

Do chemical and nutritional compounds change during the storage of Jabuticaba (*Plinia cauliflora*)?

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Abstract

Jabuticaba (*Plinia cauliflora*) is a native Brazilian Atlantic Rainforest fruit tree. Its fruits are purplish berries with a short shelf life, due to fermentative processes that begin shortly after harvest. Recently, commercial jabuticaba exploitation has intensified, justifying the application of postharvest technologies. In this context, this study aimed to evaluate the chemical characteristics and nutraceutical compounds of “ponhema” jabuticabas stored at room and cold temperatures. Chemical analyzes (soluble solids (SS), titratable acidity (TA), pH, soluble sugars, soluble pectins and pectinamethylesterase (PME) activity), and nutraceutical compounds (total anthocyanins (TA), yellow flavonoids (YF), total phenolic compounds (TFC)) were performed. A completely randomized experimental design was applied. Analyzes were performed every 2 days, at days 0, 2, 4 for the room temperature (25° C) assay and at days 0, 2, 4 and 6 for the cold temperature (13° C) experiment. Fruits stored under cold temperature presented lower acetaldehyde and ethanol contents, as well as high soluble sugar, total anthocyanin, and total phenolic compound levels. Fruits stored at room temperature displayed marked wilting and fermentation on the fifth day of storage, preventing their consumption after this period. Fruits presented a shelf-life gain of up to two days when stored at cold temperature, displaying better characteristic maintenance, such as soluble solids, titratable acidity, pH and soluble sugars, which were verified by the acetaldehyde and ethanol tests. Total anthocyanin and phenolic compound levels were higher in fruits stored at cold temperature.

Keywords: Anthocyanins; Native Brazilian Atlantic Rainforest fruit; Phenolics; PCA.

Abbreviations: SS_Soluble solids, TA_Titratable acidity, PME_Pectinamethylesterase, TA_Total anthocyanins, YF_Yellow flavonoids, TFC_Total phenolic compounds.

Introduction

Jabuticaba (*Plinia cauliflora*) is a native Brazilian Atlantic Rainforest fruit tree belonging to the Myrtaceae family. This biome is recognized as one of the richest in the world in terms of biodiversity, but it is under intense devastation caused by human activity (Donado-Pestana et al., 2015).

Three main jabuticaba species exist, namely *Plinia jaboticaba* (Vell) Berg, *Plinia cauliflora* (DC) Berg and *Plinia trunciflora* (Berg) Mattos (Citadin & Danner, 2010). *Plinia jaboticaba* it is most cultivated and known in Brazil, mainly in the Southeastern region, where medium scale productions are found. *Plinia cauliflora*, popularly known as “ponhema” jabuticaba, is less consumed in Brazil than *Plinia jaboticaba*. This fruit occurs spontaneously in most parts of Brazil, being more frequent in the southeastern and southern states. Its fruits are globose berries ranging up to 3 cm in diameter, presenting a thin purple or black skin, whitish (translucent) bittersweet and very tasty pulp, containing from 1 to 4 seeds (Lima et al., 2008).

This native fruit tree is highly productive and rustic, and its fruit can be used in various forms, consumed both fresh and processed. However, jabuticaba commercialization is limited due to high fruit perishability, with a short marketing period after harvesting due to rapid quality changes from intense moisture loss, decay and fermentation (Rufino et al., 2010; Inada et al., 2015; Vieites et al., 2011; Wu et al., 2013). A commonly applied technology is cold storage, which slows down senescence, effectively acting in maintaining produce quality and lengthening marketing periods (Brunini et al., 2004).

Maintaining native fruit quality can offer the population the opportunity to access health-beneficial compounds. Jabuticabas contain significant amounts of anthocyanins, flavonoids, galotanines, elagitanines, depsids, and other phenolic compounds, while also presenting a high antioxidant capacity and containing volatile compounds that determine their flavor (da Silva et al., 2018; Rufino et al., 2010; Inada et al., 2015; Wu et al., 2012; Wu et al., 2013).

The number of studies demonstrating the functional potential of jaboticabas has increased in the last decade, and several studies have reported that the whole fruit, the skin or leaves display the potential to reduce the risk of developing chronic diseases, mainly due to high polyphenol content, such as anthocyanins and flavonoids (Batista et al., 2014; Hsu et al., 2016).

