

Characterization of growth and development of pumpkin cv Mini Jack fruits

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ABSTRACT

The physiological changes that occur during the growth and development of Mini Jack pumpkins fruits can lead to post-harvest losses, and shorten their shelf life. Thus, this work aimed to characterize the growth and development of the pumpkin cv Mini Jack. For this, fruits were harvested at 0, 5, 10, 15 and 20 days after anthesis (DAA) and evaluated for respiratory rate, height, diameter, weight, skin color, firmness, skin and pulp thickness, concavity fruit, pH, acidity, total soluble solids, carotene in the skin and pulp, reducing sugars, non-reducing sugars, and total sugars. During the growth and development of the fruits, there was an increase in the levels of total sugars, total soluble solids, and changes in carotene levels up to 10 DAA. The levels of total carotenoids increased significantly throughout the development of the fruits reaching the highest values at 20 DAA. It was possible to verify that the respiratory rate of the fruits was high at the beginning of development presenting reduction and stabilization at 10 DAA. The weight, height and concavity of the fruits increased up to 10 DAA. Thus, the ideal point for harvesting the fruits of the Mini Jack pumpkin occurred between 10 and 15 DAA.

1. Introduction

Pumpkins belong to the Cucurbitaceae family and the *Cucurbita* genus, with about 130 species cataloged and distributed throughout the world, including wild and cultivated varieties. *Cucurbita maxima*, *Cucurbita pepo* L., *Cucurbita moschata* Duchesne ex Poir, *Cucurbita ficifolia* and *Cucurbita argyrosperma* are the most important agricultural species (Blanca et al., 2011; Valdivieso et al., 2015). Among these, *Cucurbita pepo* is the one with the greatest genetic variability (Paris, 2001; Valdivieso et al., 2015). Pumpkin fruits are commonly used in food, including in the preparation of hot or cold desserts, salted, stewed or cooked; in addition to being, in some places, also used in the decoration of environments.

Small cultivars such as the ‘Mini Jack’ (*Cucurbita moschata*) developed by the Dessert Seed Company, from the cross between the Squash Wild Nu and Jack O’Lantern pumpkins. The smaller size of these pumpkins facilitates transport, storage and are a consumption alternative for small families (Echer et al., 2014).

Despite the economic and nutritional importance of the pumpkin culture, little is known about the growth and development of the fruits. Vidigal et al. (2021) and, Marouelli et al. (2017) evaluated the growth of Tetsukabuto pumpkin as a function of different doses of nitrogen fertilization and irrigation depths. However, none of these studies described the physiological changes that occur during fruit growth and development. This knowledge makes it possible to describe the different physical, chemical, biochemical and physiological processes since the

Abbreviations: a*, green-red coordinate; b*, blue-yellow coordinate; DAA, days after anthesis; ETo, the reference evapotranspiration; h, Hue angle; RCR, the relative growth rate; TTA, total titratable acidity; TSS, total soluble solids.

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formation of the organ. This favors the proper management of the crop, avoiding economic losses with appropriate decision-making in the stages of the production chain.

The development stages of a fruit consist of growth, maturation, physiological maturity, ripening and senescence (Coombe, 1976; Luo et al., 2020). Thus, through the evaluation of these phases, it is possible to establish parameters to define the appropriate harvest point, which influences the production of fruits with higher post-harvest quality. Altmann et al. (2019) monitored the development and maturation of jaboticabeira fruits and found an increase in soluble solids, which ranged from 7.0 graus Brix in green fruits 28 days after flowering (DAPF) to 11.9 graus Brix signaling the advance of maturation. The maintenance of quality and longer storage time of fruits and vegetables are linked to the ideal harvest point of fruits and vegetables. Knowing this factor guarantees the efficient maintenance of quality and the increase in fruit consumption time (Botelho et al., 2019).

Studies that evaluate the growth pattern of pumpkin cv Mini Jack are incipient. Therefore, the objective of this work was to evaluate the respiratory rate, identify the growth pattern and characterize the physicochemical and biochemical changes that occurred during the different stages of growth and development, thus proposing the ideal point for harvesting.

2. Material and methods

2.1. Experimental area, plant material and experimental design

The study was conducted at the Experimental Farm, located in the municipality of São Cristóvão (latitude 10 55' 27" S, longitude 37 12' 01" W and altitude of 46 m).

The climate of the region is classified as As, tropical rainy, with average annual temperature around 25.2 °C, dry summer and average annual precipitation of 1300 mm, according to the Köppen classification, with precipitation concentrated between the months of April and September (Santos et al., 2009).

