



Postharvest arginine spraying delayed ripening during storage of papaya

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ABSTRACT

Purpose: Climacteric metabolism makes papaya (*Carica papaya* L) a highly perishable fruit, especially under ambient conditions. Considering that few retail outlets (markets) have a cold chain for storage, it is necessary to evaluate technologies to extend the commercial shelf life of this fruit under ambient conditions. Thus, the objective of this research was to evaluate the effect of arginine application to delay ripening and preserve the physicochemical quality of papaya during storage under ambient conditions.

Research method: Physiologically ripe 'Hawaii' papayas (stage 1) were harvested from a commercial orchard, selected (physiological injuries, pests and diseases), sanitized in a chloride solution and sprayed with solutions containing distilled water (control) and arginine (25 mg.L⁻¹) determined in preliminary tests. They were then placed on benches and kept under ambient conditions (28 ± 2 °C and 85 ± 5 % RH) for a period of 7 days with quality assessments performed daily. **Findings:** Spraying a solution containing arginine (25 mg.L⁻¹) significantly delayed the ripening of papayas, corroborated by reduced respiratory activity and ethylene production, the effects of which were observed in delayed chlorophyll loss in the peel, reduced mass loss, maintenance of firmness, reduction in total soluble solids accumulation and titratable acidity, in addition to reduced degradation of vitamin C and lycopene. **Research limitations:** Understanding the biochemical mechanism of arginine in the regulation of ripening.

Originality/Value: The results of this study provide the producer/trader with a viable and easy-to-apply technology to ensure a product with a longer marketing period and quality for the final consumer.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the main economic crops in tropical and subtropical regions, characterized by its sweet and unique flavor, high nutritional value (vitamins A, C, and E), antioxidants (carotenoids), as well as proteins, amino acids, and minerals (Zheng et al., 2021; Mabunda et al., 2023). As a climacteric fruit, papaya ripens rapidly after harvest characterized by color change, pulp softening, and rotting during storage and transportation, resulting in short shelf life and high losses (Kahawattage et al., 2023; Zhou et al., 2024).

Fruit ripening is a genetically programmed process that leads to various physiological, biochemical, and metabolic changes which irreversibly alter its sensory characteristics (flavor, aroma, appearance) (Nasir et al., 2024). In the postharvest storage of climacteric fruits, such as papaya, this process is fully regulated by increased respiratory activity and ethylene production, which has a direct impact on the conservation potential since it favors the loss of quality and senescence (Corpas et al., 2023). In this sense, inhibition of ethylene biosynthesis or action is the most common strategy used in postharvest to prolong storage, maintain fruit quality, and extend the availability of fresh products (Wei et al., 2021). The findings of these studies contribute to the development of innovative approaches and technologies to ensure the delivery of high-quality fruits to consumers while minimizing waste.

During the ripening process, accumulation of primary metabolites, such as amino acids, plays a crucial role in forming aromatic compounds in fruits (Pott et al., 2020). Research has shown that amino acid metabolism is affected during postharvest storage and has a direct effect on the ripening and senescence of vegetables (Yuan et al., 2017; Tang et al., 2020). Among the amino acids, arginine is an important nitrogen-carbon (N/C) binding amino acid in plants (Shu et al., 2020) and acts as a biosynthetic precursor of important compounds such as polyamine (PA) and nitric oxide (NO) (Mirmiran et al., 2017), which are important messenger molecules in most physiological and biochemical processes, including the regulation of ethylene biosynthesis (Wei et al., 2021). Several researchers reported that the exogenous application of arginine (immersion) favored prolonged shelf life and improvement of the antioxidant system during ripening of fruits such as apples (Wills et al., 2016), plums (Mahmoudi et al., 2022), and persimmon (Nars et al., 2022).

At retail outlets (street markets), not all traders have facilities for cold storage, and considering the perishability of papaya, especially under ambient conditions, and the potential for exploitation of amino acids in the postharvest chain, this study aimed to evaluate the effect of arginine spraying as a strategy to control ripening and preserve the quality characteristics of papaya during storage at room temperature.

MATERIALS AND METHODS

Plant material

Papayas of the Solo group, cultivar 'Hawaii' were harvested physiologically ripe in a commercial orchard located in the city of Santa Izabel, Pará, Brazil. At harvest, fruits at maturity stage 1 were considered, that is, with the epicarp completely green, free of physiological defects or affected by pests and diseases. These were wrapped in bubble wrap to avoid mechanical damage during transportation and taken in plastic boxes to the Postharvest Quality Laboratory of the Socio-Environmental Institute of Science and Technology of the Amazon (ISACTA), Belém, Pará, Brazil.