In this context, this study aimed to analyze chemical and nutraceutical compound changes during the storage of “ponhema” jaboticaba, a native Brazilian Atlantic Rainforest fruit, stored at room and cold temperatures.

Results and discussion

Acetaldehyde, ethanol, soluble solids, and titratable acidity

Fruits subjected to room temperature contained acetaldehyde levels of $658.57 \mu\text{g } 100 \text{ g}^{-1}$ on the fourth day of storage, 2.6 times higher than that produced by fruits under refrigeration. Therefore, an increased fermentation process occurred, leading to pulp deterioration, making storage impracticable for any more time (Figure 1A). Fruits subjected to the cold temperature displayed a considerable increase from the sixth day (Figure 1A). Acetaldehyde is a volatile flavor compound present in many fermented foods, important in the production of red and white wines (Osborne et al., 2006), although it becomes undesirable in fresh produce, as it may indicate the beginning of the fermentation period. Ethanol production remained constant until the fourth day of storage for fruits maintained room temperature (Figure 1B). Fruits stored at cold temperature displayed an increase in ethanol contents on the sixth day (Figure 1B). Solomon et al. (2018) reported that fruits stored at room temperature present rapid appearance changes due to intense moisture loss, leading to fruit softening two to three days after harvest. In “Ponhema” jaboticabas were still viable for consumption up to three days after harvest when maintained at room temperature, while fruits stored at cold temperature displayed a shelf-life gain of up to two days. This result is satisfactory, as it demonstrates the postponement of undesirable compound formation and the maintenance of postharvest quality.

The average soluble solids content of fruits stored at room temperature of was 13.4° Brix (Figure 2A), while fruits stored at the cold temperature displayed an increase in soluble solid content from 13.4° Brix to 15.5° Brix at the end of the storage period (Figure 2A). Vieites et al. (2011), when analyzing “Sabará” jaboticabas submitted to different temperatures observed increased soluble solid contents in fruits stored at 9° C and 12° C , respectively, and indicated that this is due to jaboticaba respiratory and climacteric metabolisms. In addition, according to Brunini et al. (2004), soluble solids contents are related to fruit flavor and maturity stage, so the higher the soluble solids content, the better their characteristic flavor.

Concerning titratable acidity (TA) (Figure 2B) $0.74 \text{ g } 100 \text{ g}^{-1}$ citric acid and $1.23 \text{ g } 100 \text{ g}^{-1}$ citric acid were observed on the fourth and sixth day of storage at room and cold temperatures, respectively. da Silva et al. (2018), analyzing “Pêndula” jaboticabas (*Plinia trunciflora*) and “Ponhema” jaboticabas (*Plinia cauliflora*) reported $0.52 \text{ g } 100 \text{ g}^{-1}$ and $1.24 \text{ g } 100 \text{ g}^{-1}$, respectively. During storage, a concentration of reduced hazardous substances for use in the Krebs cycle is noted, i.e. the production of reduced coenzymes, substrates for ATP production, as part of the energy supply for fruit metabolic activities (Oms-Oliu et al. 2011). However, during the fermentation process, titratable acidity such as ethanol

contents tend to increase. Increased titratable acidity levels and ethanol contents were observed on the sixth day of storage in fruits stored cold temperature (Figure 1B and Figure 2B). Thus, although jaboticaba may present adequate external consumption conditions, the internal fermentative process may have already begun.

pH, soluble sugars, soluble pectin and pectinamethyl-esterase activity

Fruits stored at room temperature did not display significant pH alterations (Figure 3A), while fruits stored at 13° C displayed a pH variation during the storage days from 3.3 to 3.6 (Figure 3A). Total soluble sugar contents were the same at both investigated temperatures on the first day of storage. Jaboticabas stored at room temperature displayed a gradual increase from $3.23 \text{ g } 100 \text{ g}^{-1}$ to $4.53 \text{ g } 100 \text{ g}^{-1}$, while cold jaboticabas displayed an increase from $3.66 \text{ g } 100 \text{ g}^{-1}$ to $5.12 \text{ g } 100 \text{ g}^{-1}$ on the sixth day of storage (Figure 3B).