The experimental area consisted of three ridges of 19 m long x 1.2 m wide, using a spacing of 1.0 m between plants, in a single row, totaling 60 plants. Borders were placed on the sides of the experiment containing the same pumpkin variety.

Pumpkin seeds of the cultivar Mini Jack were obtained from Isla®. The plants were obtained by sowing directly in the hole, using two seeds per hole. After germination, thinning was performed, keeping the most vigorous plants in the field. Foundation fertilization (before sowing) and top dressing (15 days after planting) were carried out. In each one, 30 ton ha⁻¹ of N (urea), 30 ton ha⁻¹ of K₂O (potassium chloride) and 30 ton ha⁻¹ of P₂O₅ (monoammonium phosphate) were used (Sobral et al., 2007). A drip system was used to irrigate the plants, the amount of water used for irrigation was calculated based on the reference evapotranspiration (ET₀) values reported by the meteorological station.

The experiment was carried out in two cycles, between the months of September and November 2021. A completely randomized design was used, with 16 replications per stage of development for the physicochemical and biochemical analyses, totaling 80 fruits at the end of the analysis. For the respiratory rate, 4 fruits were used per stage of development, at the end of the analysis, a total of 20 fruits were analyzed.

2.2. Marking the flowers and harvesting the fruits

The beginning of flowering took place one month after planting. Flower markings were made daily due to flowering irregularity and to better monitor flower/fruit growth and development. Marking was performed after anthesis, where flowers were selected and randomly marked. The selection of flowers was based on their homogeneity within each stage of development. Subsequently, after the differentiation of the fruits, the collections were started for the physicochemical, biochemical and respiratory rate analyses. Fruits that showed uniformity in size and

color during their growth and development were harvested. The harvest of the fruits took place between 7:00 and 9:00am. Fruits were collected at 0, 5, 10, 15 and 20 days after anthesis (DAA) (Fig. 1).

To determine the respiratory rate and ethylene production, it was applied the methodology of Furtado et al. (2018), with adaptations. For the analysis at development time 0, 120 mL flasks were used, 1500 mL flasks were used for development times 5 and 10, and 2000 mL flasks were used for the other times. The levels of carbon dioxide (CO₂) and ethylene released, for each development time (0, 5, 10, 15 and 20 DAA), were analyzed in four fruits, totaling 20 fruits at the end of the analysis. The fruits were placed individually in hermetically sealed jars. With the aid of a syringe, 1 mL aliquots of CO₂ and ethylene were collected after 0.5 h and 1 h of vial closure.

The analysis of CO₂ and ethylene concentration was performed in gas chromatograph (Varian, model CP-3380), equipped with a series-connected thermal conductivity (TCD) and flame ionization detector (FID) and an capillary column (RESTK, model Rt®- Q-BOND). The carrier gas was helium, with a flow of 5 mL/min. The temperatures of the column, the inlet, the TCD detector and the FID detector were 30, 200, 120 and 200 °C, respectively. The chromatographic analysis time was 120 s and the CO₂ and ethylene retention time was 60 and 102 s, respectively. The quantification of CO₂ and ethylene was performed by comparing the peak areas of the samples with the peak areas of the CO₂ and ethylene standard of known concentration, using the Galaxie Workstation software. Results were expressed in% CO₂ and in mg L⁻¹ of ethylene. After the respiratory rate analysis, the fruits were used in the physicochemical and biochemical analyses.

2.4. Physicochemical and biochemical analyzes

The growth and development of the pumpkin cv Mini Jack fruits was monitored by determining the physical, chemical and biochemical changes that occurred at each stage of development, for which the following analyzes were performed: fruit weight, relative growth rate (RCR), height, diameter, pulp and skin thickness, concavity, firmness, skin color, total soluble solids, titratable acidity, ratio, pH, reducing, non-reducing and total sugars, total carotenoids.

The individual weight of the fruits, expressed in grams, was determined on a digital scale, weighing each fruit at different stages of development.

The relative growth rate (RCR) was calculated according to the methodology of Cairo et al. (2008) and the following equation was used:

$$RCR = \frac{\ln P_2 - \ln P_1}{t_2 - t_1}$$

where: LnP1= Neperian logarithm of weight 1, LnP2= Neperian logarithm of weight 2, t1= time 1, t2= time 2.

The height, diameter, thickness of the pulp and skin and the concavity of the fruits were observed with the aid of a digital caliper with a precision of 0.1 mm. For the last four variables mentioned, measurements were performed in the equatorial region of the fruits. Measurements were performed on each fruit throughout its development and values expressed in millimeters.

Firmness was measured using a 53205TR benchtop digital penetrometer (Fruit Pressure Tester, Italy) with an 8 mm probe. To measure firmness, the epicarp of the fruits was removed. Measurements were performed in the equatorial region of the fruits, with three readings for each fruit and results expressed in Newton (N).