Postharvest treatments and storage

In the laboratory, the fruits (200 units) were sanitized in a chloride solution (150 mg.L^{-1}) for 10 minutes, rinsed in running water, and allowed to dry under ambient conditions. Subsequently, they were randomly divided into two batches placed on masonry benches, and subjected to the following treatments. One batch of fruits (100 units) represented the control treatment and was sprayed with distilled water, the other batch (100 units) was sprayed with an arginine solution at 25 mg.L^{-1} (Sigma Aldrich) was selected after preliminary tests with concentrations ranging from 5 to 50 mg.L^{-1} , where concentrations lower than 25 mg.L^{-1} had no potential effect on ripening, and when higher than 35 mg.L^{-1} , they negatively affected the cell structure (softening). Both spray solutions (control and arginine) contained Tween 20 at 0.1 g.L^{-1} (Sigma Aldrich) as a wetting agent. After spraying, the fruits were kept under ambient conditions ($28 \pm 2 \text{ }^\circ\text{C}$ and $85 \pm 5 \%$ relative humidity) for 7 days.

Physicochemical quality assessment

The fruits of each treatment were assessed daily (in triplicate) for:

Respiratory activity and ethylene production: For the determinations, 10 fruits with known mass (average 584.11 g) were individually placed in hermetic glass containers with a capacity of 2000 mL, previously exposed to the temperature and humidity conditions of the experiment. One hour after closing the vials, 1 mL gas samples were collected from the vials through a silicone septum, using a syringe suitable for chromatography (Hamilton, Gastight, Nevada, USA). The gas samples were analyzed in a Thermo Finnigan Trace 2000GC gas chromatograph. The chromatograph was equipped with a 2 m long Porapack N capillary column set at $80 \text{ }^\circ\text{C}$, with hydrogen as carrier gas (40 mL min^{-1}). A methanator at $350 \text{ }^\circ\text{C}$ was used for respiration (CO_2) analyses. Gas samples were analyzed by a flame ionization detector at $250 \text{ }^\circ\text{C}$. Respiration and ethylene production were determined by the difference between the initial (when the vials were closed) and final (after 1 h) gas concentrations, expressed in $\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ and $\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$, respectively.

Fresh mass loss: Determined by weighing the fruits on an analytical scale (Mars, model AS 2000, São Paulo, Brazil) at the beginning of the experiment (initial weight) and again at each period of exposure to ethanol vaporization (final weight). The results were expressed as a percentage (percentage) based on the following equation (1):

$$\text{FML (percentage)} = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \times 100 \quad (1)$$

Firmness: It was determined with a digital penetrometer (53200 TR Turoni, Italy), taking 2 readings on opposite sides in the region of greatest diameter of the fruits. A thin layer of the skin was removed from the papayas, then it was placed firmly on the bench and the 8 mm tip was inserted with constant force until the fruit was pierced. The results were expressed in Newtons (N).

Peel and pulp color: Determined with the aid of a colorimeter (Minolta CR-300, Osaka, Japan), taking two readings per fruit in the region of greatest diameter of the fruits. The results were expressed in hue angle (h°).

Total soluble solids: To determine total soluble solids (TSS), approximately three drops of fruit pulp juice were evaluated in a digital refractometer with automatic temperature correction (Atago PR-101, Atago Co Ltda., Tokyo, Japan). The results were expressed in $^\circ\text{Brix}$ (AOAC, 2020).

Titrateable acidity: To determine titrateable acidity (TA), approximately 10 g of fruit pulp juice were taken and 90 mL of distilled water added. Titration was performed with 0.1 M sodium hydroxide (NaOH) solution to pH 8.1. The results were expressed in g of citric acid per 100 g of pulp (AOAC, 2020).

pH: Determined in 10 g of juice using a portable Gehaka potentiometer, model PG2000, and the results expressed in pH units.

TSS/TA ratio: Determined by the quotient between the TSS and the TA content.

Vitamin C content: To determine the ascorbic acid content, approximately 5 g of the pulp was homogenized with 25 mL of 1% oxalic acid, followed by centrifugation at 4,000 xg for 10 minutes. The collected extract (1 mL) was diluted in oxalic acid (4 mL) and titration was performed with a 2,6-dichlorophenol-indophenol solution (AOAC, 2020), and the results were expressed in mg of ascorbic acid per 100 g of pulp.