This behavior was similar to that described for SS in fruits stored at both temperatures. Soluble sugar contents may interfere with fruit flavor, where fruits stored at cold temperature presented better shelf life quality compared to fruits stored at room temperature under the conditions applied in the present study. Becker et al. (2015), when analyzing “Sabará” jaboticabas detected total soluble sugars in the skin and pulp of 8.16 and $4.86 \text{ g } 100 \text{ g}^{-1}$, respectively. Enzymes and pectins are present in the fruit cell wall and influence fruit texture during the storage period. Pectinamethyl-esterase (PME) acts on the cell wall and may contribute to fruit softening, determining texture and leading to loss of viscosity, while also contributing to the formation of gels (Van Buggenhout et al., 2009). Regardless of temperature, PME activity is intense as soon as jaboticabas are harvested, contributing to the rapid senescence of this fruit. Enzymatic PME activity decreased from $3836.84 \text{ U}^{-1} \text{ g}^{-1} \text{ min}^{-1}$ (first day of storage) to $3015.30 \text{ U}^{-1} \text{ g}^{-1} \text{ min}^{-1}$ (fourth day of storage) in fruits stored at room temperature, while at cold temperature PME activity decreased from $3836.84 \text{ U}^{-1} \text{ g}^{-1} \text{ min}^{-1}$ (first day of storage) to $3302.88 \text{ U}^{-1} \text{ g}^{-1} \text{ min}^{-1}$ (sixth day of storage). Statistical differences were observed between storage days for both temperatures ($P < 0.05$) (Figure 4A). PME activity appears to be a significant influence concerning postharvest jaboticaba quality. PME activity has been positively correlated to loss of quality in guava fruits (Gill et al., 2016).

Regarding soluble pectins, no statistically significant differences were observed between fruits stored at both temperatures (Figure 4B). However, fruits stored at cold temperature presented higher pectin solubilization contents ($151.38 \text{ mg } 100 \text{ g}^{-1}$ of galacturonic acid) on the sixth day of storage compared to fruits stored at room temperature ($134.12 \text{ mg } 100 \text{ g}^{-1}$ of galacturonic acid) on the fourth day of storage. In contrast, Garcia et al. (2019) reported an increase in soluble pectin contents during cold jaboticaba storage.

Antunes, Gonçalves & Trevisan (2006), when analyzing blackberry fruits, reported higher pectin solubilization percentages in the cold environment than at room temperature, indicating the possibility of enzymatic inactivation, in particular due to the disruption of fruit tissues with juice release. In addition, even though the cold environment presented higher soluble pectin percentages, it

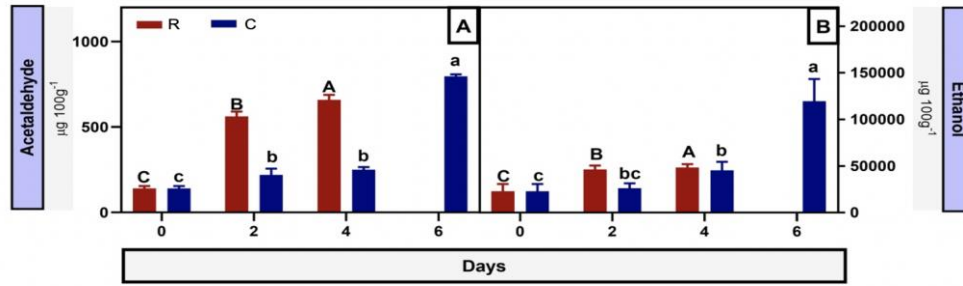


Figure 1. Acetaldehyde (A) and ethanol (B) contents in “Ponhema” jaboticabas stored at room (R) ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold (C) ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) temperatures. Different upper- and lower-case letters in each column indicate statistically significant differences for the room and cold temperatures, respectively.

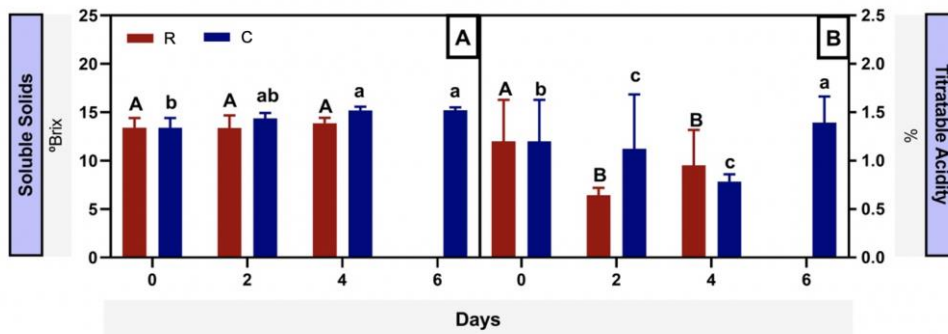


Figure 2. Soluble solids (A) and titratable acidity (B) in “Ponhema” jaboticabas stored at room (R) ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold (C) ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) temperatures. Different upper- and lower-case letters in each column indicate statistically significant differences for the room and cold temperatures, respectively.

