Review Paper

The hydrothermal treatment associated with calcium chloride improve banana cv. 'Prata Gorutuba' quality modulating primary metabolism

Abstract

The low 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. In addition, the combination with another postharvest treatment aiming to maintain the fruit quality and increase the banana shelf life seems to be an alternative to improve banana postharvest attributes. Therefore, the combination of $CaCl_2$ and hydrothermal treatment can be an important alternative to improve banana fruit quality and influence 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% and 3% (w/v) of $CaCl_2$. However, the fruit storage at control condition have shown lower fresh weight loss (%), followed by total soluble solids and sugars content. Our study showed that, fruit firmness, titratable acidity, skin brightness and hue angle were not significantly influenced by the treatments. However, banana fruits when treated only by hydrothermal treatment maintained better postharvest quality trait when compared to the fruits of the hydrothermal treatment associated with calcium chloride. The hydrothermal treatment when combined together with $CaCl_2$ can have influence on the banana fruit quality affecting skin color and primary metabolism such as sugar and starch content.

Key-words: Calcium chloride, fruit quality, central metabolism, Postharvest

1. INTRODUCTION

The fruit quality is an important characteristic to be considered during commercialization, mainly when the fruit is directly designated to consumption freshly as in case, banana fruits. Noteworthy, problems associated with the production and

high domestic consumption and postharvest diseases, the main restrictive factor for exportation market is the low postharvest management technology of fruits Lichtember and Lichtemberg, 2011). In addition, one of the most widely used techniques used to guarantee increased shelf life in several climacteric fruits is the is the refrigeration system. These effects is mainly due influences on metabolic rates, affecting respiratory process, which in turn delays ripening, leading increases in the shelf life of these fruits De Oliveira *et al*., (2017). 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 (Nuyen *et al*.,2003). Furthermore, according De Morais *et al*., (2012), the refrigeration techniques alone is not sufficient to maintain fruit quality for long periods, and it is necessary to use other conservation techniques to improve the performance, such as edible coating (Hernámdez-Munõz *et al.,* 2008), hydrothermal treatment (Salvador-Figueroa et al., 2011), chloride calcium treatment (Hernámdez-Munõz *et al.,* 2008).

The hydrothermal treatment used to control pests attacks and diseases incidences in various fruits (Moraes *et al*., 2006; Ribeiro *et al*., 2018; Salvador-Figueroa *et al*., 2011). As a consequence, this technique provides an additional advantage to the maintenance of the quality standard of the fruits such as inducing protein specialized in protein repair. Moreover, in our knowledge, the postharvest characterization of banana fruit cv. 'Prata Gorutuba' still remains poorly understood, mainly associating hydrothermal treatment with calcium chloride. The hydrothermal treatment is well known to affect the fruit metabolism and increases thermo-tolerance (Lurie, 1998). Remarkable, enabling storage at a lower temperature than the one normally recommended, thus providing greater fruit conservation (Schirra *et al*., 2004). The calcium ions play 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-linking free carboxyl groups on adjacent poligalacturonase chains present in the middle lamella of the plant cell wall contributing to cell–cell adhesion and cohesion (Brummell & Harpster, 2001). Postharvest treatment with calcium salts have been effective in controlling several

physiological disorders, reducing the incidence of fungal pathogens and improving the fruit quality (Barman et al., 2018; Madani et al., 2014; Silva et al., 2015).

The association between between hydrothermal treatment and calcium chloride has been investigated in different fruits such as pineapple (*Annas comosus*) (Youryoun *et al*., 2018), Atemoya (*Annona cherimola Mill* × *A. squamosa* L.) (Rasai *et al*., 1994), Fig fruit (*Ficus carica*) (Irfan et al., 2013) and papaya (Ayón-Reyba *et al*., 2017; Madani *et al*., 2016 and Madani *et al*., 2014). Taken together, in our understanding, the results are still poorly understood and so far to be completely understood. Therefore, we our research has proved a physiological mechanism involved the low hydrothermal treatment in association with calcium in to maintenance of banana fruit quality and extends shelf life. Once, it is already known that hydrothermal treatment when investigated singly in banana fruit has provided better physical, chemical and phytosanitary quality of fruits (Ribeiro *et al*., 2018). 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. MATERIAL 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 483m. 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 (PBMH & PIF, 2006). 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 fruits with mechanical lesions. After selection, 4 banana fruits per banana bunch were kept, 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, five different calcium chloride concentration (0, 1, 2, 3 and 4% w/v) during 7 times of treatment.