Lycopene content: Determined according to the methodology described by Sadler et al. (1990) with modifications. The sample (1.0 g) was homogenized with 50 mL of the hexane/acetone/ethanol solution (2:1:1, v/v/v) and left under stirring for 30 minutes. The homogenate was then transferred to a separatory funnel and 10 mL of distilled water was added. The solution was separated into a polar fraction (35 mL) and a nonpolar fraction (25 mL), the latter containing lycopene. The extract was collected and the absorbance of the solution in hexane was measured at 472 nm. The conversion of absorbance into lycopene concentration was based on the extinction coefficient of 3.450 specific for the pigment in hexane and the results were expressed in $\mu\text{g}\cdot\text{g}^{-1}$ of sample.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design (CRD) under a 2×8 factorial arrangements, with two treatments (spraying with and without arginine) and eight evaluation times (0, 1, 2, 3, 4, 5, 6 and 7 days) with three replicates and the experimental plot composed of 3 fruits. The data were submitted to ANOVA and the means compared with each other by the Tukey test at the 5% probability level using the R software.

RESULTS

Treatment of fruits with arginine resulted in lower respiratory activity (CO_2) and ethylene production (C_2H_4) compared to untreated fruits (control). Considering the storage period, the peak production of CO_2 and C_2H_4 occurred at 4 days in the control fruits ($61.1 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ and $6.93 \text{ }\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) and at 6 days in those treated with the arginine ($66.5 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ and $6.41 \text{ }\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) (Fig. 1A and B), respectively.

The mass loss was 24.97% greater in the control fruits compared to those sprayed with arginine, especially after the 3rd and 5th day of storage, respectively (Fig. 2A). Firmness reduced by 85.30% compared to the initial day, especially in the control fruits after 4 days of storage. In the papayas sprayed with arginine, the most significant reduction occurred after the 5th day, but considering the entire storage period, they were 56.25% firmer than the control fruits (Fig. 2B).

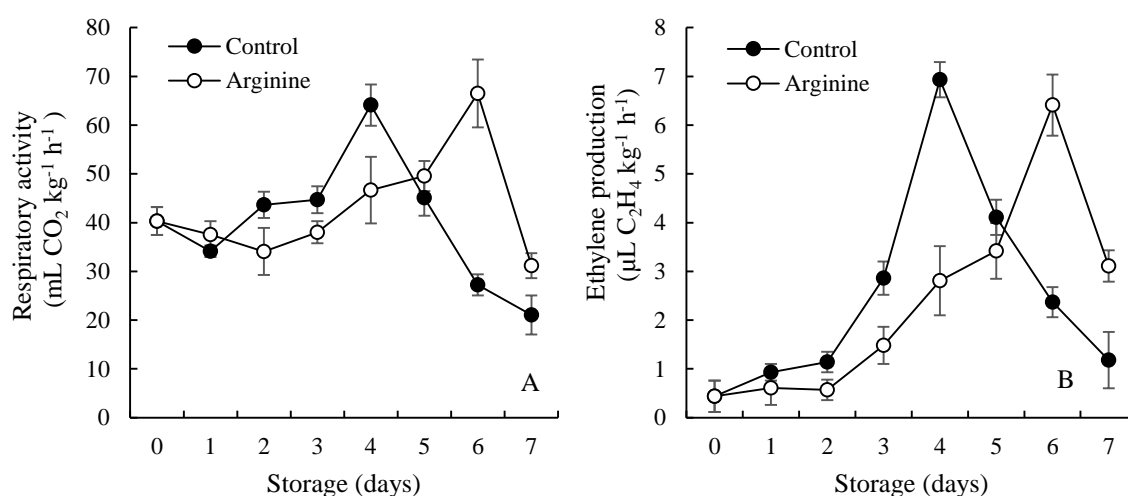


Fig. 1. Respiration and ethylene production in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature ($28 \pm 1 \text{ }^\circ\text{C}$) with 85-90% RH for 7 days.

