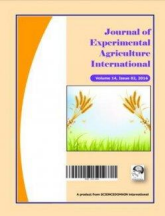

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Physiological and Biochemical Responses of Banana 'Prata-Anã' Subjected to Hydrothermal Treatment

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JMDSP and GPM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. The authors FSA, MLMR, MODJ, LGCDQ and SNAF managed the analyses of the study. Authors EHM and PFSA managed the literature searches and assisted in the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The objective of this study was to assess the effect of hydrothermal treatment applied to banana 'Prata-Anã' before refrigerated storage.
Study Design: The employed experimental design was the completely randomized type (CRD) and composed of 4 repeats with 4 fruits, in a 5 x 4 +1 factorial scheme, with five storage days (days 1, 2, 3, 4 and 5) at 25°C after removal from the refrigerated chamber, four immersion temperatures and one control (fruits without hydrothermal treatment).

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Study Location and Duration: The experiment was conducted in the Post-Harvest Physiology Laboratory of the State University of Montes Claros between March and June 2017.
Methodology: The bananas were harvested and separated into 4-fruit bouquets, which were immersed in water at 50, 52, 54 and 56°C for 2 minutes; fruits without hydrothermal treatment were used as control. Each bouquet was placed in polyolefin membrane packs that were then contained in boxes and stored in chambers at 10°C and RH of 80% for 25 days. After the 25 days, the fruits were taken out of the chamber and stored at 25°C. During the 25°C storage, physical and chemical analyses were performed.
Results: On the day of removal from the refrigerated chamber, all bananas, regardless of treatment, presented a yellowish color. The temperature of 56°C resulted in greater fresh mass loss and higher solute extravasation values. Lower chilling index values and higher vitamin C values were found as the immersion temperature increased.
Conclusion: The full ripening process of banana 'Prata-Anã' occurred after removal from the refrigerated chamber in all hydrothermal treatments and with lower chilling index values.

Keywords: Musa spp.; hydrothermal therapy; post-harvest; preservation.

1. INTRODUCTION

After harvest, bananas are usually exposed to low storage temperatures so that their respiration, ripening and senescence are delayed, increasing their shelf life and marketing period. However, tropical and sub-tropical fruits and vegetables are sensitive to low temperatures and may develop chilling injuries if the temperature is too low or if low temperature conditions are maintained for a long period of time. The incidence of chilling, a physiological disorder, limits the use of low temperature storage. Cold-sensitive fruits and vegetables suffer cell membrane damage and produce reactive oxygen species (ROS), which causes oxidative stress.

Currently, post-harvest technologies are employed to inhibit or reduce chilling injury (CI) in sensitive fruits and vegetables of high commercial interest. Use of thermal treatment is considered as an ecological, environment-friendly technology against CI. Post-harvest thermal treatments change the normal protein synthesis and cellular metabolism program during thermal stress. When thermal stress is imposed, there is a rapid disassociation of polyribosomes, and the protein synthesis is briefly interrupted and then resumed with a new set of proteins, including HSPs (heat shock proteins) [1]. The consequence of this change is that normal ripening processes are inhibited, and if the fruit is exposed to low temperature, the inhibition persists for a while. When the fruit is exposed to room temperature after refrigerated storage, ripening is resumed. Therefore, post-harvest thermal treatments can regulate the ripening rate of commodities, in addition to preventing post-harvest storage disorders [2,3].

In order to extend the post-harvest life and maintain the quality of bananas 'Prata-Anã', the objective of this study was to assess the effect of hydrothermal treatment applied to them before refrigerated storage.

2. METHODOLOGY

Bananas 'Prata-Anã' cultivated in the municipality of Nova Porteirinha, Minas Gerais, Brazil, at 15°41'21.4'' South latitude and 43°16'23.3'' West longitude were harvested on March 28, 2017. The fruits were harvested at stage 1, according to the Von Loesecke ripening scale [4]. The bananas were decoupled from their branches and immersed in a water tank at room temperature for latex coagulation, as well as field heat and harvest residue removal. After this procedure, they were placed inside plastic boxes and carried to the State University of Montes Claros' Post-Harvest Physiology Laboratory, Janaúba campus, Minas Gerais.