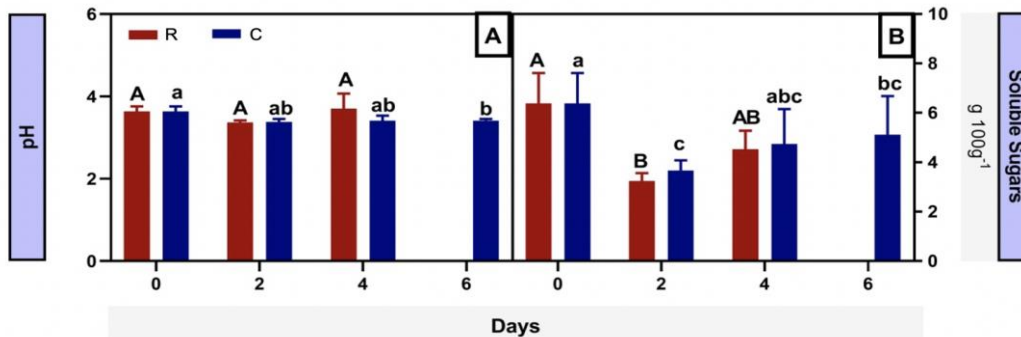


Figure 3. pH (A) and soluble sugars (B) in “Ponhema” jaboticabas stored at room (R) ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold (C) ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) temperatures. Different upper- and lower-case letters in each column indicate statistically significant differences for the room and cold temperatures, respectively.

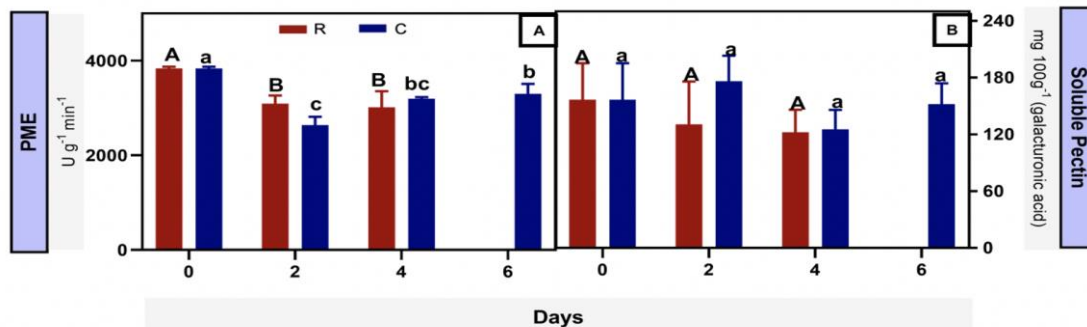


Figure 4. Pectinamethylesterase (PME) activity (A) and soluble pectin (B) in “Ponhema” jaboticabas stored at room (R) ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold (C) ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) temperatures. Different upper- and lower-case letters in each column indicate statistically significant differences for the room and cold temperatures, respectively.

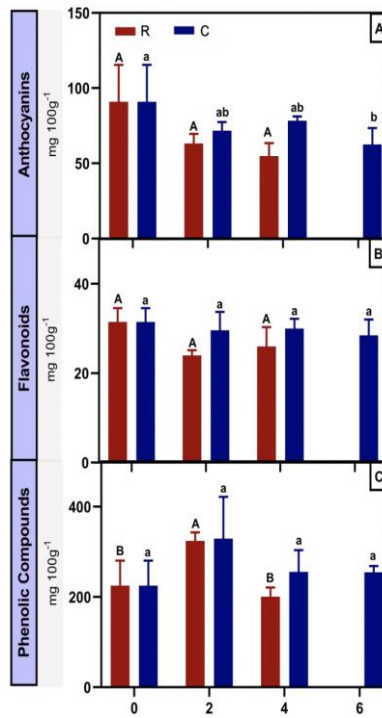


Figure 5. Total anthocyanins (A), yellow flavonoids (B) and total phenolic compounds (B) in “Ponhema” jaboticabas stored at room (R) ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and cold (C) ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) temperatures. Different upper- and lower-case letters in each column indicate statistically significant differences for the room and cold temperatures, respectively.