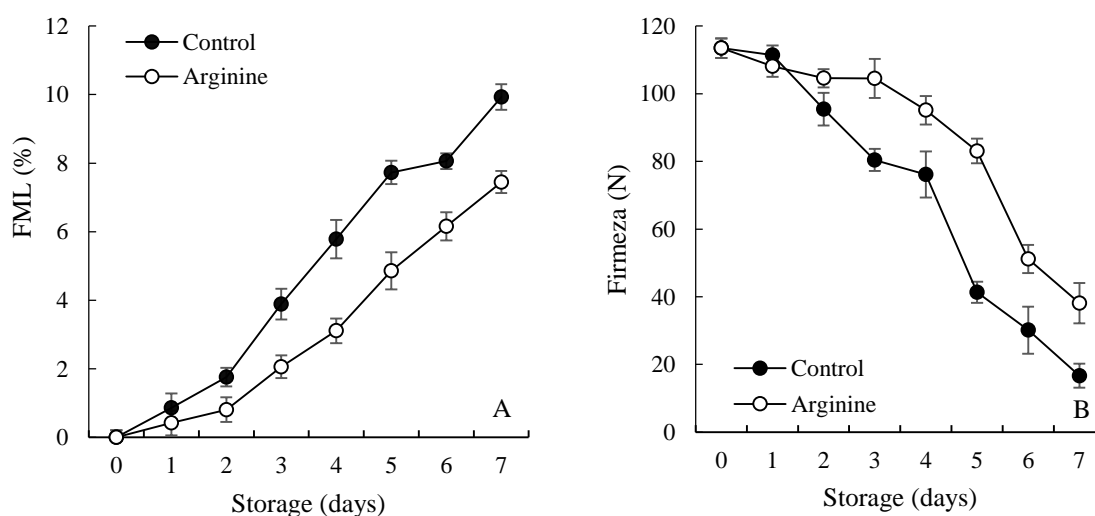


Fig. 2. Fresh mass loss (A) and firmness (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature ($28 \pm 1 \text{ }^\circ\text{C}$) with 85-90% RH for 7 days.

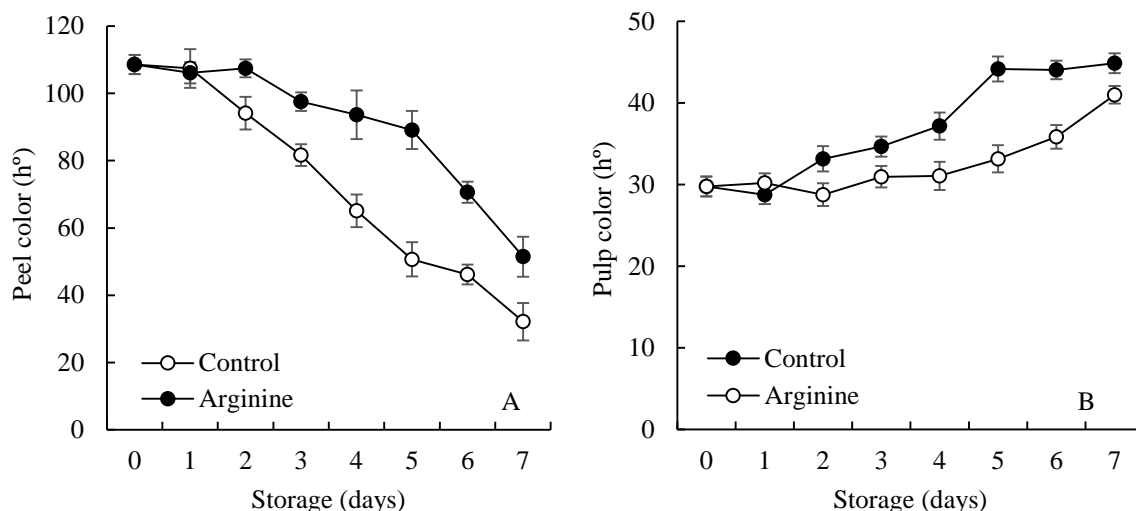


Fig. 3. Peel color (h°) (A), and pulp color (B) in ‘Hawaii’ papayas sprayed or not with arginine and stored at room temperature (28 ± 1 °C) with 85-90% RH for 7 days.

Regarding skin color, untreated fruits showed color change at 2 days (yellow lines) with a change from green to yellow in the order of 13.36% in relation to the initial day, while for the same period, the change was only 1.12% in papayas sprayed with arginine, indicating greener fruits. At 6 days, the control fruits were completely ripe (yellow) with an average value of 46.13 h°, while the same value (51.47 h°) was only observed at 7 days in fruits sprayed with arginine (Fig. 3A, Fig. 7). In the pulp, there was an increase in average values with storage time; however, the control fruits showed higher values, mainly from the 4th day (44.16 h°), while in those treated with arginine, this increase was only significant after the 6th day (41.01 h°) (Fig. 3B, Fig. 7).