In the laboratory, bananas with no injuries and that met the standards were selected and separated into 4-fruit bouquets. The latter were washed again in running water and neutral detergent at 0.2% for removal of latex residues. Then, for treatment composition, the bouquets were immersed in water heated up at 50, 52, 54 and 56°C for 2 minutes with the aid of SOLAB water-curing bath, model SL-154, with water circulation and digital display for temperature monitoring. Control fruits were those without hydrothermal treatment.

Immediately after each hydrothermal treatment, the fruits were immersed in cold water at ± 20°C for 2 minutes to stop the heating. After application of each hydrothermal treatment and immersion in cold water, the fruits were immersed in an Imazalil fungicide (Magnate®) solution for 3 minutes at a concentration of $2mL.L^{-1}$. Subsequently, the fruits were placed on benches to be dried in ambient air. The control fruits were only immersed in the fungicide solution.

Each bouquet was placed in 'Vegetalpack' polyolefin membrane packs with a thickness of 25 micrometers and density of 0.9987 g/cm³. The air inside the pack was removed to the maximum with the aid of a vacuum cleaner, and the pack was sealed with a string. The packs were placed inside standard cardboard boxes for export, with a 18-kg capacity, and stored in refrigeration chambers at 10°C ± 1°C and relative humidity of 80% ± 5% for 25 days.

After all 25 days of storage, the fruits were taken out of the refrigeration chamber and stored at 25°C on benches in the laboratory to simulate the marketing period.

The employed experimental design was the completely randomized type (CRD), composed of 4 repeats with 4 fruits, in a 5 x 4 + 1 factorial scheme, with five storage days (day 1, 2, 3, 4 and 5) at 25°C after removal from the refrigerated chambers, four immersion temperatures and one control (fruits without hydrothermal treatment).

After the fruits were taken out of the chamber, during storage at 25°C, the following variables were analyzed daily:

- Fresh mass loss: Each bouquet was weighed on GEHAKA electronic scale, model BK6000. Results were expressed as percentage (%) of fresh mass loss.
- Soluble solids (SS): SS quantification was performed on Reichert digital bench refractometer (AR200), which provided results in °Brix.
- Titratable Acidity (TA): TA was determined according to AOAC methodology [5]. Results were expressed as g of malic acid per 100 g of $pulp^{-1}$.
- Ripening Index: The "ratio" (ripening index) was obtained by dividing the nominal value of soluble solids (°Brix) by the titratable acidity value.
- Solute extravasation: The analysis was carried out according to methodology described by Serek [6]. Results were expressed as percentage.
- Chilling Index: A 1-5 grade scale was used, which was described by Nguyen [7], based on the browning intensity on the peel surface.
- Color Analysis (lightness, °hue and chroma): Color was determined by means of a Color Flex colorimeter, model No 45/0, with direct reflectance reading of coordinates L* (lightness), coordinate a* and b* of the Hunterlab Universal Software colorimetric system. a* and b* values provided hue angle ($°hue= tg^{-1}$ (b*/a*)) and chroma ($C^*= (a^{*2} + b^{*2})^{0,5}$) values, with results being expressed as (°).
- Fruit Firmness: It was determined with a Brookfield texturometer, model CT3 10K. Results were expressed as Newton (N).
- Vitamin C: Ascorbic acid content was determined by titrimetry. Results were expressed as mg of ascorbic acid per 100 g^{-1} of pulp [8].
- Color index: The fruits' color evolution was determined with the aid of a visual color scale according to PBMH & PIF classification norms [4].
- Peel Total Phenolic Compounds: They were determined by Fu's method [9]. Results were expressed in milligrams of gallic acid equivalent (GAE) per 100 g of peel.
- Peel Carotenoids: Determined on spectrophotometer with acetone 80% according to Lichtenthaler's equations [10] and expressed as µg. g of peel.

Data were subjected to analysis of variance, and the significance of the interactions between the tested factors was verified, with further slicing for significant results. Polynomial regression models were adjusted, and significant models with higher coefficient of determination and that better explained the biological phenomenon were selected. Dunnett's test was performed as well, at a 5% probability, to compare data on control fruits and on those subjected to the hydrothermal treatments. Data analysis processing used software SISVAR [11], while software SAS was used for Dunnett's test.