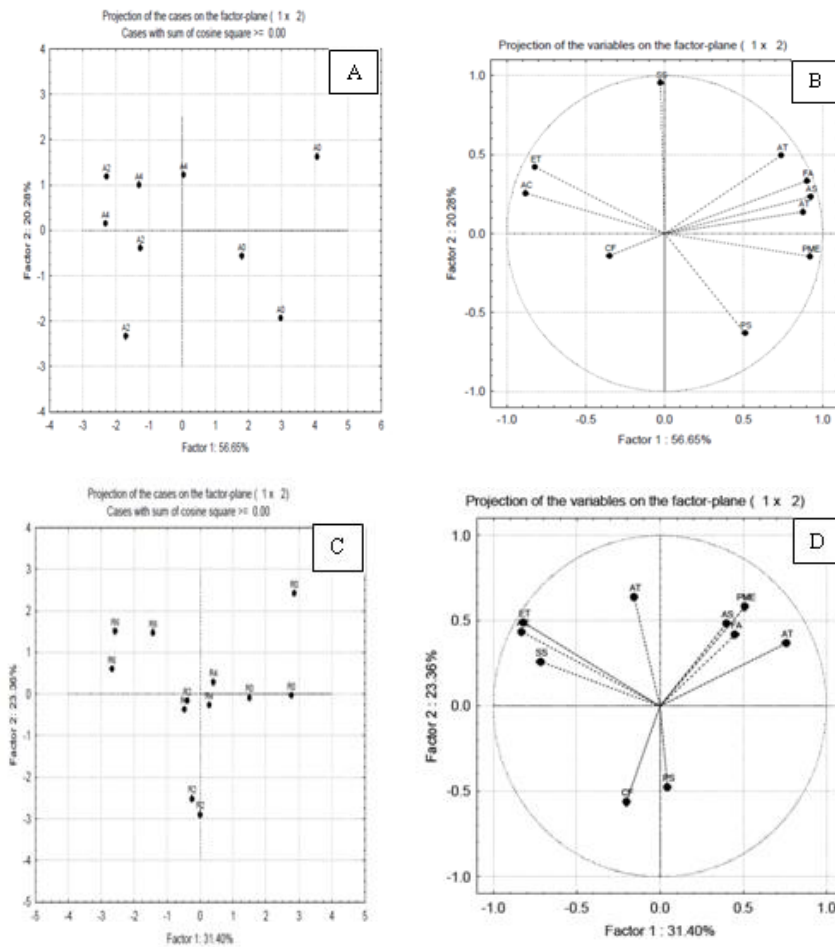


Figure 6. Principal component analysis of fruits in “Ponhema” jaboticabas stored at room ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) (A and B) and cold ($13^{\circ}\text{C} \pm 1^{\circ}\text{C}$) (C and D) Labels: AC = Acetaldehyde; ET = Ethanol; TA = titratable acidity; SS = soluble solids; AS = soluble sugars; YF = yellow flavonoids = ATT = anthocyanins; FC = phenolic compounds; SP = soluble pectin; PME = Pectinamethylesterase.

was more suitable for fruit conservation, leading to the maintenance of adequate characteristics for longer marketing periods. Vieites et al. (2011) observed similar behavior for jaboticabas stored at different temperatures.

Total anthocyanins, yellow flavonoids and phenolic compounds

Anthocyanins are phenolic compounds responsible for the red, blue and violet colors in fruits, and are abundant in jaboticabas (Calloni et al., 2015). Total anthocyanin contents (Figure 5A) were better maintained in fruits stored at 13 °C, with average contents of 62.56 mg 100 g⁻¹ on the sixth day of storage, while fruits stored at room temperature contained 54.87 mg 100 g⁻¹ total anthocyanin contents. Fruits stored at room temperature displayed higher variations in total anthocyanin contents during the entire storage period, probably due to respiratory processes. Thus, it is clear that the cold temperature was more effective for the maintenance of this bioactive compound.