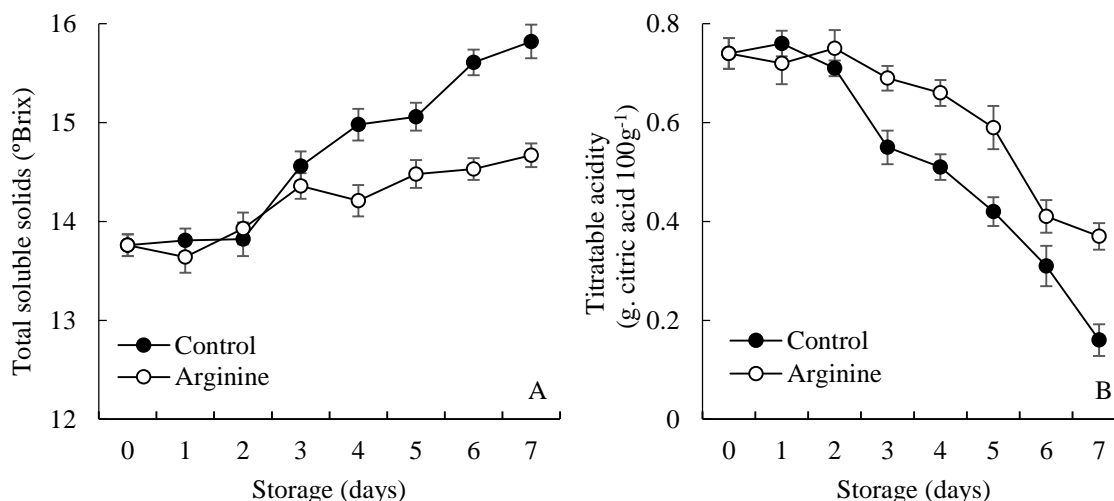


Fig. 4. Total soluble solids (A) and titratable acidity (B) in ‘Hawaii’ papayas sprayed or not with arginine and stored at room temperature (28 ± 1 °C) with 85-90% RH for 7 days.

There was little variation in the total soluble solids (TSS) content during storage, especially in papayas treated with arginine, whose increase was only 6.02% in relation to the initial day when compared to 13.21% in the control fruits (Fig. 4A). Regarding titratable acidity (TA), there were decreases in the average values with storage time, especially in untreated fruits immediately after the 2nd day of evaluation, with an average value of 0.16 g of citric acid 100 g⁻¹ after 7 days. In papayas sprayed with arginine, the most significant reduction only occurred after 4 days of storage, and the average content was 56.75% higher in relation to the control fruits on the last day of evaluation (Fig. 4B).

The ratio (TSS/TA) increased considerably during storage, regardless of the treatment. In the control fruits, this increase was around 77.74% and was more pronounced from the 3rd day onwards, while in those sprayed with arginine, the increase was 70.84%, especially from the 5th day of storage (Fig. 5A). The average pH values increased by 22.93% over the storage time. This increase was more evident in the control fruits after 4 days, reaching an average value of 6.23 after 7 days. On the other hand, in the papayas sprayed with arginine, stability was observed between day 0 (4.67) and the 4th day of storage (4.81), followed by an increase and an average value of 5.89 after 7 days (Fig. 5B).

The vitamin C content increased by 21.85% during storage. In the control fruits, the maximum peak was obtained at 6 days (71.15 mg.100 g⁻¹) with subsequent stability. In the fruits sprayed with arginine, the highest content (64.43 mg.100 g⁻¹) was observed at 7 days, approximately 7.74% lower than in the untreated fruits (Fig. 6A). Regarding the lycopene content, an increase of 53.55% occurred in the control fruits between day 0 (11.56 µg.g⁻¹) and the fifth day of storage (24.89 µg.g⁻¹), followed by a decline. On the other hand, a gradual increase was observed until the 4th day in the papayas sprayed with arginine (14.62%), followed by the rise and highest peak (25.17 µg.g⁻¹) at 7 days of evaluation (Fig. 6B).

