respective wavelengths. Ascorbic acid (after oxidation to dehydroascorbic acid) was determined by the colorimetric method

using 2,4 dinitrophenylhydrazine, according to Strohecker & Henning (1967).

Titrate acidity (TA) was determined by titration with a solution of 0.1 N NaOH using phenolphthaleine as an indicator; 20 g of the pulp were diluted in 100 mL of distilled water. The results were expressed as the percentage of citric acid in the solution (Instituto Adolfo Lutz, 1985); pH was determined using a Schott Handylab pH meter (Lavras, Brazil), according to the AOAC technique (Association of Official Agricultural Chemists, 1990). Soluble solids (SS) were determined by refractometry using a ATAGO PR-100 digital refractometer (Lavras, Brazil) with an automatic temperature compensation of 25 °C (Association of Official Agricultural Chemists, 1990).

### 2.3 Respiratory activity and ethylene production

In order to determine the respiratory activity and ethylene production, fruits at the 8th (56 days), 9th (63 days), 10th (70 days), 11th (77 days), and 12th weeks (84 days) after anthesis ( $n = 5$  fruits per group) were used. Each fruit was individually placed into a 590 mL flask at 20 °C in the absence of light. The flask was tight-sealed for 1 hour, and the analyses were performed every 6 hours for 3 days. After this period of time, gas samples were withdrawn through a silicone septum and the respiratory rate was determined by ethylene and CO<sub>2</sub> quantification. The content of CO<sub>2</sub> was determined directly in the flask using a PBI Dansensor gas analyzer (Model 9900) (Lavras, Brazil), and the results were expressed in mL CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>. Gas samples were taken from 10 mL vacuum tubes for 5 minutes for ethylene analysis; 0.5 mL of the gaseous samples were removed from the tubes and injected into the gas chromatograph (Varian Chrompack CP-3800 model fitted with flame ionization detector) (Lavras, Brazil) under the following conditions: Porapak Q packed column; injector temperature 250 °C; detector temperature 280 °C; column programmed with an initial temperature of 90 °C, which was increased after the initial 4.5 minutes at a rate of 100 °C per minute until it reached 220 °C to clean the column; draft gas nitrogen with column flush and pressure of 20 mL min.<sup>-1</sup> and 0.1 psi, respectively. The results were expressed in  $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$  based on the area of a standard of 90  $\mu\text{L kg}^{-1} \text{ h}^{-1}$ .

### 2.4 Statistical analysis

Statistical analysis was conducted using SISVAR statistical software (Ferreira, 2000). After the analysis of variance, significance level of the F test was determined. The means of the experimental period (weeks) were submitted to the polynomial regression, in which the models were selected according to the significance of F test of each model and according to the determination coefficient.

## 3 Results and discussion

### 3.1 Growth and development of the pequi fruit

Pequi fruit flowering started in September in Itumirim, Minas Gerais, Brazil, and its peak took place in November, coinciding with the rainy season. Fruit set was observed at first in January, with peak production in February, but some ripe fruits still were found in early March. According to Almeida et al. (1998), pequi

fruit flowering occurs from August to November, coinciding with the rainy period, and fruit set takes place from November to February. In a study on the Brazilian *cerrado*, Araújo (1995) reported that the flowering period took place before the onset of the rainy season, and the development and maturation of the fruits took place before the rainy period.

It took 12 weeks (84 days) from flower opening at anthesis until harvest for the pequi fruit to develop, which was determined when the fruit, still on the mother plant, exhibited cracks in the epicarp (peel). The period of time between anthesis and ripening could vary according to different fruit species. The life cycle of the fruits begins, in general, with fertilization, which is followed by steps such as set, growth, and maturation, including the ripening and senescence phase (Biale & Young, 1964).