The cyanidine group is the most abundant anthocyanin in jaboticabas (Inada et al., 2018; Inada et al., 2015). Studies have correlated the performance of this compound with antioxidant capacity in both *in vitro* and *in vivo* experiments (Wu et al., 2013; Inada et al., 2015). Yellow flavonoid contents were determined as 28.22 mg 100 g⁻¹ and 30.16 mg 100 g⁻¹ for fruits stored at the assessed room and cold temperatures, respectively (Figure 5B). Fruits stored at room temperature displayed a greater loss of this pigment when compared to fruits subjected to refrigeration. In contrast, the contents reported by Rufino et al. (2010) were higher, at 147 mg 100 g⁻¹.

Regarding total phenolic compounds, contents decreased at both temperatures after the second day of storage (Figure 5B). However, the loss was more significant (324.19 mg GAE 100 g⁻¹ on the second storage day to 200.81 mg GAE 100 g⁻¹ on the fourth storage day) in fruits stored at 25 °C compared to fruits stored at 13 °C, whose contents remained almost constant between the fourth (255.71 mg GAE 100 g⁻¹) and the sixth day of storage (254.71 mg GAE 100 g⁻¹). Phenolic compound levels in jaboticabas fall sharply while the fruit is in a senescence state. Vieites et al. (2011) indicated a set of chemical and enzymatic changes in phenol compounds, resulting in the maturation process, as being responsible for the decrease of the phenolic compounds in jaboticabas. In addition, the refrigeration temperature was proven efficient in conserving these contents compared to room temperature.

Principal component analysis concerning the temperature assays

The Principal Component Analysis (PCA) carried out for jaboticabas stored at room temperature explained 76.93% of the data variation, 56.65% in the first factor and 20.28% in the second (Figures 6A and B). The initial storage day was correlated to titratable acidity, soluble sugars, yellow flavonoids, total anthocyanins, pectinamethylase and soluble pectin, which displayed the most significance. On days 2 and 4, a correlation was observed between the decreasing levels of phenolic compounds. Acetaldehyde, ethanol and soluble solids were observed in the same quadrant, demonstrating that these characteristics were significant concerning the senescence metabolism of jaboticabas stored at room temperature.

The Principal Component Analysis (PCA) carried out for jaboticabas stored at cold temperature explained 54.76% of

the data variation, 31.40% in the first factor and 23.36% in the second (Figures 6C and D). The first factor was related to the initial storage day and the second and fourth storage days, with soluble sugars, pectinamethylase activity, soluble pectin, yellow flavonoids, total anthocyanins and phenolic compounds displaying the most significance. The second component was characterized by the sixth day of storage, whose acetaldehyde and ethanol contents, as well as soluble solids contents and titratable acidity, formed a cluster. The PCAs for room and cold temperature indicated changes in jaboticaba quality during storage, mainly concerning the fermentation process and acetaldehyde and ethanol production. Similar data were reported by Wall, Miller & Siderhurst (2018) in *Morinda citrifolia* L fruits, native to India, whose fully ripe and fermented samples were clearly separated from the other pre-mature stages, forming two clusters, with the three pre-mature stages grouped as the third cluster.

Material and methods

Chemicals and reagents

All solutions were prepared with analytical reagents and ultrapure water (Milli-Q, Millipore, Bedford, MA, USA). The following reagents were purchased from Sigma-Aldrich (St. Louis, CO, USA): sodium hydroxide (NaOH), hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate, ethanol, acetaldehyde, soluble sugars and galacturonic acid.

Fruit sampling

"Ponhema" jaboticabas (*Plinia cauliflora*, trees about seventy years old) were harvested manually at the mature stage (purple skin) in Piracicaba, São Paulo, Brazil.

Fruit storage

The fruits were placed in 14.5 cm wide, 24.2 cm long and 5.6 cm high trays. Thirty fruits were used for each day and storage condition, i.e. 90 and 120 fruits, for the room and cold tests, respectively. The trays were placed in rows and stored at room temperature of 25 °C ± 1 °C and cold temperature of 13 °C ± 1 °C, at a relative humidity of 85%.

Analyses

Analyses were performed every 2 days, at days 0, 2, 4 for the room temperature assay and at days 0, 2, 4 and 6 for the cold temperature experiment. For the experiments, the seeds were removed, and the whole fruits were homogenized.

