The Hydrothermal Treatment Associated with Calcium Chloride Improve Banana cv. ‘Prata Gorutuba’ Quality Modulating Primary Metabolism

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors EAP, MOJ and WBS designed the study, performed the statistical analysis, wrote the first draft of the manuscript. Authors FSA, RRSA, EHM and JMSP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The temperature is one of the most used techniques to maintain fruit quality over long time storage and consequently the fruit respiratory metabolism is directly influenced. The combined use of CaCl2 and hydrothermal treatment can be an important alternative to improve banana fruit quality and influences the ratio starch/sugar and consequently quality traits. Based on the hypothesis the calcium and hydrothermal works synergistically modulating banana primary metabolism and skin color changes. The starch content and chrome parameters were kept in higher values at 2% e 3% (w/v) of CaCl2. However, the fruit storage at control condition have shown lower fresh weight loss (%), followed by total soluble solids and sugars content. In addition, our study showed that, fruit firmness, titratable acidity, skin brightness and hue angle were not significantly influenced by the

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1. INTRODUCTION

The fruit quality is an important characteristic to be considered during commercialization, mainly when the fruit is directly designated to consumption in natura as in case, banana fruits. Additionally, problems associated to production and high domestic consumption, the main restrictive factor for exportation market is the low postharvest management technology of fruits [1]. Additionally, according with [2] one of the most widely used techniques that has guaranteed effects on large scale conservation is refrigeration. This effect is mainly due to the alteration in metabolic rates, affecting the respiratory process, which in turn delays ripening in climacteric fruits, which leads to an increase in the shelf life of these fruits [3]. However, bananas fruit when stored at temperatures below 13 °C, there is a classical disorders associated with chilling injury, called chilling, causing the skin browning, affecting the visually quality-trait which leads to lower acceptance by consumers [4]. Furthermore, according [5], the refrigeration techniques alone is not sufficient to maintain fruit quality for long periods, and it is necessary to use other conservation techniques such as edible coating [6], hydrothermal treatment [7], chloride calcium treatment [8-9].

The hydrothermal treatment is an increasingly used technique for the control of pests and diseases in fruits [10-12] and as consequence, provides as an additional advantage the maintenance of the quality standard of the fruits. Moreover, in our knowledge, the postharvest characterization of banana fruit cv. ‘Prata Gorutuba’ still remain poor understood, mainly associating hydrothermal treatment associated with calcium chloride. The hydrothermal treatment is well known to affect the fruit metabolism and increases thermo-tolerance [13]. Remarkable, enabling store at a lower temperature than the one normally recommended, thus providing greater fruit conservation [14]. The positive effects is usually guaranteed by heat-shock proteins (HSP), acting as molecular chaperones, maintaining the spatial structure of other proteins under stresses such as an increase in temperature [15].

The calcium ions plays a crucial role in plant cell physiology. They are important intracellular massagers and can act as a mediator to hormones. Additionally, calcium plays an essential role in the membranes and cell wall structural maintenance, mainly cross-link free carboxyl groups on adjacent poligalacturonase chains present in the middle lamella of the plant cell wall contributing to cell–cell adhesion and cohesion [16]. Postharvest treatment with calcium salts have been effective in controlling several physiological disorders, reducing the incidence of fungal pathogens and improving the fruit quality [17-19].

The associated use between hydrothermal treatment and calcium chloride has been investigated in different fruits such as pineapple (Ananas comosus) [20], Atemoya (Annona cherimola Mill × A. squamosa L.) [21], fig fruit (Ficus carica) [22] and papaya [23-25]. Taken together, in our understanding, the results are still poorly understood and so far to be completely understood. Therefore, we truly believe that, news studies in banana could increase knowledge and raise new questions for future research. Once, is already know that hydrothermal treatment when investigated singly in banana fruit has provided better physical, chemical and phytosanitary quality of fruits [26]. Considering what was addressed, our research aimed to evaluate the application of calcium chloride after hydrothermal treatment on postharvest quality maintenance of banana fruit cv. ‘Prata Gorutuba’.