3. RESULTS AND DISCUSSION

Fresh mass loss was significantly higher for fruits subjected to the temperatures of 54 and 56°C compared to control (Table 1). The hydrothermal

treatment with these temperatures may have eliminated waxy cuticles from the surface of the fruit, which cause a greater water loss to the environment. The waxy cuticle that covers the plant surface is an effective barrier against water motion [12]. Post-harvest water loss in tropical fruits depends on the fruit, the cultivar, pre-harvest conditions, vapor-pressure deficit, peel damages, post-harvest thermal treatments, and presence of overlays or wrappers [13].

The temperature of 56°C also promoted a greater cell solute extravasation on all assessment days and negatively affected fruit lightness and chromaticity on the last assessment day, with control differing significantly, which indicates changes in the composition and permeability of the cellular membrane and in the wax that composes the protecting cuticle of the fruit (Table 1). However, the temperatures of 54 and 56°C were effective in increasing the amount of vitamin C on the first assessment day, after the fruits were taken out of the refrigerated chamber, in addition to reducing chilling index symptoms on the third and fourth days. These temperatures probably induced an

Table 1. Treatment means and Dunnett's test for fresh mass loss (ML, %), solute extravasation (SE, % of total), lightness (LI, °), chroma (CR, °), vitamin C (VC, mg asc. acid 100 g pulp^{-1}), chilling injury (CI) in bananas 'Prata-Anã' subjected to hydrothermal treatment and stored for 25 days at 10°C

Days	T °C	Assessed variables					
		ML	SE	LI	CR	VC	CI
1	50	1.5	29.1	59.5	48.8	7.1	1.2
1	52	1.3	25.2	57.4	47.3	8.0	1.5
1	54	2.0*	31.3	57.9	45.0	8.9*	1.0
1	56	2.6*	33.2*	61.5	48.1	9.1*	1.0
1	TEST[2]	1.3	24.4	58.9	46.4	7.1	1.2
	CV	13.4	14.8	4.5	7.4	9.9	34.0
2	50	1.6	23.3	60.5	47.8	6.9	1.2
2	52	1.5	21.4	61.9	44.0	7.6	1.5
2	54	2.1*	25.5	60.4	43.9	7.7	1.2
2	56	2.7*	35.6*	54.9	43.7	9.6	1.0
2	TEST[2]	1.4	23.2	59.2	47.2	7.7	1.7
	CV	12.4	14.2	6.9	8.6	13.1	34.5
3	50	1.8	29.6	58.9	45.4	7.3	2.7
3	52	1.7	31.1	62.7	42.6	71	1.7*
3	54	2.4*	31.9	59.9	47.2	7.8	1.2*
3	56	2.9*	36.9*	59.1	45.3	8.9	1.7*
3	TEST[2]	1.6	26.9	54.5	44.5	7.7	3.0
	CV	11.4	10.1	10.7	8.3	10.3	27.5
4	50	2.0	30.7	56.4	43.2	8.2	2.2
4	52	1.9	29.3	55.6	44.4	7.7	2.2
4	54	2.6*	31.2	54.8	46.8	7.8	2.0*
4	56	3.1*	34.1*	53.8	44.9	7.7	2.0*
4	TEST[2]	1.9	27.7	62.5	45.6	7.3	3.0
	CV	10.3	9.9	9.7	9.9	8.6	21.0
5	50	2.0	41.1	59.1	44.6	6.8	2.7
5	52	1.9	33.5	57.9	45.5	6.2	2.7
5	54	2.6*	30.6	55.9	45.3	6.8	2.2
5	56	3.1*	46.8*	50.5*	39.8*	8.3	2.2
5	TEST[2]	1.9	25.4	60.9	46.5	6.9	3.0
	CV	10.0	23.8	7.1	6.5	20.1	17.2

Means followed by an asterisk (*) differ from control by Dunnett's test (P<0.05) for each storage day at 25°C. 1Storage days at 25°C after refrigerated storage. 2 Control: Fruits without hydrothermal treatment

increase of reactive oxygen species, stimulating the metabolism route of vitamin C, which has a high antioxidant potential in the cell and possibly reduced the damages caused by the chilling index.