The color of the bark was measured from the values of the Hue angle (°h). The pitch angle h starts on the +a* axis and is given in degrees; where 0° (red), 90° (yellow), 180° (green) and 270° (blue) (Konica Minolta®) (McGuire, 1992). Values were obtained using a CR-400 colorimeter (Minolta Sensing Inc., Japan). Three readings were performed in the equatorial region of the fruits.

Total soluble solids (TSS) of the pulp were determined through direct reading of the cell juice extracted by a manual pressing and read using a

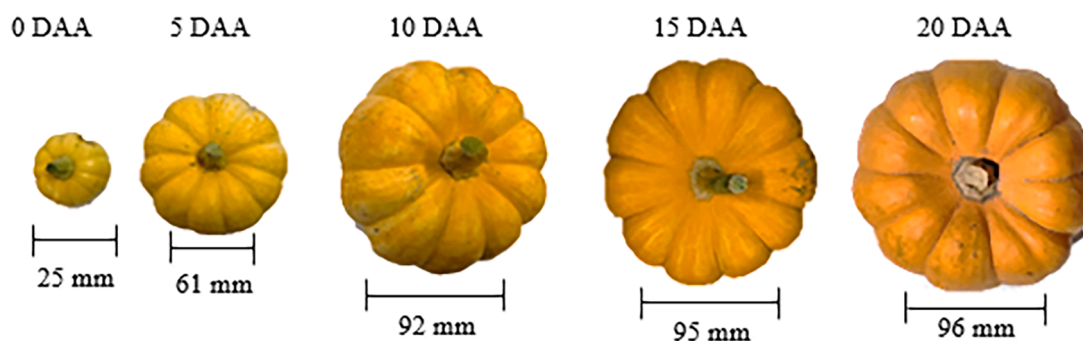


Fig. 1. Mini Jack pumpkin fruits (*Cucurbita pepo* L.) at 0, 5, 10, 15 and 20 days after anthesis. September to November 2021.

digital refractometer model HI (Hanna Instruments, Romania) according to the method proposed by Association of official analytical chemists (AOAC, 2000) the values obtained were expressed in%.

The pH was obtained by reading 5 ml of pumpkin extract with the aid of a digital pH meter according to the method proposed by the Instituto Adolfo Lutz (IAL) (1985). Total titratable acidity (TTA) was determined by titration with 0.1 mol/L sodium hydroxide (NaOH) solution of 5 ml of extract diluted in 25 ml of water, the TTA results were expressed in grams of malic acid/100 ml (IAL, 1985). The ratio was obtained through the ratio between TSS and TTA (TSS/TTA).

The analysis of reducing sugars was performed according to the Dinitrosalicylic acid method proposed by Miller (1959). The extract was prepared using 2.0 ml of pumpkin extract, diluted in 100 ml of distilled water. An aliquot of 0.04 mL of the extract was mixed with 1.1 mL of water and 1.0 mL of Dinitrosalicylic acid solution to obtain the samples, followed by shaking and incubation in a water bath at 100 °C for 5 min, was performed in a spectrophotometer at 540 nm, in spectrometer SP1105, using glucose as a reference to obtain the standard curve, the values obtained were expressed in%.

Total sugars were determined by the Anthrone method (Yem & Willis, 1954). The extract was obtained by diluting 20 µL of pumpkin extract in 9.98 mL of distilled water. The samples were prepared in an ice bath, adding 1 mL of the extract, 4.0 mL of the 0.1 % anthrone solution to a tube, followed by stirring and resting in a water bath at 100 °C for 3 min. Samples were read in a spectrophotometer at 620 nm, in spectrometer SP1105, using glucose as a reference to obtain the standard curve.

Total carotenoids were determined and calculated according to the method proposed by Lichtenthaler (1987). The following equation was used to calculate the total carotenoid content:

$$\text{Total carotene} = (1000A_{470} - 1.91Ca - 95.15Cb) / 225$$

The values obtained of the total carotenoids were expressed in µg/100 g.

2.5. Data analysis

The results of the analysis were submitted to the analysis of the minimum significant difference in a multiple comparison test, in which differences between two treatments greater than the sum of two standard deviations were considered significant at 5 % probability (Mawele Shamaila et al., 1992).

3. Results

3.1. Physical changes in the development and growth of Mini Jack pumpkin fruits

Initially, a rapid increase in the weight of the Mini Jack pumpkin fruits was observed, where at stage 0 of development the harvested fruits weighed an average of 8 g, fruits harvested at ten DAA presented

average weight values of 258 g. Subsequently, it was observed that the values referring to the weight of the fruits stabilized, reaching 287 g at 15 DAA (Fig. 2A).