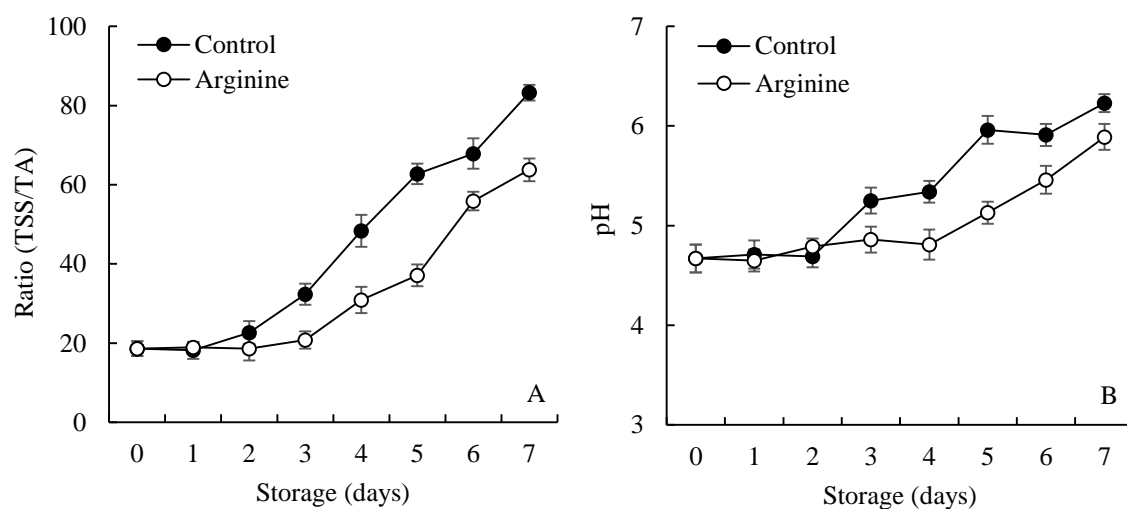


Fig. 5. TSS/TA ratio (A) and pH (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ± 1 °C) with 85-90% RH for 7 days.

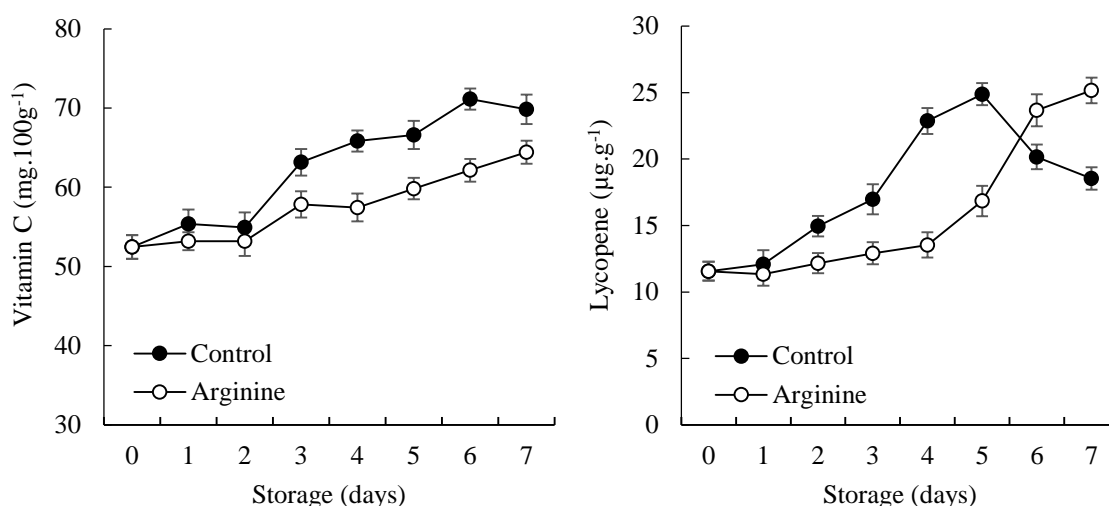


Fig. 6. Vitamin C (A) and lycopene (B) in 'Hawaii' papayas sprayed or not with arginine and stored at room temperature (28 ± 1 °C) with 85-90% RH for 7 days.

DISCUSSION

In postharvest, controlling respiratory activity is the main strategy to delay the physiological (transpiration) and biochemical (ethylene production) transformations that induce ripening. In this study, the delay in ripening characterized by lower respiratory activity and ethylene production in papayas sprayed with arginine (Fig. 1A and B) may be associated with both the role of this arginine in serving as a reserve source of nitrogen for plants, thereby regulating fruit senescence (Zhang et al., 2014), as well as with the biosynthetic capacity of molecules such as nitric oxide and polyamines that in their free forms may have anti-ripening and senescence properties (Gao et al., 2009).