The following variables did not present significant differences in relation to control: soluble solids, pH, titratable acidity, ripening index, ^{o}hue, pulp firmness, fruit firmness with peel, color index, phenolic compounds, and carotenoids; therefore, the hydrothermal treatment did not affect the fruits' ripening process.

Analyzing only fruits subjected to hydrothermal treatments, the fresh mass loss variable presented isolated effect for assessment days and hydrothermal treatments, with a linear mass loss variation of 1.87% to 2.45% throughout assessments (Fig. 1-A). The highest fresh mass loss percentage was 2.90%, found in fruits subjected to 56^{o}C (Fig. 2-B). The highest fresh mass loss detected in the present study is considered low (<5%) and did not cause apparent withering in the banana peel, which did not lower the product's marketing value.

According to Kechinski, C. P. et al. [14], hydrothermal treatment changed the natural wax layer of papaya, producing a more even cover, with fewer cracks; crystalloids, typical wax layer structures, formed on the surface of the treated fruits. However, this study observed that a higher immersion temperature favored a greater fresh mass loss, likely due to the removal of the fruits' natural thin wax layer, favoring the transpiration of the free water present inside the cells.

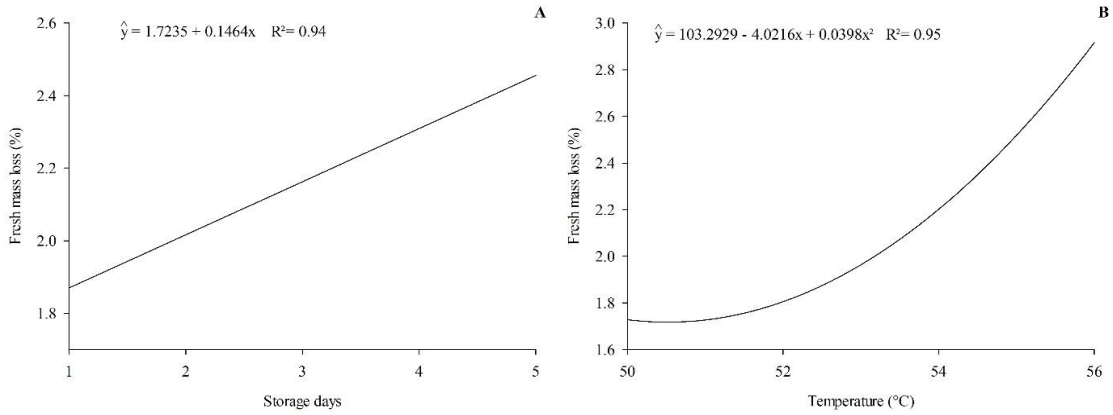

Fig. 1. Fresh mass loss (%) of banana 'Prata -Anã' subjected to hydrothermal treatment and stored at 10^{o}C for 25 days, as a function of storage days at 25^{o}C and immersion temperatures (previous hydrothermal treatment)

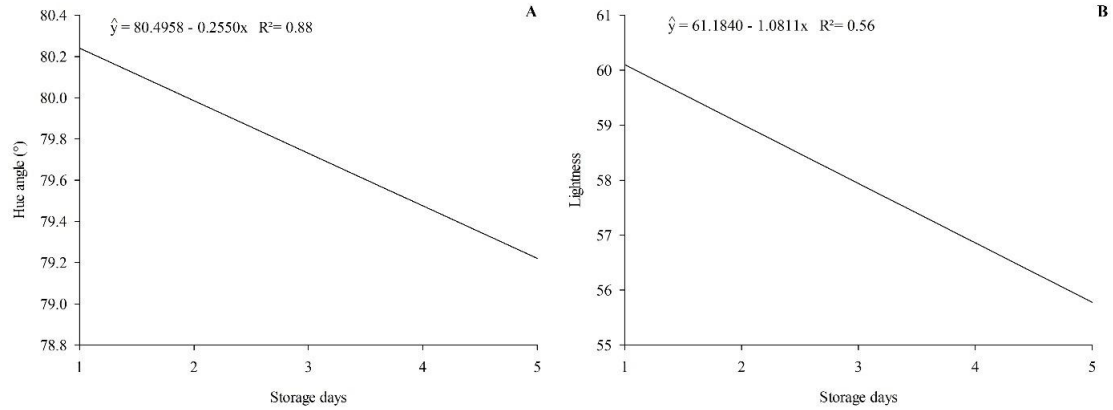

Fig. 2. Hue angle and lightness of the peel of banana 'Prata-Anã' subjected to hydrothermal treatment and stored at 10^{o}C for 25 days, as a function of storage days at 25^{o}C, after removal from refrigeration