Similarly to what was verified with the accumulation of fresh fruit mass, there was an increase in the values referring to concavity, height and in the thickness of the fruit pulp in the first 10 days of development (Fig. 2B and D). After this period, the values of these variables stabilized. Fruits harvested soon after anthesis (0 DAA) showed less concavity, at 15 DAA, the fruits showed values around 68.91 mm, indicating an increase of 389.5 % in relation to 0 DAA (Fig. 2B).

These changes demonstrated that pumpkin cv Mini Jack fruits show a marked growth in the first ten DAA, stabilizing after this period. Regarding the height and diameter of the pumpkin cv Mini Jack fruits (Fig. 2C), it was found that those harvested shortly after anthesis (0 DAA) had an approximate size of 20.61 mm.

The increase in fruit height occurred until the tenth day of evaluation, with an average of 51.38 mm. The fruits had larger diameters at 15 DAA, with an average of 95 and 95 mm. At ten DAA, a rapid increase in pulp thickness was also observed (Fig. 2D), indicated by the increase in this variable by approximately 525 % when compared to zero and ten DAA. However, the shell thickness (Fig. 2D) continued to increase until 20 DAA, when it presented an average of 1.23 mm. (Fig. 2D).

Regarding the relative growth rate of the Mini Jack pumpkin, it was found to follow a simple sigmoid pattern (Fig. 2A) (Coombe, 1976). The pumpkin cv Mini Jack fruits showed a relative growth rate (RRR) of 4.69 g day⁻¹ at ten DAA (Fig. 2E). After this period, as well as the weight and the concavity, the values referring to the RGR of the fruits also stabilized, thus showing the end of the growth stage of the Mini Jack pumpkin. The fruits showed an initial respiratory rate of 767 mg CO₂ kg⁻¹ h⁻¹, which decreased markedly at 5, 10, 15 and 20 DAA, reaching 451 mg CO₂ kg⁻¹ h⁻¹ at 15 DAA (Fig. 2E). From 15 to 20 DAA, there was stability in this rate (Fig. 2E). Under the experimental conditions used, ethylene production was not detected during growth and development.

The fruits had their firmness increased during development. The lowest values for this variable (0.60N) were observed right after anthesis (0 DAA) (Fig. 2F) and the highest (14.57N) at 20 DAA (Fig. 2F).

The observed values of the Hue angle of the pumpkin cv Mini Jack fruits decreased with advancing development (Fig. 3).

Initially, the value of the Hue angle was 84.67°, close to the yellow color. However, as the fruits developed, the Hue angle gradually decreased, reaching the lowest values at 20 DAA (72.8). This indicated a change in the shade of the bark, from a less intense yellow to more intense shades of yellow (Fig. 3). The hue angle (°h) allows accurate visualization of the change in the color of the fruit peel (Silva et al., 2008). The values found allowed us to conclude that with the advancement of development, the Mini Jack pumpkin fruits showed a more intense yellow tone ($h = 80^\circ$).