Pequi fruit set and growth started with pollination and fertilization of the flower, followed by a fast growth of the ovary walls, which peaked at the initial set of the fruit at 7 days (1<sup>st</sup> week) after anthesis. The ovary development continued for 84 days (12th week). It is known that fruit growth starts in the floral primordium (Hulme, 1970). Some hormones can be involved in fruit growth; auxins are the main hormones responsible for the growth of the ovary wall and other floral parts that will turn into fruit (Biale & Young, 1964).

The set of the internal mesocarp (edible pulp) and the kernel (seed) of pequi fruit was observed from the 2<sup>nd</sup> week (14 days) and 3<sup>rd</sup> week (21 days) after anthesis, respectively. The removal of the kernel occurred at 56 days (8th week), when an increased rigidity of the stone was observed in that stage of fruit development, making it possible to remove the whole kernel at that time. The woody endocarp (thorns) was noticed on the 4th and 8th weeks; they were already stiff and completely set. The yellowish color typical of the internal mesocarp could be observed from the 9th week (63 days) after anthesis.

There was a significant increase in the mass, longitudinal diameter, and transverse diameter of the pequi fruit for the 84 days during its development. The pequi fruit showed an increased mass during its development, from an average of

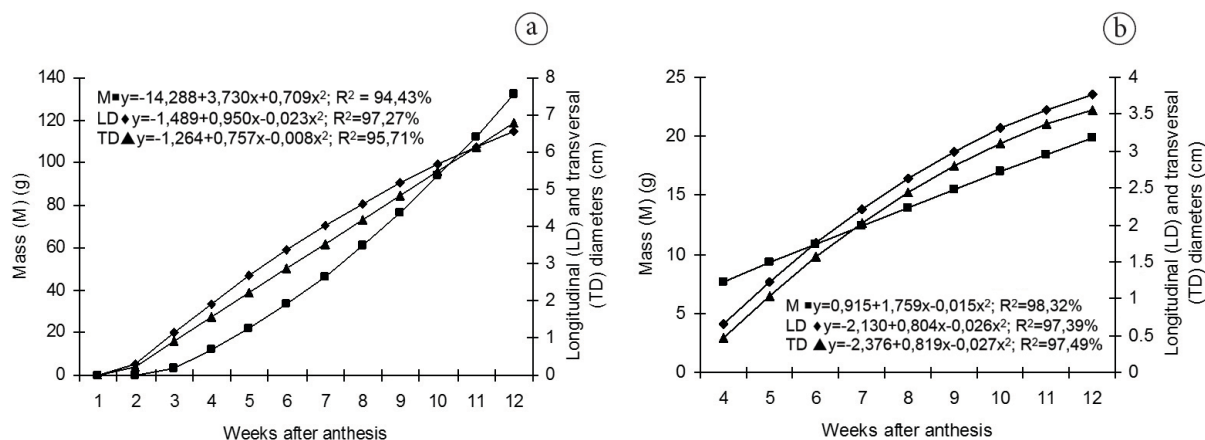
0.10 g at the early set of the fruit (1<sup>st</sup> week) to 119.26 g at the end of the development period, at 84 days (12th week) (Figure 1a).

The maximum values of longitudinal (6.26 cm) and transverse (6.21 cm) diameters were found at the 12th week of its development (Figure 1a).

The mass of the pequi fruit stone ranged from 7.71 to 19.33 g from 28 to 84 days after anthesis, respectively (Figure 2b). The longitudinal and transverse diameters values were 3.65 and 3.40 cm, respectively, at the 12th week after anthesis (Figure 1b).

The variables shown in Figure 1 revealed a pattern of accumulative growth during fruit development. The growth pattern of pequi exhibits a simple sigmoid pattern, as shown by those variables. Therefore, one can distinguish the developmental periods of the fruit. Growth was slow from the early developmental stages of the fruits up to about 14 days (2nd week) after the opening of the flower. From that period onwards, there was a fast growth which continued up to 84 days (12th week). Based on the longitudinal diameter of the pequi fruit, growth rate reduced at 77 days (11th week) up to the end of the experimental period at 84 days (12th week) after anthesis, at fruit harvest. The simple sigmoid growth curve is characterized by fresh weight and fruit diameter, and it has three developmental stages: an initial slow growth (one to two weeks after pollination), a rapid increase in size (for about 3 weeks), and a final decline in the growth rate (until maturation) (Hulme, 1970). This growth pattern has also been reported for apple, pear, dates, pineapple, avocado, mango, pineapple, banana, strawberry, melon, and orange (Biale & Young, 1964; Gortner et al., 1967; Coombe, 1976).