As shown by Fig. 2-A, the °hue variable presented decreasing linear effect due to chlorophyll degradation over the storage days at 25°C, ranging from 80.24° to 79.22°, that is, the bananas were yellow already (close to scale 5) since the 1st day after removal from the refrigerating chamber. This variable had no effect as to hydrothermal treatment. Perhaps, the temperatures used in the experiment were not efficient in inactivating the activity of chlorophyllases, this is why the bananas 'Prata-Anã' lost their green color.

According to [15], ripening inhibition by heat can be mediated by the effect of heat on the ripening hormone, ethylene. Besides, the inactivation of the activity of chlorophyll-degrading enzymes, as a result of the hot water treatment, is also a possible reason for reducing degreening. [16] describe that the action mechanism of ethylene consists of it binding to a receptor molecule located in the endoplasmic reticulum membrane, which, through phosphorylation, triggers a signal transduction cascade until the activation of enzyme-encoding genes, which mediate physical and biochemical changes that follow the ripening process of fruits.

Lightness (L*) values decreased over the storage days, presenting isolated effect for storage at 25°C, that is, the bananas lost their brightness throughout this period (Fig. 2-B). According to [17], lightness (L*) indicates how much the sample changes its color to a darker or lighter shade. [18] found L* value reductions associated to the occurrence of chilling injuries in bananas 'Cavendish'. In the present study, a light degree of chilling on banana 'Prata-Anã' peel caused bright loss, decreasing lightness values.

The chilling index presented isolated significant effect for storage days and hydrothermal treatments, with an increase from 1.08 to 2.49 over the storage days at 25°C (Fig. 3-A) and decrease from 2.05 to 1.53 as the immersion temperatures were raised (Fig. 3-B). Presumably, the hydrothermal treatment dropped the chilling index due to the phenomenon known as cross-protection, in which a stress induced by the hydrothermal treatment probably caused the accumulation of ROS-inactivating enzymes and molecular chaperones, which persisted in the banana during the refrigerated storage at 10°C and for a while after removal from the cold chamber, attenuating chilling on the banana 'Prata-Anã' peel. Moreover, a change in the composition of membrane lipids may also have happened due to an increase in the activity of desaturases, increasing the proportion of unsaturated lipids, causing the membranes to remain fluid under very low temperatures and protecting the bananas against cold-related damages.

Wang, H. et al. [19] found that, for immersion of bananas 'Cavendish' in hot water (52°C for 3 minutes), at intervals of 0.5 to 24 hours at 20°C before refrigerated storage at 7°C for 5 days, all fruits treated with hot water presented lower chilling injury indexes compared to control fruits (unheated). According to the same authors, the effect of thermal treatments on cold resistance induction may be explained by a "cross-adaptation" scenario, in which, with sub-lethal