2. MATERIALS AND METHODS

2.1 Fruit Material and Environmental Conditions

The fruits were purchased from the commercial plantation located in Mocambinho, Jaíba, Minas
Gerais, Brazil. The area is located in the extreme north of the state of Minas Gerais, at 15° 12' south latitude and 43° 47' west longitude, with an altitude of 483 m. The bunches were harvested twenty weeks after the inflorescence emission, at stage 2 (green with yellow dashes) of maturation according to the scale of Von Loesecke [27]. After harvest, the fruits were packed in plastic boxes lined and covered with papers and transported to the laboratory. The banana fruits were selected according to their visual appearance, discarding damaged fruits as well as with mechanical lesions. After selection, were kept 4 banana fruit per banana bunch and then sterilized and washed. The fruits were treated with fungicide Imazalil (Magnate®) according to the manufacturer's recommendations. The statistical experimental design was completely randomized involving two main factors such as 5 chlorite calcium concentration (0, 1, 2, 3 and 4% w) under 7 times under treatment with 4 repetition which, was considered 4 fruits per repetition.

2.2 Postharvest Treatment

The hydrothermal treatment was performed in a water bath at 54°C, during 5 min under immersion (time defined in preliminary experiments). The fruits were then cooled in room temperature water (± 25°C) for min 5 min. After this period, the fruits were treated with calcium chloride by immersion during more 5 min in concentrations of 0 (Control), 1, 2, 3 and 4% CaCl₂ (w/v). The fruits were dried on benches in room temperature and then stored in low density polyethylene packages of 16μm at 13.5 ± 1°C and relative humidity (RH) 85±5 % during 30 days and samples were collected every 5 days during 35 days (7 time-points).

2.3 Fruit Fresh Weigh Loss (%)

The banana fruit fresh weigh loss was determined as previously described by [28-29]. The banana fruits were weighted at the beginning of the experiment after coating and air-drying, and thereafter each day during the storage period. The fresh weight loss was expressed as percentage loss of the initial total.

2.4 Physical Quality Attributes

The skin color of the fruit was performed using colorimeter Color Flex 45/0(2200), stdzMode:45/0 with direct reflection reflectance of the coordinates L* (brightness) a* (red or green tonality color range) and b* (yellow or blue tonality range) according with Hunterlab Universal Software system. From the L*, a* and b* values, were determined the hue angle (°h) and the saturation Chrome index (C°). The fruit firmness was performed using a digital texturometer (Brookfield model CT3 10 KG) with 4 mm Ø. The evaluation made in two equidistant regions on opposite sides. The firmness was measured as the maximum penetration force expressed in Newton (N).

2.5 Determination of Chemical Quality Parameters

After physical measurement, in order to understand the effects hydrothermal treatment associated with CaCl₂, we performed the soluble solids concentration (SSC) and titratable acidity (TA), pH, starch and sugars content. The SSC was used a hand refractometer (Model N-3000E, Atoag, Japan), calibrated with distilled water prior the readings. The titratable acidity (TA) analyzed according to the method of [30]. The results of TA were according malic acid content per 100 g⁻¹ of banana pulp. The pH was measured by using pH meter Crison MicropH 2001 (Crison Instruments SA, Barcelona, Spain).

2.6 Physiological Parameters

The soluble sugars were determined by the anthrone method [31]. The quantification of starch was carried out according to the method described by [32] and dosages were made by the anthrone method [33]. The starch was obtained by spectrophotometry, with reading at 510 nm, according to the method described by [34].

2.7 Pectinmethylesterase Activity (PME)

The enzymatic extraction was according with [35], with modification as described in details by [36]. The PME activity determination as according [37]. The PME activity unit was according with the enzyme capacity to catalyze the pectin demethylation by 1nM of NaOH consumption by fresh weight fruit during 1 minute of reaction.