During pequi fruit development, changes in the color of its husk and stone were observed by the values of the coordinates  $L^*$ ,  $a^*$  and  $b^*$ . The  $L^*$  and  $a^*$  values exhibited a quadratic behavior, increasing gradually over the fruit development (Figure 2a). The relationship between these two parameters indicates the loss of the brightness of the green color with fruit growth, changing from dark-green ( $L^*=4.65$  and  $a^*=-24.82$ ) to light green ( $L^*=36.80$  and  $a^*=-5.86$ ). The green color results from the presence of chlorophyll, and green color loss is associated with



**Figure 1.** Adjusted mean values and regression equation of the mass and longitudinal and transverse diameters of the pequi fruit (*C. brasiliense*) (a); mass and longitudinal and transverse diameters of the stone (internal mesocarp along with the woody mesocarp and seed) (b) during fruit development.



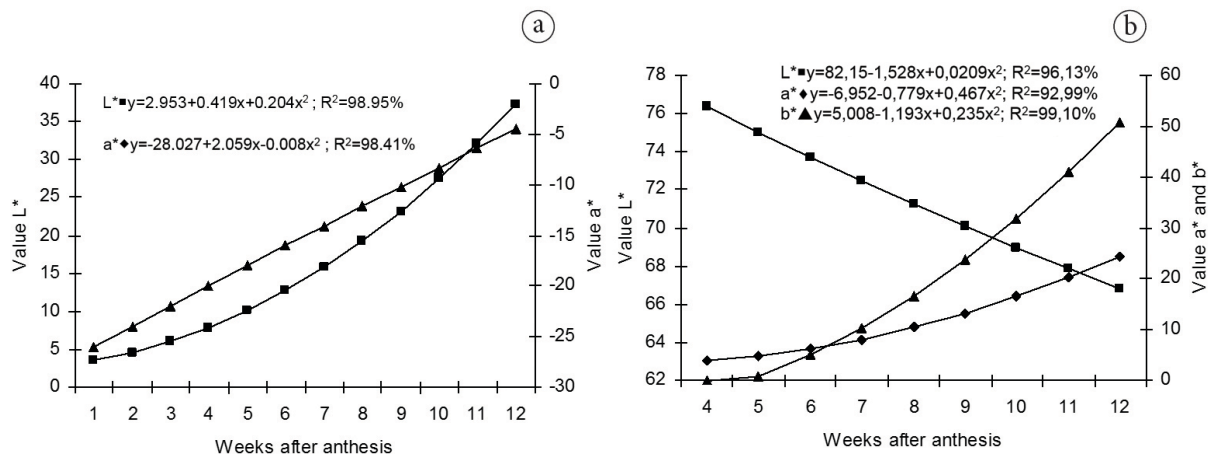
its degradation. The loss of that pigment is due to its structural decomposition, owing to several factors that can affect separately or jointly, including decreases in pH that are mainly caused by the accumulation of organic acids in the vacuoles, oxidative systems, and chlorophylases (Chitarra & Chitarra, 2005).

The most drastic change observed during the development of pequi fruit stone was its pigmentation, characterized by the yellowing observed from the ninth week onwards (63 days) after the flower bud opening; such color change was intensified during the stone development. Coordinate  $L^*$  decreased with the development of the internal mesocarp, indicating pulp browning. Yellowing was marked by the increase of the value  $b^*$  during fruit development. The average values of  $L^*$  and  $b^*$  ranged from 75.87 to 67.05 and 1.05 to 45.63, respectively. Variable  $a^*$  also increased with the growth of pequi fruit stone (Figure 2b).