Physiological water loss is one of the many postharvest disorders in the fresh fruit industry and is usually associated with transpiration and storage conditions. Generally, most vegetables lose quality when they reach a water loss greater than 5% of their initial weight, favoring processes such as wilting that leads to loss of commercial weight and fruit texture, induction of browning, reduction of flavor, acceleration of senescence and greater fluidity of the cell membrane that becomes more susceptible to attack by microorganisms and *chilling injury* (Lufu et al., 2020). In this study, this effect on quality loss could be observed in control fruits that presented mass loss greater than 5% after 4 days of storage, corroborated by the reduction in firmness. On the other hand, the delay in ripening caused by arginine treatment allowed a lower transpiration rate resulting in less mass (water) loss and consequent greater firmness during storage (Fig. 2A and B).

In climacteric fruits, the color change characterized by the loss of chlorophyll and synthesis of pigments (carotenoids, flavonoids) marks the beginning of ripening. In this study, there was a delay of 1 day for the onset of visual changes in the color of the peel and pulp and up to 2 days for the complete ripening of papayas sprayed with arginine compared to control fruits (Fig. 3A, B and C). Similarly, treatment by immersing tomatoes in an arginine solution significantly delayed the change from green to red during storage (Yu et al., 2024).

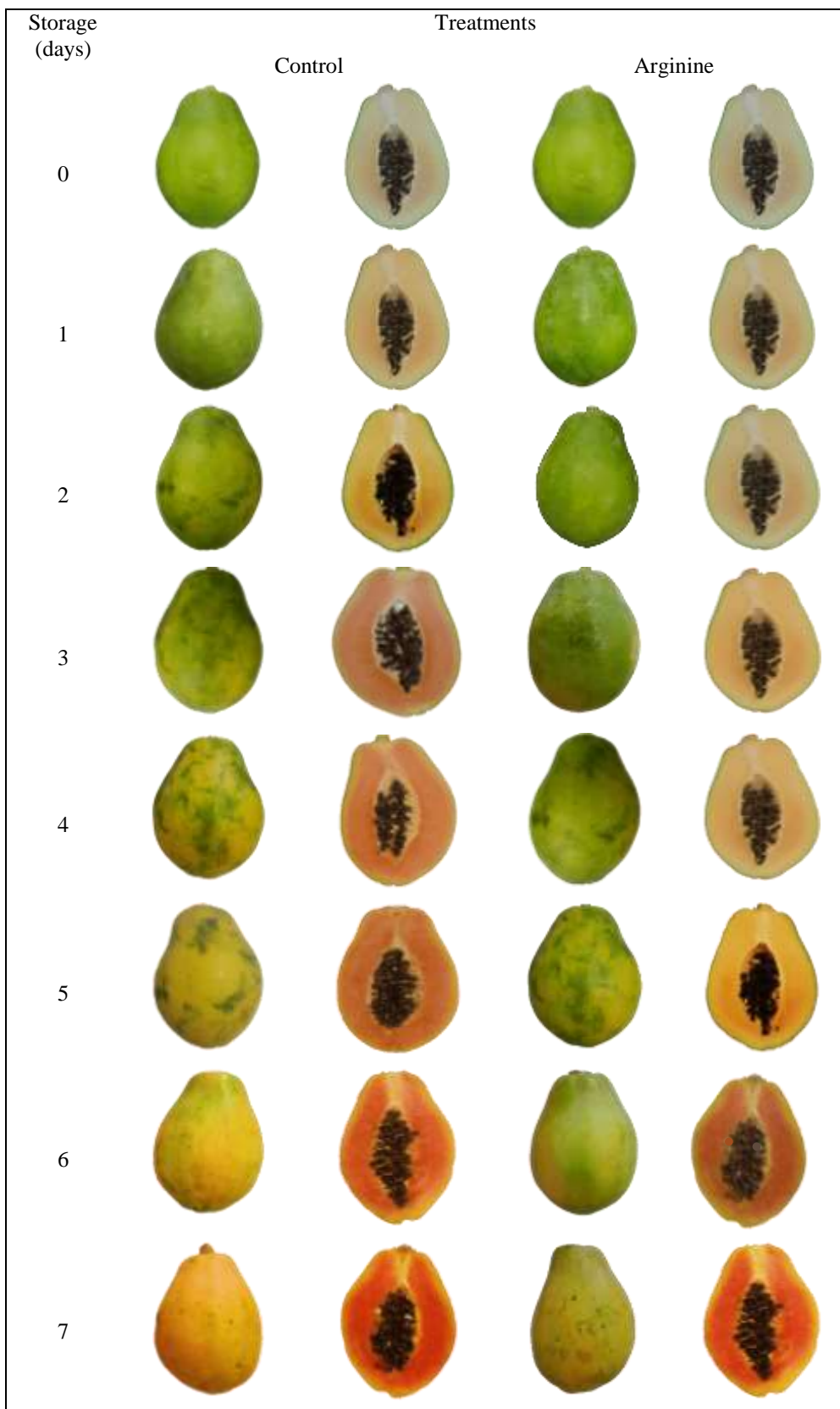


Fig. 7. Peel and pulp appearance quality of ‘Hawaii’ papayas sprayed or not (control) with arginine and stored at room temperature (28 ± 1 °C) with 85-90% RH for 7 days.