2.8 Experimental Design and Statistical Analyses

The experiment was performed in a completely randomized design (CRD), with four repetition. Statistical analyzes were performed using the Sisvar software [38].
3. RESULTS AND DISCUSSION

3.1 The Role of Hydrothermal Treatment and CaCl_2 in the Physical Attributes in Banana Fruits

The ripening are a complex process genetically programmed, culminating in dramatic changes, mainly in color and fruit texture [39]. In order to characterize better the role of hydrothermal treatment and CaCl_2 in the physical attributes in banana fruits we measured some physical attributes. The fresh weight loss (FW) were significantly affected (p<0.05) by days after storage and also by CaCl_2 concentration. The FW loss was increased during fruit ripening over storage time (Fig. 1A). The FW loss average at 30 days after storage was around 1.23% independently of treatment concentration. The increases in FW partially explained by respiration and fruit transpiration and seems to be the major determinant of storage life and quality of banana [40]. Furthermore, according with [41], the FW loss range admissible is around 5 and 10%. Our results in the meantime did not reach 5%, which did not compromise the final fruit quality at 30 days of storage. As the CaCl_2 concentration increased, the FW loss increased proportionally (Fig. 1B), which presented 0.45% and 0.85% of FW loss to 0 and 4% (w/v) CaCl_2, respectively. Interestingly, control treatment (0%) and 1% showed significantly smaller averaged of FW loss. Similar results were observed in guavas cv. Cortibel by [42]. The effect of the salt present on banana’s fruit skin surface can cause dehydration, increasing the FW loss during the fruit storage [43].

According with skin brighthless (L*), as an important fruit/vegetable postharvest parameter once is possible to identify the visual appearence. The L* values range from 0 for fully black samples to 100 for totally white samples, the lower values indicate opaque shell (No brighthless) and higher values indicate brighter fruits [44]. Our results have showed significantly effects (P<0.05) of storage times under banana’s fruit L*. During fruit ripening, the L* was increased (45.82) at 0 day after treatment to 58.47 at 30 days after treatment storage at 13.5°C (Fig. 2A), which are indicating increases in fruit brighthless over fruit ripening which are significantly correlated to skin pigments (Chlorophyll and Carotenoids) changes [45] which can be influenced by ethylene [46]. Our results is in agreement with previously observed by [47]. As to L*, h* was significantly affected by fruit ripening without changes by treatment (Fig. 2B). However, no effect was observed by hydrothermal treatment and neither by calcium concentration. As expected, h* decreased during fruit ripening and remained within the angular range of the green color until around the 15th day of storage with 93.76 °, which shows that there was delay in the ripening process. The changes of coloration occurring during the ripening of the fruits are related to the degradation and / or biosynthesis of pigments [48]. In the banana, chlorophyll degradation (green color) is intense during maturation, showing the pre-existence of carotenoid pigments (yellow to orange color), while the synthesis of other pigments is performed at relatively low levels [49]. Chromaticity is an objective specification of the quality of a color regardless of its luminance [50]. Chroma values around (0) zero represent neutral colors, while values close to 60 express intense colors and it means ripe fruits [51]. Chroma values slightly increased during storage period and was also significantly influenced by CaCl_2 treatments (Fig.3). Although a linear increase of Chroma was observed during fruit ripening over storage time, fruits treated with 2 and 3% of CaCl_2 had a lower color intensity of the skin color with average of Chroma of 37.96 and 37.75, respectively (Fig. 3B).

The fruit firmness as expected was influenced significantly (P<0.05) by fruit ripening over storage period (Fig. 4). However, the CaCl_2 treatment, independently of the concentration has not influenced the fruit firmness. Our results has shown a oscillation in the fruit firmness values with 36.03 N at 5 days after storage period and increases up to 50N (15th day after storage), followed by a drastic reduction at 30 days after storage (29.23 N) (Fig.4). In our understanding, this variation may be explained due an unevenness in banana’s fruit ripening. The firmness loss in fruits is generally associated with the action of pectinolytic enzymes which leads to destabilization of the cell wall. According with [52], mean values of firmness can be found in banana cv. ‘Prata-Anã’ ranged from 32.8 N ~ 40 N at 25 days of refrigerated storage under 13.5 °C. The fruit firmness is generally associated with the integrity of the cell wall, the middle lamella and the cellular turgor, which both are directly dependent of water potential [53-54]. Therefore, losses in mass due to dehydration and respiration, very common during storage, decreases turgidity, affecting fruit firmness. According to Bleinroth [55], the loss of firmness...
of the fruit is an unavoidable characteristic in the ripening process, which is caused by the progressive cell wall solubilisation.