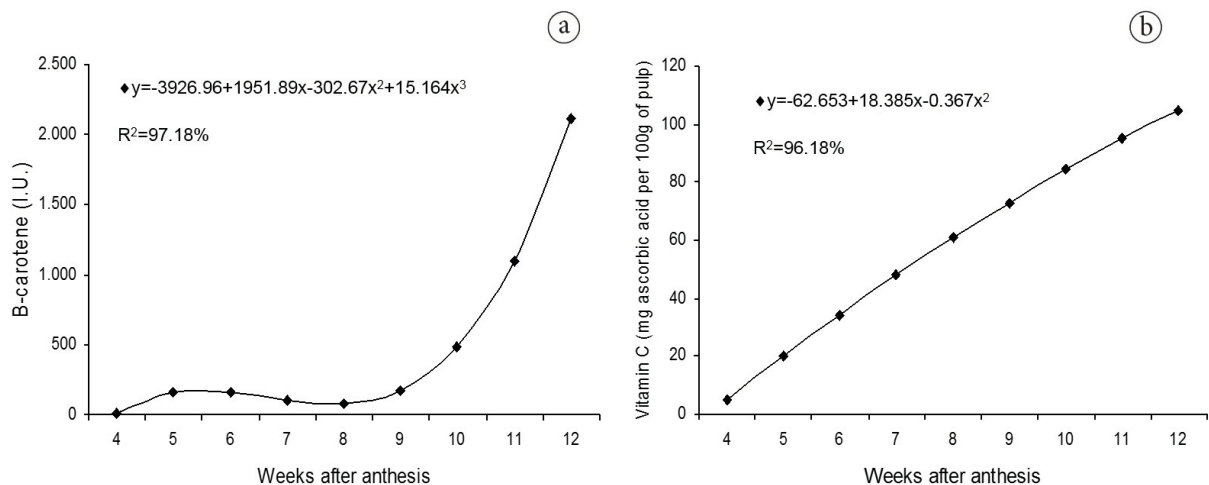
The variable  $\beta$ -carotene in the internal mesocarp (edible part without thorns) proved unstable up to 53 days (the 8th week), with a significant increase instability up to 84 days (the

12th week), reaching the contents of 2,235.66 I.U. (Figure 3a). These data are consistent with that of coordinate  $b^*$  (Figure 2b), allowing us to associate the increase in the yellow pigmentation with the synthesis of that carotenoid. The changes in fruit color during its maturation can be due to chlorophyll degradation with the gradual formation of pigments, including carotenoids (such as that in the banana), or simply due to the synthesis of these carotenoids (Brady, 1987).

There was an increase in the content of ascorbic acid, and the maximum value of 98.84 mg of ascorbic acid per 100 g of pulp (Figure 3b) was reached at 84 days (the 12th week) after anthesis. Similar results have been reported by Teotia et al. (1970) and Dhillon et al. (1987), who found the highest amounts of ascorbic acid in the late stages of guava maturation. The biosynthesis of ascorbic acid in plants is not completely understood. According to Smirnoff et al. (2001), ascorbic acid synthesis in plants begins with several precursors, including D-mannose and L-galactose, which arise preferentially via D-galacturonic acid.



**Figure 2.** Adjusted average values and regression equations of coordinates  $L^*$  and  $a^*$  of the peel (a) and coordinates  $L^*$ ,  $a^*$ , and  $b^*$  of the stone (internal mesocarp including the woody endocarp and seed) (b) of the pequi fruit (*C. brasiliense*) during fruit development.



**Figure 3.** Adjusted mean values and regression equations of  $\beta$ -carotene (a) and vitamin C (b) of the internal mesocarp (edible pulp) of the pequi fruit (*C. brasiliense*) during its development.