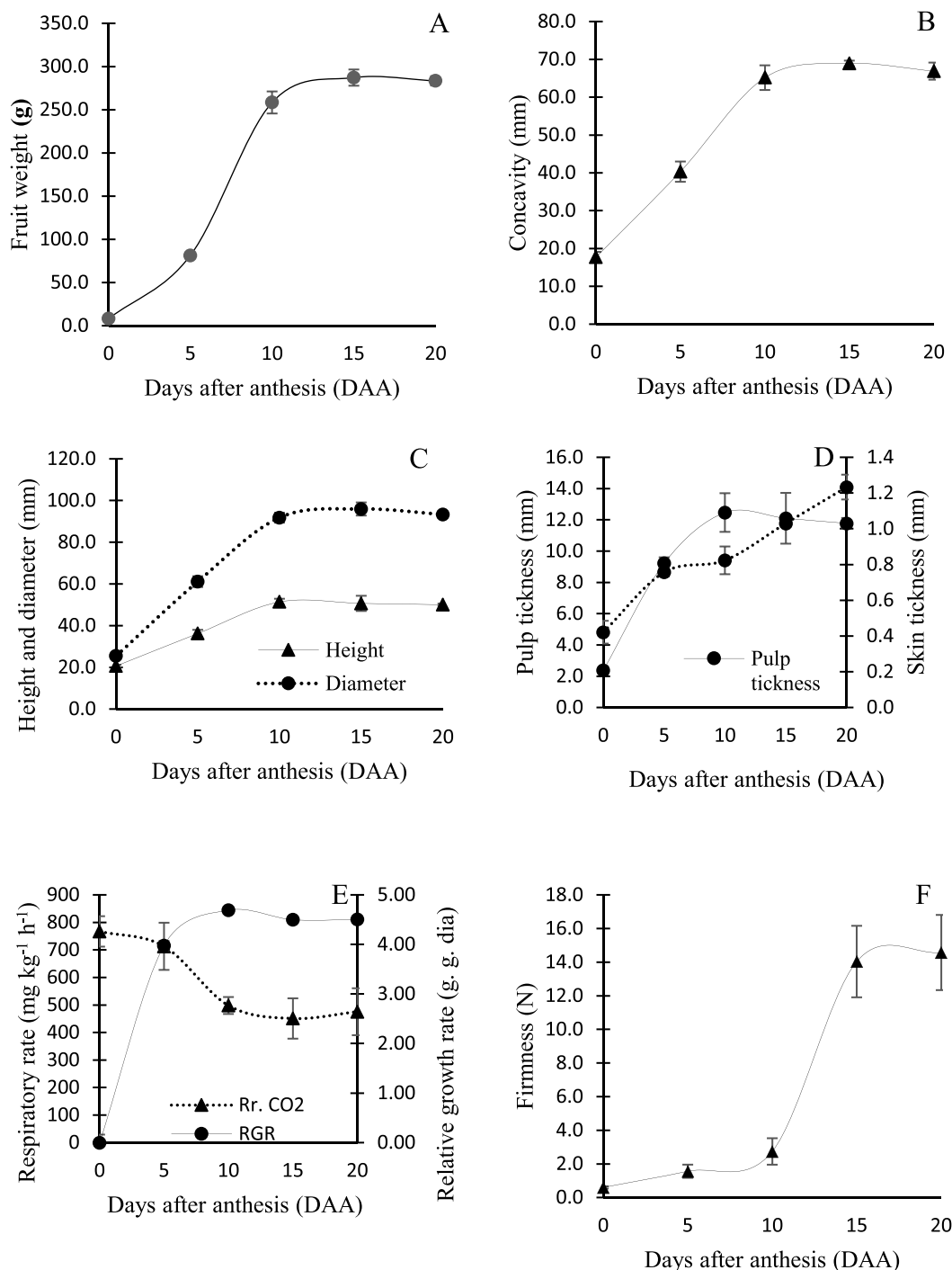


Fig. 2. Fruit weight (A), concavity (B), fruit height and diameter (C), pulp and skin thickness (D), respiratory rate and relative growth rate (E) and fruit firmness (F) as a function of growth and fruit development of the Mini Jack pumpkin.

3.2. Biochemical and chemical changes in Mini Jack pumpkin fruits during growth and development

The chemical and biochemical parameters of pumpkin cv Mini Jack fruits are shown in Fig. 4.

The levels of total carotenoids in the peel increased significantly throughout the development of the evaluated fruits (Fig. 4A), starting from 0.70 $\mu\text{g } 100 \text{ g}^{-1}$ on the day of anthesis (0 DAA), reaching the highest values of this variable at 20 DAA, when it was around 0.450 $\mu\text{g } 100 \text{ g}^{-1}$. Regarding the sugars present in fruits, in general, they decreased with the advancement of development (Fig. 4B). Initially, the

fruits harvested soon after anthesis (0 DAA) presented, on average, 2.10 %, 0.42 % and 1.76 % of total, reducing and non-reducing sugars, respectively. At 20 DAA, lower levels of total, reducing and non-reducing sugars were observed: 1.46 %, 0.20 % and 1.28 %.

The pH values remained around 7.00 and 7.76 up to 20 DAA (Fig. 4C). The lowest pH values were verified at five DAA (7.00). After this period, there was an increase in this variable, and at 20 DAA its highest value was found, 7.76. On the other hand, a drop in TTA levels was verified when comparing the zero and fifth DAA 0.325 and 0.122 respectively (Fig. 4D), the drop in TTA values in this period may be associated with the use of organic acids in the breathing process of the