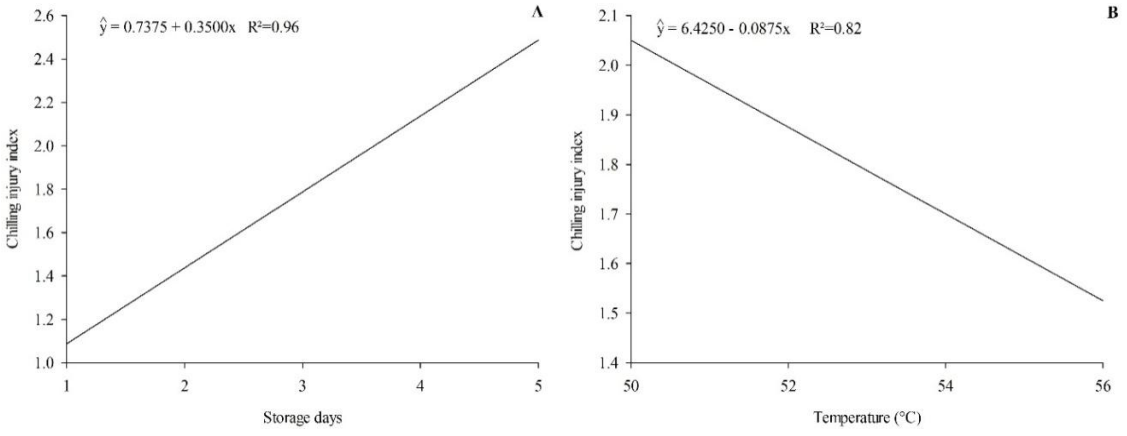

Fig. 3. Chilling injury index on the peel of banana 'Prata-Anã' subjected to hydrothermal treatment and stored at 10°C for 25 days, as a function of storage days at 25°C, after removal from refrigeration and immersion temperatures (previous hydrothermal treatment)

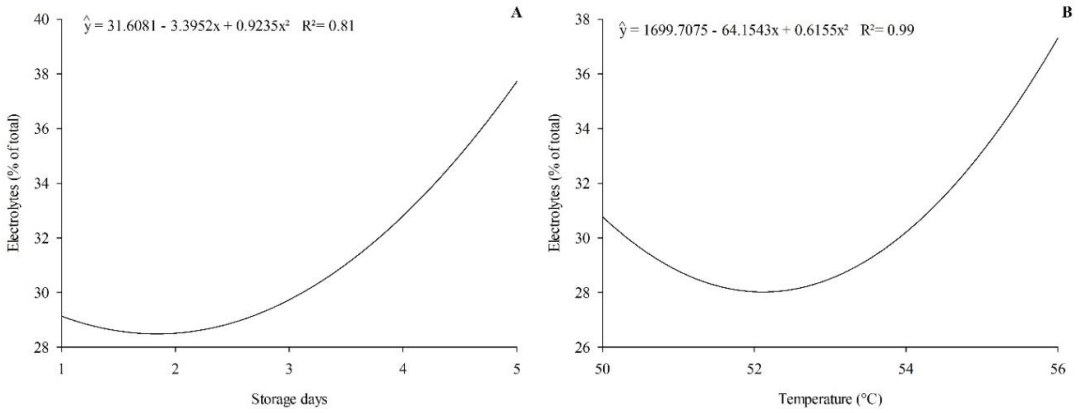

Fig. 4. Solute extravasation (% of extravasated electrolytes in relation to total cell electrolytes) of the peel of bananas 'Prata-Anã' subjected to hydrothermal and stored at 10°C for 25 days, as a function of storage days at 25°C after removal from refrigeration and immersion temperatures (previous hydrothermal treatment)

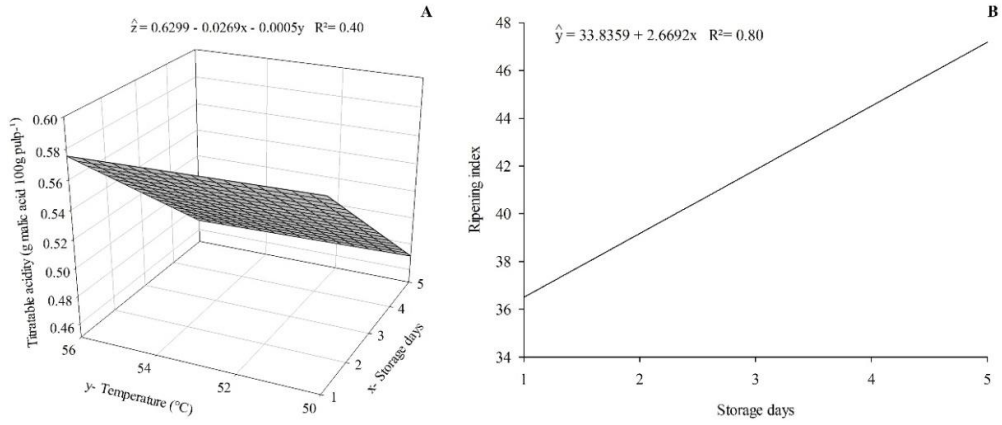

Fig. 5. Titratable acidity (g of malic acid per 100 g of pulp^{-1}) and pulp ripening index of banana 'Prata-Anã' stored at 10°C for 25 days, as a function of different immersion temperatures (previous hydrothermal treatment) and storage days at 25°C, after removal from refrigeration

heating, many plant species develop resistance to cold and to other types of stress.