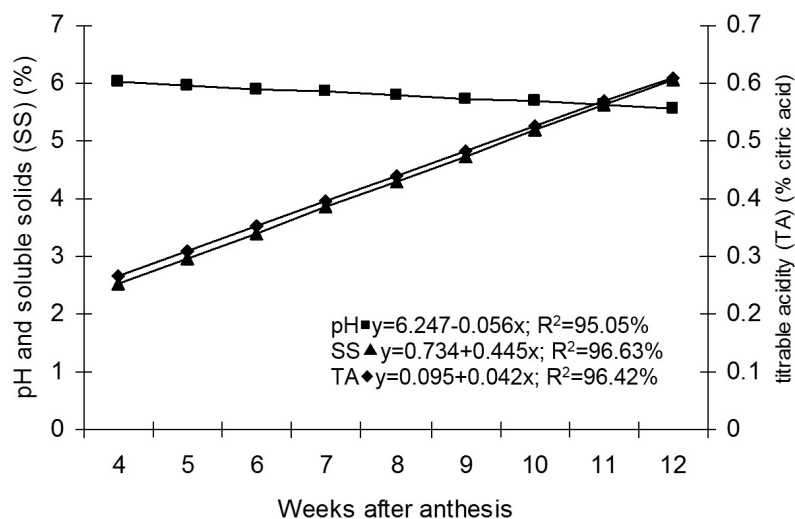
TA showed low values during the development of pequi fruit, with an early value of citric acid of 0.26% at 28 days (the 4th week) after anthesis. The amount of citric acid increased fast with fruit growth and reached a maximum value in the final developmental stage, 0.60% at 84 days (the 12th week). An opposite but consistent behavior was found for pH, which decreased with the development of the fruit pulp (Figure 4). Organic acids are intermediary compounds of fruit respiratory metabolism and are stored in the vacuoles of the cell; they are fundamental for the synthesis of phenolic compounds, such as lipids and volatile aromas (Brady, 1987). Acidity content tends to decrease during the maturation process due to the acid oxidation in the tricarboxylic acid cycle as a result of respiration (Biale & Young, 1964). The AT of the Pequi fruit varies from 0.9% to 2.0% of citric acid, depending on the maturation stage of the fruit (Vilas Boas, 2004).

SS increased gradually with the development of the fruit, ranging from 2.83% at the 4th week to 5.90% at the 12th week after anthesis (Figure 4). Soluble solids represent the water-soluble compounds present in the fruit, including sugars, vitamins,

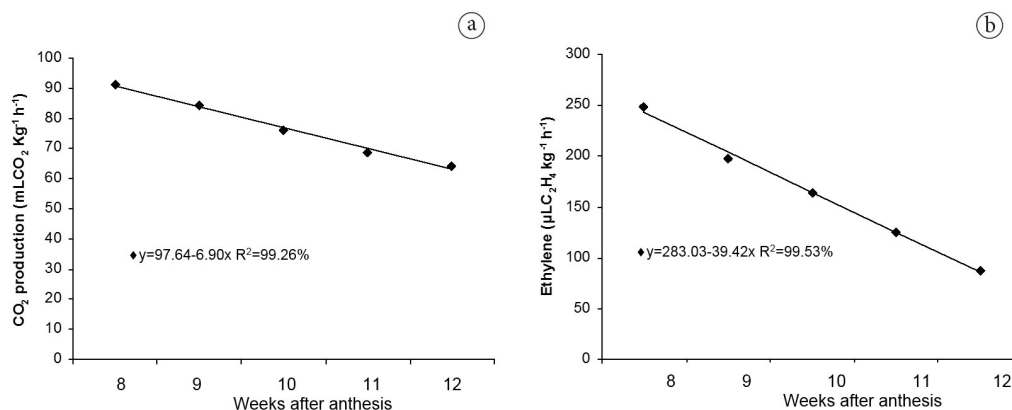
acids, aminoacids, and a few pectins. The SS content depends upon the maturation stage in which the fruit is collected and generally increases during maturation by the biosynthesis or degradation of polysaccharides (Chitarra & Chitarra, 2005). Sugar accumulation is associated with the development of the fully edible (ripe) form of the fruit. The same sugars can be derived from the sap of the plant and starch degradation (Vilas Boas, 2004). According to Vilas Boas (2004), SS content in the pequi fruit pulp depends on its maturation stage at the time of harvest, and this content can vary from 5.0% to 9.0%.