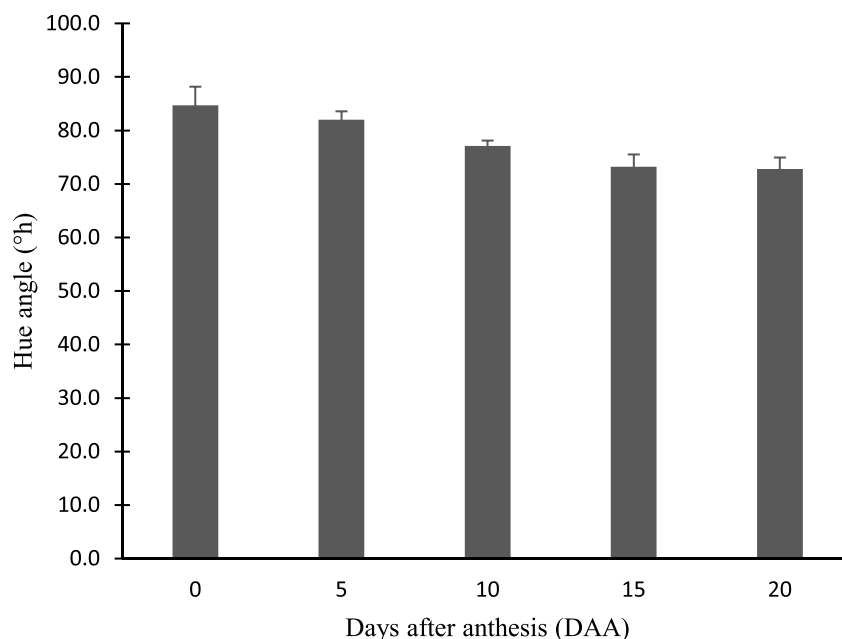


Fig. 3. Hue angle along the fruit development of the Mini Jack squash.

animals. Mini Jack pumpkin fruits. In the 20 DAA, the values found referring to TTA were 0.244. The TSS content increased with fruit development time (Fig. 4D). At zero DAA the fruits showed average values of 3.56 %, higher values were observed at 15 DAA, where the values found were 6.1 %. The lowest values referring to the ratio of the pumpkin cv Mini Jack fruits were observed immediately after anthesis (0 DAA) 10.98. The highest values were found at the five DAA 33.67 where it is also possible to observe the lowest value referring to acidity, thus contributing to the higher ratio value.

4. Discussions

Fruit development involves changes in numerous characteristics, whether physical, chemical or physiological, which can be influenced by external and internal factors (Coombe, 1976). These changes can be divided into phases or stages of development.

The process of fruit development is characterized by the growth stage where the greatest physical changes occur, mainly related to changes in the dimensions of the fruits, followed by the maturation process where the chemical changes related to the synthesis and degradation of some compounds responsible for the flavor and appearance of the fruits, at ripening the fruits have a pleasant flavor and are ready to be consumed and senescence where the degradation processes are greater than the synthesis processes causing the death of the fruit (Luo et al., 2020)

According to the results obtained, it is verified that the first phase of the development of the pumpkin cv Mini Jack can be characterized by the accelerated increase in weight, height, diameter and concavity, it can be seen that these changes are concentrated between zero and ten DAA (Fig. 2). Fruit growth, which is an irreversible increase in size, is affected by a combination of cell division and expansion (Taiz & Zeiger, 2002).

Coombe (1976) stated that during fruit growth, the time interval from anthesis to physiological maturity, there is an orderly development of a large number of plant tissues, with cell division completed at the time of anthesis or during the beginning of the period. of growth, and that the cell volume is the one that represents the greatest contribution to the total expansion of the fruit. The change in diameters is fundamentally produced by the action of phytohormones such as indoleacetic acid and gibberellins, which are ultimately synthesized because of photosynthesis and plant respiration (Wang et al., 2020).

In addition, the growth of the pumpkin cv Mini Jack can be characterized by the high respiratory rate observed at 0 DAA (Fig. 2E), confirming the accelerated metabolism possibly caused by the numerous physical, chemical and physiological changes that occurred in the observed period.

Analyzing the data on relative growth rate, respiratory rate (Fig. 2E) and the non-detection of ethylene during the growth and development of the pumpkin cv Mini Jack, it can be inferred that the fruits present the behavior of non-climacteric fruits (Iannetta et al., 2006). In climacteric fruits, there is a peak in respiration and an increase in the autocatalytic production of ethylene, while in non-climacteric fruits these changes are not observed (Farcuh et al., 2019). Knowledge of respiratory activity and ethylene production rate in fruits is an extremely important attribute for fruit ripening. Information about the physiological changes that occur during fruit growth and development can result in an increase in shelf life and help maintain the quality of these products (De Corato, 2020).

It was observed that the firmness of the evaluated fruits increased until the 15 DAA (Fig. 2F), these results indicate according to Coombe (1976) the second phase of the development, which involves the growth and elongation of the fruits. For the pumpkin cv Mini Jack this phase was extended from 10 DAA to 15 DAA. During cell elongation, secondary wall polymers are constantly synthesized, while the primary wall expands (Coombe, 1976; Taiz & Zeiger, 2002). Giving more firmness to the fruits.