Chemical analyses

Acetaldehyde and ethanol

Acetaldehyde and ethanol were determined according to the adapted method reported by Davis & Chace Junior (1969). Ethanol and acetaldehyde standards were prepared with 1 mL of each samples containing from 100 to 3000 µg of ethanol and 5 to 43 µg of acetaldehyde placed in 40 mL flasks and maintained in a water bath at 50 °C for 30 minutes. Subsequently, 1 mL of the free vial space was collected with a 2.5 mL Gastight Hamilton syringe and injected into a gas chromatograph equipped with a flame ionization detector (FID) and a 1.8 m Porapack N column, in order to establish the standard curve. The acetaldehyde and ethanol contents of each sample were calculated by correlating the respective chromatographic areas with those

obtained in the standard curves. Results were expressed as $\mu\text{g } 100 \text{ g}^{-1}$ fresh weight (f.w.).

Soluble solids (SS), titratable acidity (TA) and pH

SS were quantified using an Abtato PR-101 digital Palette refractometer and the results were expressed as °Brix (AOAC, 2012). Titratable acidity (TA) was measured by diluting 1 mL of the fruit juice with 80 mL of distilled water and titrated with 0.1 M NaOH to a pH 8.2 endpoint using a Metrohm 862 compact titanium sampler (Herisau, Switzerland), according to the methodology described by Carvalho et al. (1990). The results were expressed as $\text{g } 100 \text{ g}^{-1}$ of citric acid. The pH was measured using a potentiometer from liquefied samples, according to the methodology described by Carvalho et al. (1990).

Soluble sugars, soluble pectins and pectinamethylesterase (PME) activity

Soluble sugars were determined by spectrophotometry at 490 nm using a standard glucose curve (1%) ranging from 10 to 90 mg, as described by Dubois et al. (1956). The results were expressed as $\text{g } 100 \text{ g}^{-1}$ f.w. of glucose. Soluble pectins were extracted according to McCready & McComb (1952), and quantified by the Bitter and Muir (1962) technique, with results expressed as $\text{mg } 100 \text{ g}^{-1}$ of galacturonic acid f.w. Pectinamethylesterase (PME) activity was determined according to Jen & Robinson (1984), where one unit of PME was defined as the amount of enzyme capable of catalyzing pectin demethylation corresponding to the NaOH consumption per gram of fresh pulp per minute. Results were expressed as units per gram of fresh fruit per minute ($\text{U min}^{-1} \text{g}^{-1}$ f.w.).

Nutraceutical compounds

Total anthocyanins (TA) and yellow flavonoids (YF)

Anthocyanins and yellow flavonoids were determined following the methodology reported by Francis (1982) with modifications. A total of 1 g of pulp was used for 50 mL of the extraction solution (ethanol P.A: 1.5 M HCl - 85:15) and stored under refrigeration for 12 hours. The samples were then filtered through Whatman No. 1 filter papers and the filtrate were used for anthocyanin and yellow flavonoid quantitation. Values were expressed as $\text{mg } 100 \text{ g}^{-1}$ f.w.

Total phenolic compounds (TFC)

Total phenolic compounds (TFC) were determined by the Folin-Ciocalteu method described by Woisky & Salatino (1998). Fresh samples (1g) were extracted with methanol (80% v/v) and filtered through filter paper and mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10), 2 mL of a 4% (w/v) sodium carbonate solution and maintained in the dark for 2 h. Absorbances were determined at 740 nm on a Biochrom Libra S22 spectrophotometer. Results were expressed as $\text{mg } 100 \text{ g}^{-1}$ gallic acid equivalents (GAE) f.w.

Data analyses

A completely randomized experimental design was applied. For the $25 \text{ }^\circ\text{C} \pm 1^\circ\text{C}$ assay, an initial sampling + two samples (2 and 4 days) was performed, while an initial sampling + three samples (2, 4, 6 days) were performed for the $13 \text{ }^\circ\text{C} \pm 1^\circ\text{C}$ assay. Three replicates with ten fruits were used for each storage day. The results were submitted to an individual variance analysis for each storage temperature. Means were compared by Tukey's test at a 5% probability of error using the SAS 9.4 software. A principal component analysis was applied using the Statistica software.

Conclusions

Fruits stored at cold temperature presented a shelf-life gain of up to two days, with better soluble solid, titratable acidity, pH and soluble sugar maintenance as verified by the acetaldehyde and ethanol tests. Total anthocyanins and phenolic compounds were higher in fruits stored at cold temperature, while the difference between the two temperatures tested was not relevant for yellow flavonoids.

Conflict of interest

The authors have no conflict of interest to declare. Also, no part of this paper is available in any format in any media before publication in AJCS.

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