### 3.2 Respiratory activity and ethylene production

During the developmental stages of pequi fruit, decreased respiratory activity and ethylene production were found, and at the 8th week (56 days after anthesis) showed ethylene production about three times higher than that of the fruits at the 12th week ( $248.73 \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$ ) (Figure 5b). Respiratory activity decreased with fruit development, varying from  $91.12$  to  $64.3 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  at the 12th week (84 days after anthesis) (Figure 5a).



**Figure 4.** Adjusted mean values and regression equations of pH, TA, and SS of the internal mesocarp (internal pulp) of the pequi fruit (*C. brasiliense*) during its development.



**Figure 5.** Adjusted mean values and regression equations of respiratory activity ( $\text{CO}_2$  production) (a) and ethylene production (b) of the pequi fruit (*C. brasiliense*) during its development.

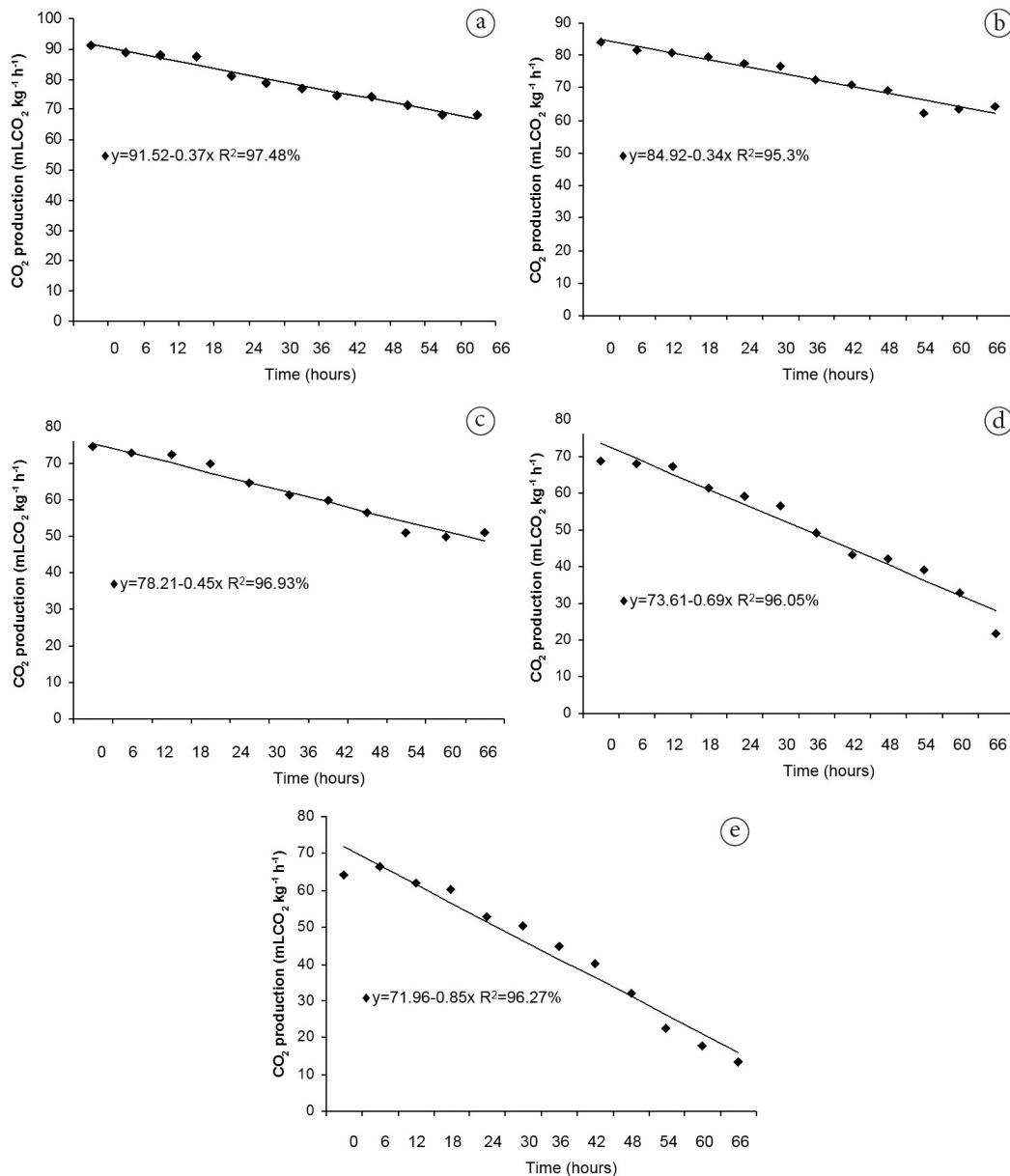
Respiratory activity was also determined at five different developmental stages of pequi fruit; specifically, it occurred every 6 hours for 3 days. Reduced values of  $\text{CO}_2$  were observed over the experimental period regardless of the development stage investigated (Figures 6a, b, c, d, and e).