3.2 The Role of Hydrothermal Treatment and CaCl₂ in the Chemical Attributes in Banana Fruits

The SSC were significantly (P<0.05) affected by ripening process as well as to CaCl₂ treatment associated with hydrothermal pretreatment (Fig.5). The SSC increased linearly during ripening process when stored until 30 days at 13.5°C (Fig.5). Additionally, SSC increased proportionally with the concentrations of CaCl₂ (Fig. 5). The lowest SSC were observed in control (0% CaCl₂, 13.64ºBrix) and in opposite, 4% of CaCl₂ increased the SSC (13.31ºBrix). This results suggest that, hydrothermal (control) treatment delayed the conversion of the starch to sugars and that the hydrothermal treatments associated to the higher concentrations of calcium chloride had higher soluble solids contents, therefore they were in a more mature maturation stage. The banana’s SSC increases to a maximum of 27% in the ripening process, with a small decrease when the fruit is very ripe/senescent stage [56]. Remarkable, [57] showed that banana fruit cv. ‘Prata’ hydrothermal treatment at 50°C, 3’ and 8’ storage at room temperature (25°C) increased SSC around 23 ~ 23.5 ºBrix, receptivity.

![Fig. 1. The effect of hydrothermal treatment and CaCl₂ on fresh weight loss during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. ‘Prata Gorutuba’ Values are presented as means ± SE (n=4)](image)

![Fig. 2. The effect of hydrothermal treatment and CaCl₂ on Brightness (A) and Hue Angle (B) during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. ‘Prata Gorutuba’ Values are presented as means ± SE (n=4)](image)
There is no significantly difference in TA under CaCl$_2$ associated with hydrothermal treatment. However, the ripening process affected significantly the TA concentration over fruit storage (Fig.6). The acidity can be used as a point of reference for fruit ripening, which is attributed mainly to organic acids. Organic acids are used as a substrate during fruit respiration, leading ATP production in the mitochondrial electron transport chain (mETC) [58]. Our results showed an increased concentration during ripening process which can be partially explained by organic acid production by mainly TCA cycle pathway in the mitochondria in comparison with com a degradation by respiration process. According with Bleinroth [59], banana fruit in green stage are characterized by low acidity which can increase during fruit ripening reaching maximum values in senescence.
Fig. 5. The effect of hydrothermal treatment and CaCl$_2$ on SSC during 30 days after treatment at 13.5 $\pm$ 1 °C and RH85 $\pm$ 5% in banana fruit cv. ‘Prata Gorutuba’

Values are presented as means ± SE (n=4)

Fig. 6. The effect of hydrothermal treatment and CaCl$_2$ on TA during 30 days after treatment at 13.5 $\pm$ 1 °C and RH85 $\pm$ 5% in banana fruit cv. ‘Prata Gorutuba’

Values are presented as means ± SE (n=4)

The pH variable showed a significant interaction ($P<0.05$) between days after storage and CaCl$_2$ concentration. During the storage time, the banana pulp pH was reduced in all treatment (Fig. 7). Complementary, 30 days after storage all pH were similar for all treatment with pH average 4.05, 4.05, 4.05, 4.04 and 4.04 in the 0, 1, 2, 3 and 4% CaCl$_2$ (w/v). Our results were in close agreement with previously observed by [60] in banana cv. ‘Prata-Anã’. The reduction in pH values over ripening is partially explained by increases in sugar contents with decreasing in TA/SSC ratio [61].