Solute extravasation increased over the days after the fruits were taken out of the refrigerated chamber, regardless of immersion temperature (Fig. 4-A). In contrast, those subjected to 56°C presented higher solute extravasation values (Fig. 4-B). It is believed that all fruits were already in the post-climacteric phase and, thus, the cellular membranes had already lost their selective permeability. Electrolyte loss by the fruits is linked to ripening and senescence. The treatment at 56°C may have affected the plasmatic membrane of the cells, causing a greater oxidative stress derived from an imbalance between reactive oxygen species, enzymes and antioxidant compounds, resulting in the peroxidation of lipids of the plasmatic membrane by free radicals and in increased intracellular material extravasation, favoring the bananas' senescence.

Titratable acidity decreased over the assessment days at the respective immersion temperatures (Fig. 5-A), with significant interaction being observed between the analyzed factors. The treatments showed minimal difference and behaved similarly. Titratable acidity content ranged on average from 0.58 to 0.47 g of malic acid. 100 g of pulp^{-1} from the first to the fifth assessment days, respectively, indicating that the fruits were already ripe since the first storage day at 25°C. Because the fruits were already

ripe, accumulated organic acids probably began to be used in respiration, which decreased acidity during storage at 25°C after removal from the refrigerated chamber.

According to [20], pulp pH and titratable acidity are important attributes in post-harvest ripening analysis of bananas. Usually, when fruits are harvested unripe, pulp pH is high; as they ripen, pH decreases. Organic acid levels in a fruit may significantly affect its flavor. Normally for bananas, the pulp has higher acidity during ripening, and the main organic acids present are malic, citric and oxalic. With ripening and senescence, acidity decreases, presumably due to use as respiratory substrates.

Due to high soluble solid content (mean of 21.3 °Brix) and lower titratable acidity, there was a sharp linear increase in the "ratio" ripening index over the storage days (Fig. 5-B), regardless of immersion temperature. The increase in "ratio" during ripening is related to the flavor of fruits and is a more representative index than the isolated measurement of soluble solids or acidity [21]. According to the results in this study, different treatments with hot water did not affect the ripening index of banana 'Prata-Anã'. Therefore, the flavor of banana 'Prata-Anã' can be preserved in the treatment with hot water at 56°C for 2 minutes.

Vitamin C values presented linear effect for storage days, ranging from 8.32 to 7.25 mg of malic ascorbic acid per 100 g of pulp^{-1} (Fig. 6-A). The highest value was found for the hydrothermal treatment at 56°C (8.53 mg of ascorbic acid per 100 g of pulp^{-1}), regardless of assessment day (Fig. 6-B). Vitamin C index reduction may be attributed to ascorbic acid oxidation as the fruit ripens; however, the treatment at 56°C kept the fruit with a higher amount of antioxidants.

Bananas treated at 50°C for 10 minutes presented significant differences as to ascorbic acid (AA) content, with the latter being higher for bananas without thermal treatment (control) [22]. This confirms that stress during treatment with hot water, before refrigerated storage, can activate the antioxidant system in fruits during refrigerated storage and, as a result, antioxidant contents may rise to lower oxidative stress as the banana ripens.

Carotenoids dropped from 11.93 to 9.08 µg per g of peel^{-1} during storage at 25°C (Fig. 7-A). In line with [20], carotenoid levels (xanthophylls and carotenes) remained approximately constant at 8 µg/g of fresh weight in ripe bananas.

In 61 banana varieties, the mean total carotenoid content was 4.33 µg g^{-1}; among triploids, the means were 3.05 µg g^{-1} (AAA), 9.31 µg g^{-1} (AAB) and 3.15 µg g^{1}(ABB) [23]. These values are similar to those of the present study with bananas 'Prata-Anã' in group AAB.

The phenolic compounds variable presented interaction between storage days and immersion temperature. Phenolic compounds decreased during storage at 25°C for all immersion temperatures, with the highest values being obtained in fruits subjected to the temperature of 56°C – 70.61 and 33.35 mg of GAE^{-1} per 100 g of peel on the first and last storage days at 25°C, respectively (Fig. 7-B). [24] observed a reduction in phenolic compound content throughout ripening in different banana varieties.