Fruit firmness is an especially essential attribute for fleshy fruits and should be extensively investigated because it influences both harvest and post-harvest shelf life (Lago et al., 2022). However, the mechanisms responsible for the change in fruit firmness are not fully understood. Available information on changes in pectin, hemicellulose and cellulose fractions varies with each fruit (Fischer & Bennett, 1991).

Nutritional and genetic factors can affect fruit firmness. In addition, it can also vary depending on the cohesiveness, shape, size and turgidity of the cells that make up the fruit tissue. Firmness is also an important characteristic in determining the ideal point of fruit harvest and directly influences the fruit storage potential (Lara et al., 2019).

Changes in fruit color are indicative of the ripening process of fruits and vegetables (Jacobó-Valenzuela et al., 2011). This attribute, along with others, are used by consumers in the process of choosing products (Lu et al., 2021). However, through this study it was found that the color

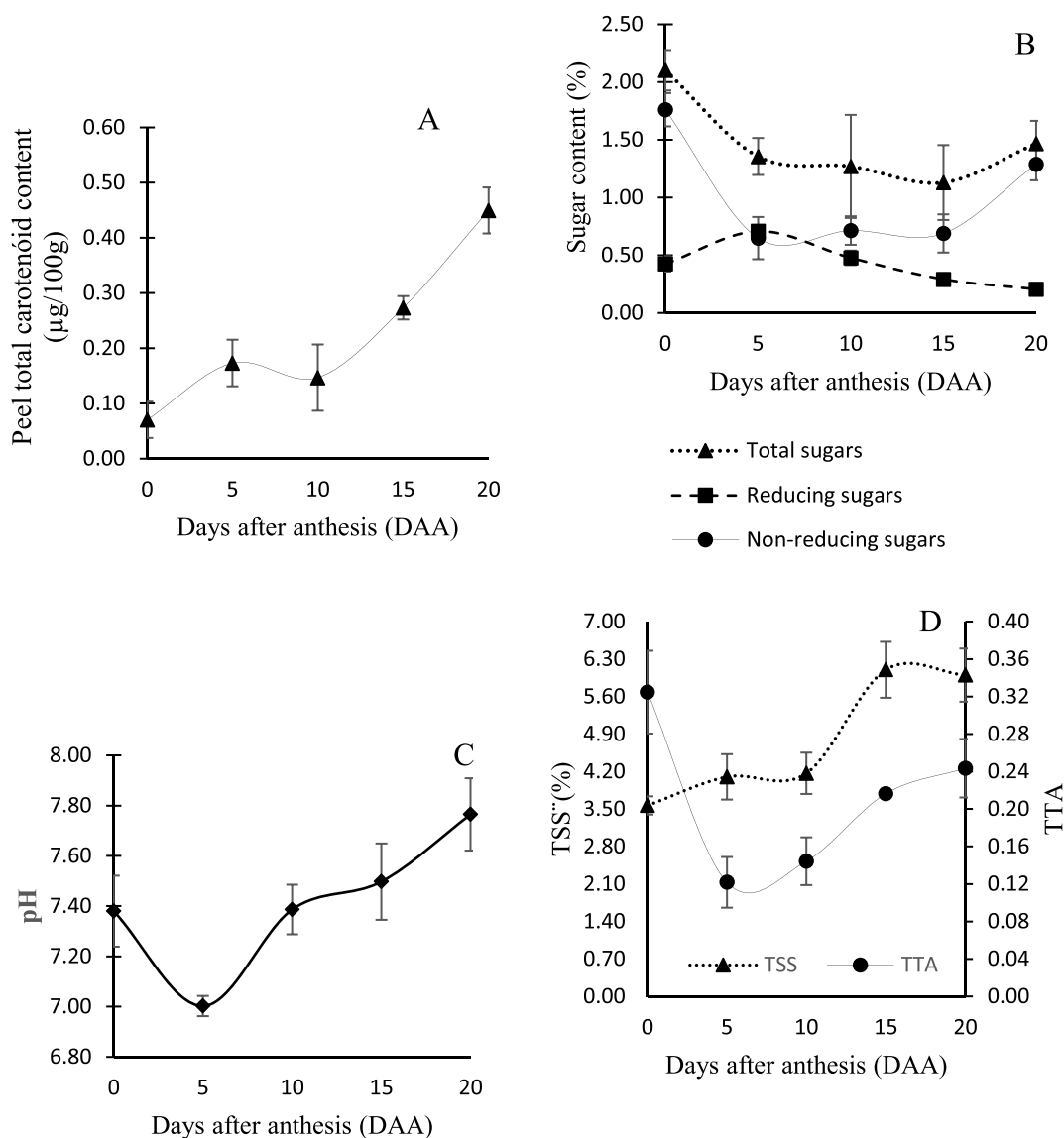


Fig. 4. Peel total carotenoid content (A), total, reducing and non-reducing sugars (B), pH (C), and TSS and TTA (D) as a function of the growth and development of pumpkin Mini Jack fruits.

variable is not the ideal indicator for decision making regarding the ripening of pumpkin cv Mini Jack fruits, given the low variation of this variable throughout development (Fig. 3). On the other hand, the low variation in the values of the Hue angle confers an important characteristic for the pumpkin cv Mini Jack fruits, as it provides the maintenance of freshness for longer periods of storage.