The starch content was significantly affected the ripening process as well by CaCl$_2$ associated with hydrothermal treatment (Fig. 8). As expected, the starch content decreased during ripening process independently of the treatment (Fig. 8A). Interestingly, each single day of storage, the starch content were decreased around 1.217% and the 1% of CaCl$_2$ treatment
After 30 days of storage, Control (0% CaCl₂) showed starch content around 2% while, when treated with 4% CaCl₂, the starch content was around 4.05%, indicating an efficient process to reduce degradation of starch and reducing the starch conversion to sugars. The starch content was higher in the higher concentrations of calcium chloride, probably due to the lower respiratory rate and higher stabilization of the pectic connections promoted by calcium [62]. According with Ali et al.; [63], the fruit softening occurs due to deterioration of structural and non-structural carbohydrates such as, cell wall and/or starch oxidation, resulting in an increase in the sugars content. In banana fruit softening were reported by a coordinated degradation of pectic, hemicellulosic polysaccharides in the cell wall and starch [64-65]. In banana, several gene is are involved in starch-to-sugars conversions during ripening process has been reported, including the amylases such as MAmy, Ma-bms, Maisa and MaDEBs [66-69]. During banana ripening, No amadurecimento da banana one of the most notable changes is the conversion of starch to simple sugars such as glucose and fructose (8-10%) and sucrose (10-20%) [70].

In order to better understand the role of hydrothermal treatment associated with CaCl₂ in sugar content, we evaluated the reducing, non-reducing and total sugar accumulated during fruit ripening (Fig.8B, C and D). The total sugar (Fig.8B) increased significantly during ripening process over storage time with a daily increase of 0.998%. Interestingly, the highest total sugar levels observed were those when treated with increasing CaCl₂. The sugar content determines the degree of sweetness of the banana and together with the acidity, is a measure more directly correlated with the taste quality [71]. The reducing sugar and non-reducing sugar as total sugar were significantly affected by ripening process over storage time as well as by CaCl₂ treatment (Fig.8 C and D). Both sugar has their concentration increased during storage with increases in 0.7738% and 0.204% to reducing and non-reducing sugars respectively for each day of evaluation. According with previously observed by [72], 23.6% of reducing sugars and 1.3% of non-reducing sugar.

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\hat{y} = 5.3715 - 0.0439D - 0.0030C \quad R^2 = 0.79
\]

Fig. 7. The effect of hydrothermal treatment and CaCl₂ on pH during 30 days after treatment at 13.5 ± 1°C and RH85 ± 5% in banana fruit cv. ‘Prata Gorutuba’

*Values are presented as means ± SE (n=4)*
Fig. 8. The effect of hydrothermal treatment and CaCl₂ on Starch (A), Total Sugar (B), Reducing sugar (C) and Non-reducing sugar (D) during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. ‘Prata Gorutuba’

Values are presented as means ± SE (n=4)

3.3 The PME Activity

To further understand the effect of chloride calcium on fruit firmness we have measured the pectinmethylesterase on banana’s fruit previously treated with hydrothermal with subsequence different CaCl₂ concentration (Fig.9). The PME activity started increasing sharply after hydrothermal and CaCl₂ treatment until 10 days after storage, reaching up to 2-fold in comparison with the initial value for all the treatments (Fig.9). Surprisingly, no difference was observed in its activity independently of CaCl₂ concentration (Fig.9). PME activity started decreasing gradually after day and by day 20 was as low as on day 0. Therefore, in can suggest that the treatment as previously observed in fruit firmness does not provide a
Fig. 9. The effect of different calcium chloride concentration on pectinmethylesterase (PME) activity during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. ‘Prata Gorutuba’

Values are presented as means ± SE (n=4)

beneficial effect on cell wall degradation in banana fruit cv. ‘Prata Gorutuba’. A similar pattern was observed during banana ripening with increases followed by decreasing on enzyme activity in banana pulp over ripening stage [73]. Differently as we observed in our study, a common answer is an inhibition on PME activity under calcium application as previously showed in different fruits such as in apples [74], sweet pepper (Capsicum annum L.) [75]. According with Almeida et al. [76], changes in the cell wall-related enzymes such as PME, during fruit ripening is dependent of pH apoplast-variation. We suggest that with a few changes in banana pH as reported in Fig.7, the PME activity has not affected by CaCl₂ after hydrothermal treatment.

4. CONCLUSION

The hydrothermal treatment increases post-harvest quality of the banana ‘Prata Gorutuba’ when associated with calcium chloride. The hydrothermal treatment and immersion in calcium chloride maintained the green coloration of the fruits until fifteen days of storage, without compromising the flavor.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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