Ripening also promotes physicochemical changes, such as the synthesis and degradation of sugars and organic acids, which directly impact the flavor and aroma of the fruits. Normally, the accumulation of sugars (TSS) is linked to the degradation of some compounds that make up the cell wall (starch), for example, while the reduction of organic acids (TA) is explained by their use as substrates in respiratory metabolism and as a carbon source for the synthesis of new compounds, especially the volatile compounds responsible for aroma (Sanches et al., 2021). The balance of the TSS/TA ratio will define the flavor, since the higher the SS content and the lower the TA content, the better the flavor ratio. However, spraying papayas with arginine resulted in less TSS accumulation (Fig. 4A), in the degradation of organic acids (Fig. 4B), in the pH variation, and the flavor ratio of the fruits (Fig. 5A and B), respectively. These results corroborate the effect of this amino acid in delaying ripening, without compromising quality during storage. In broccoli (Sun et al., 2024) and tomatoes (Yu et al., 2024), treatment with arginine (5 mM) suppressed the activities of the main enzymes involved in ethylene biosynthesis, delaying ripening, TSS accumulation, and TA degradation, resulting in a longer shelf life. Furthermore, the continuous increase in TSS and reduction in TA and the better flavor balance (TSS/TA) indicate that arginine does not interfere with the sensory quality of papaya during storage.

The vitamin C (AsA) content of the fruit depends on many factors, including cultivar, ripening stage, orchard management, harvest time, and acidity. In addition, the duration and conditions of postharvest storage influence the AsA content even before processing (Arabia et al., 2024). Normally, the AsA content decreases during storage, since as an antioxidant molecule, L-ascorbic acid is used as a reducing agent to decrease the oxidative effect caused by reactive oxygen species (ROS) produced by respiratory metabolism. Thus, the higher the ripening stage, the lower the AsA content (Macedo et al., 2023). However, in this study, the increase in AsA content was proportional to the advancement in ripening (Fig. 6A), corroborating the literature reports that ascorbic acid biosynthesis occurs in papayas during storage (Selvaraj et al., 1982; Costa et al., 2010; Mendy et al., 2019). One of the AsA biosynthesis pathways occurs through the D-galacturonic acid route, an important compound that acts in the degradation of cell wall pectins. In this case, considering that ripening is a metabolic process that leads to senescence, the increase in AsA content in papayas may be the result of modification of the cell wall that would provide substrate for AsA biosynthesis, corroborated by the loss of fruit firmness (Fig. 2B) during storage. In this sense, the lower accumulation of AsA in fruits treated with arginine (Fig. 6A) is a reflection of the ripening control exerted by this molecule on fruit metabolism.

Lycopene is the most abundant carotenoid found in papaya pulp and, although it does not have vitamin A activity, it contributed to the red color of the fully ripe fruit, corroborated by the increase in concentration and color with storage time (Fig. 6B). In papayas sprayed with arginine, the lower accumulation of lycopene is related to the delay in ripening, that is, in the synthesis of the red color that was only achieved 2 days later in relation to the control fruits (Fig. 6B). In this study, the lycopene content ($11.56 - 25.17 \mu\text{g}\cdot\text{g}^{-1}$) was close to those reported by Laurora et al. (2021) in 'Hawaii' papaya, whose levels ranged from 10.89 (green) to $27.70 \mu\text{g}\cdot\text{g}^{-1}$ (ripe), suggesting that arginine does not interfere in the biosynthesis of this pigment.

CONCLUSION

Arginine treatment via spraying is a promising, accessible and low-cost technology to delay the ripening of Hawaii papaya (2 days), considering the physicochemical quality aspects, such

as delay in the climacteric peak, less mass loss, greater firmness, less evolution of the yellow color, less accumulation of TSS, vitamin C and degradation of lycopene.

Conflict of interest

The authors have no conflict of interest to report.

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