Carotenoids are the source of pumpkin's color and provide some of the biggest nutritional benefits of eating pumpkin. The primary carotenoids in *C. pepo* are β -carotene and lutein (Azevedo and Rodriguez, 2007; Murkovic et al., 2002). Variations in total carotenoid contents can be attributed to several factors including stage of maturation, environmental factors, cultivar and growing conditions. Sousa et al. (2012), when studying twelve accessions of *Cucurbita moschata* in relation to storage days, obtained average values of total carotenoids from 14.93 to 153.15 $\mu\text{g } 100 \text{ g}^{-1}$, values higher than those found in the pumpkin cv Mini Jack fruits (Fig. 4).

Soluble sugars in the fruits of Cucurbitaceae cultures are mainly glucose (reducing sugar), fructose (reducing sugar) and sucrose (non-reducing sugar). The contents and proportions of the three sugars in different species and varieties are very different (Wang et al., 2020). In the *C. moschata* and *C. pepo* varieties, sucrose determines the sweetness

of the fruit (Corrigan et al., 2000).

Reducing sugars contribute almost 100 % of the total sugar content in the initial phase of fruit development (Long et al., 2004). However, sucrose can reach up to 50 % of the total sugars in the final stage of maturation, with an approximate proportion of 25 % for glucose and 25 % for fructose (Long et al., 2004). Pumpkin cv Mini Jack fruits initially had a low total sugar content, which may be related to the fact that in the initial stage of fruit development (Fig. 4B). The sugar content is generally low and increases slowly during maturation and ripening. As possibly the sugars produced by photosynthesis in the leaves at the early stage of development are transported to the fruit is broken down into glucose and fructose to synthesize starch and to provide energy for rapid fruit growth (Wang et al., 2020). In addition, the sugar contents can be related to external and internal factors, such as Moranga fruit (*C. maxima* cv. Duch) it has an average of 2.2 to 3.8 % of reducing sugars (Oliveira et al., 2013).

The soluble solids content indicates the amount, in grams, of solids that are dissolved in the juice or pulp. They are measured in°Brix, being used as an indirect measure of the sugar content and increase with maturation through synthetic processes or by the degradation of polysaccharides. The increase in the content of soluble solids in the pulp can

be used as one of the parameters for the ideal point of harvest, and characterize the third and last stages of the development of the pumpkin cv Mini Jack fruits that extended up to 15 DAA (Fig. 4D), totaling a cycle of 46 days after sowing, different from the results of Echer et al. (2014), concerning the fruits harvested 65 days after sowing, carried out in a seedling production tray. They found mean TSS and TTA values of 3.64 % and 0.126 %, that is, values lower than those found in this research.

5. Conclusion

Mini Jack pumpkin fruits exhibited non-climacteric fruit behavior. The most significant physical-chemical changes associated with fruit growth occurred within the initial ten days, marked by a decline in respiratory rate and an augmentation in fruit firmness and color. Throughout the fruit development phase, there was an elevation in carotenoid content in the peel and pH of the pulp, alongside a reduction in TTA and fruit sugars. Our study emphasizes that the optimal harvest point lies between 10 and 15 DAA (days after anthesis).

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Ethics Approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

CRedit authorship contribution statement

Genilza Almeida da Graça: Writing – original draft, Methodology, Formal analysis, Data curation. **Pryanka Thuyra Nascimento Fontes:** Writing – review & editing, Supervision. **Alysson Caetano Soares:** Methodology, Formal analysis. **Mônica Silva de Jesus:** Formal analysis. **Patrícia Nogueira Matos:** Methodology. **Maria Terezinha Santos Leite Neta:** Writing – review & editing. **Adriano do Nascimento Simões:** Writing – original draft, Validation, Resources. **Luciana Marques de Carvalho:** Writing – original draft, Supervision, Resources. **Luiz Fernando Ganassali de Oliveira Júnior:** Validation, Resources, Investigation. **Marcelo Augusto Gutierrez Carnellosi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Aline de Almeida Vasconcelos:** Writing – review & editing, Writing – original draft, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Further reading

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