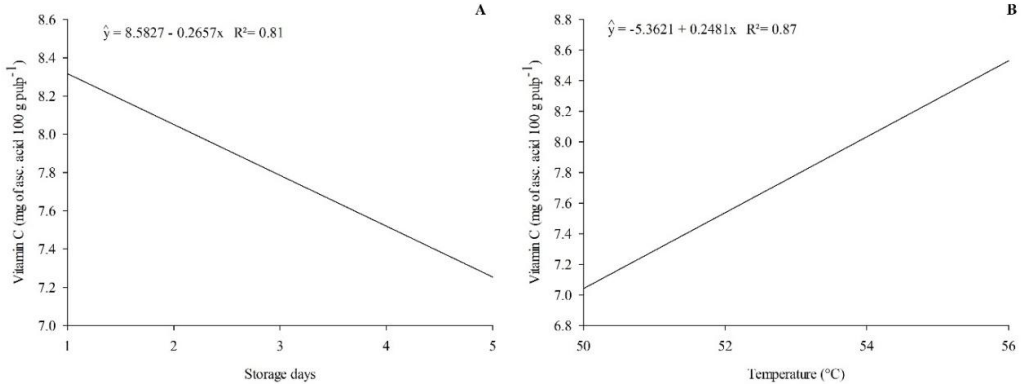

Fig. 6. Vitamin C (mg of ascorbic acid 100 g of pulp^{-1}) of banana 'Prata-Anã' subjected to hydrothermal treatment and stored at 10°C for 25 days, as a function of storage days at 25°C, fter removal from refrigeration and immersion temperatures (previous hydrothermal treatment)

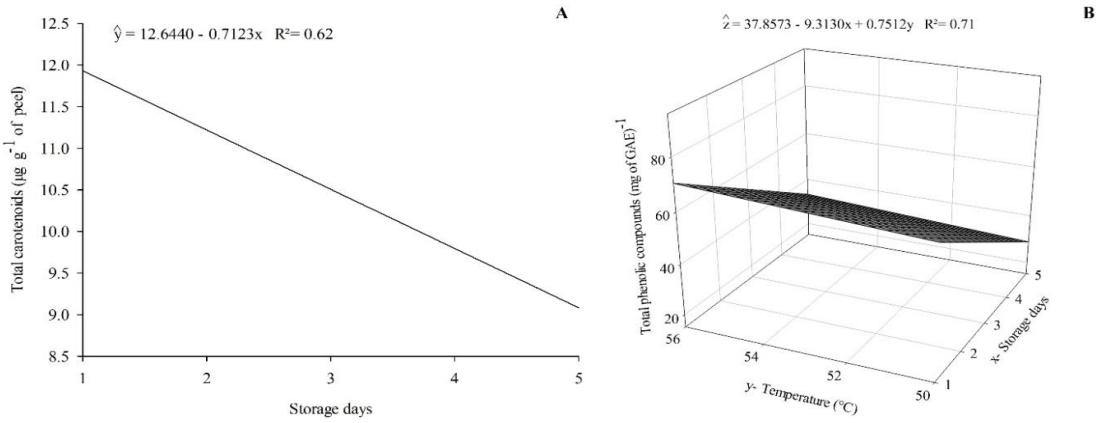

Fig. 7. Carotenoids (µg per g of peel^{-1}). Total phenolic compounds (mg of GAE per 100 g of peel^{-1}) of banana 'Prata-Anã' subjected to hydrothermal and stored at 10°C for 25 days, as a function of storage days at 25°C and different immersion temperatures (previous hydrothermal treatment)

4. CONCLUSIONS

The hydrothermal treatment at 54°C kept vitamin C content high in the pulp and delayed chilling on the peel of bananas 'Prata-Anã', being the most indicated treatment. The treatment at 56°C, although it kept vitamin C values high and decreased chilling, it presented low lightness and chroma values. The hydrothermal treatment was not effective in delaying ripening during refrigerated storage, and the complete ripening process occurred after removal from the refrigeration chamber.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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