Kader (2002) provided a classification for fruits and vegetables according to their respiratory rate; vegetables with a respiratory activity higher than  $60 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  have an extremely high respiratory rate. Pequi fruit had  $\text{CO}_2$  production around  $64.3 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  at the 12th week after anthesis; thus, it is considered as a fruit with an extremely high respiratory rate. High respiratory activity results in a reduced storage life of plant

products. Conversely, lower respiratory rate will increase the postharvest life of the product (Vilas Boas, 1999).

The rates of  $\text{CO}_2$  and ethylene production (Figures 5 and 6) showed a non-climacteric fruit behavior, with a decrease of these variables during the maturation stage. In this case, ripening occurs only if the fruit is attached to the plant, which is different from what occurs to climacteric fruits, which are able to continue ripening after harvest (Biale & Young, 1964).

According to the physical and chemical parameters evaluated, pequi fruit maturation can occur from the 9th week onwards (63 days), after anthesis. During this period of time, the synthesis of  $\beta$ -caroten and the significant increases in the contents of



**Figure 6.** Adjusted mean values and regression equations of respiratory activity ( $\text{CO}_2$  production) performed every 6 hours for 6 days at different developmental stages of pequi fruit (*C. brasiliense*). (a) 8th week – 56 days; (b) 9th week – 63 days; (c) 10th week – 70 days; (d) 11th week – 77 days; and (e) 12th week – 84 days

vitamin C, titrable acidity, and soluble solids occurs before complete maturation of the fruit. Gortner et al. (1967) define maturation as the sequence of biochemical, physiological, and structural changes in fruits that mature them to an edible form.

## 4 Conclusions

Based on the parameters of fresh mass and longitudinal and transverse diameters of the whole fruit and stone, it can be said that it took 84 days (12 weeks) for pequi fruits (*C. brasiliense*) from the Southern part of Minas Gerais state to develop reaching full maturity exhibiting a growth pattern similar to the simple sigmoid curve. Pequi is a fruit with an extremely high respiratory pattern; thus, it can be classified as a non-climateric fruit due to the decrease in CO<sub>2</sub> and ethylene production during its development. The maturation stage of pequi fruit began at the 9th week (63 days), and the fruit can be collected at the 12th week (84 days), a time period that coincided with the maximum contents of  $\beta$ -carotene, vitamin C, titrable acidity, and soluble solids.

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