2.2. *Postharvest treatment*

The hydrothermal treatment was performed in a water bath at 54°C, for 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 for additional 5 min in concentrations of 0 (Control), 1, 2, 3 and 4% $CaCl_2$ (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 % for a duration of 30 days and samples were collected every 5 days for 35 days (7 time-points).

2.3. *Fruit fresh weight loss (%)*

The banana fruit fresh weigh loss was determined as previously described by Hernández-Munõz *et al*., (2008) and Batista-Silva *et al*., (2018). 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 fruit`s skin color analysis was performed using a 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 $CaCl_2$, we performed the soluble solids concentration (SSC) and titratable acidity (TA), pH, starch and sugars content. For the SSC determination, a handheld refractometer (Model N-3000E, Atago, Japan), calibrated with distilled water prior the readings was used. The titratable acidity (TA) was analyzed according to the method of (Ranganna, 1986). The results of TA were according to 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 according Dubois *et al*., (1956). The quantification of starch was carried out according to the method described by Yemm and Willis (1954). The starch was obtained by spectrophotometry, with reading at 510 nm, according to the method described by Nelson (1944).

2.7. Pectinmethylesterase activity (PME)

The enzymatic extraction was according with Buescher and Furmanski (1978), with modification as described in details by (Vilas-Boas & Kader, 2006). The PME activity determination according Hultin *et al*., (1966).

2.8. Experimental design and statistical analyses

The experiment was performed in a completely randomized design (CRD), with four repetitions. Statistical analyzes were performed using the Sisvar software (Ferreira, 2011).

3. RESULTS AND DISCUSSION

2.9. The role of hydrothermal treatment and $CaCl_2$ in the physical attributes in banana fruits.

Ripening is a complex process genetically programmed, culminating in dramatic changes, mainly in color and fruit texture (Osório *et al*., 2013). 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) was significantly affected (p<0.05) by days after storage and also by $CaCl_2$ concentration. The FW loss 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 increase in FW partially explained by respiration and fruit transpiration and seems to be the major determinant of storage life and quality of banana (Hailu *et al*., 2013). Furthermore, according with Kader (2008), 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 was observed in guavas cv. Cortibel by Terra Werner *et al*., (2009). The effect of the salt present on banana's fruit skin surface can cause dehydration, increasing the FW loss during the fruit storage (Azzolini *et al*., 2004).

According with skin brighthless (L^*), as an important fruit/vegetable postharvest parameter once is possible to identify the visual appearance. 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 (Lancaster *et al*., 1997). Our results have shown significant effects (P<0.05) of storage times under banana's fruit L^*. During fruit ripening, the L^* increased (45.82) at 0 day after treatment to 58.47 at 30 days after treatment storage at 13.5ºC (Fig. 2A), which are indicating increases in fruit brighthness over fruit ripening which are significantly correlated to skin pigments (Chlorophyll and Carotenoids) changes (Song *et al*., 1997) which can be influenced by ethylene (Kajuna *et al*., 1998). Our

192 results is in agreement with previously observed results by Castricini *et al*., (2015).
193 As to *L*, h** was significantly affected by fruit ripening without changes by treatment
194 (Fig. 2B). However, no effect was observed by hydrothermal treatment and neither by
195 calcium concentration. As expected, *h** decreased during fruit ripening and remained
196 within the angular range of the green color until around the 15th day of storage with
197 93.76 °, which shows that there was delay in the ripening process. The changes of
198 coloration occurring during the ripening of the fruits are related to the degradation
199 and / or biosynthesis of pigments (Gross, 2012). In the banana, chlorophyll
200 degradation (green color) is intense during maturation, showing the pre-existence of
201 carotenoid pigments (yellow to orange color), while the synthesis of other pigments is
202 performed at relatively low levels (Silva *et al*., 2006). Chromaticity is an objective
203 specification of the quality of a color regardless of its luminance (Hunt, 1977).
204 Chroma values around (0) zero represent neutral colors, while values close to 60
205 express intense colors and it means ripe fruits (Mendonça et al., 2003). Chroma
206 values slightly increased during storage period and was also significantly influenced
207 by $CaCl_2$ treatments (Fig.3). Although a linear increase of Chroma was observed
208 during fruit ripening over storage time, fruits treated with 2 and 3% of $CaCl_2$ had a
209 lower color intensity of the skin color with average of Chroma of 37.96 and 37.75,
210 respectively (Figure 3B).
211 The fruit firmness as expected was influenced significantly (P<0.05) by fruit
212 ripening over storage period (Fig. 4). However, the $CaCl_2$ treatment, independently of
213 the concentration has not influenced the fruit firmness. Our results has shown a
214 oscillation in the fruit firmness values with 36.03 N at 5 days after storage period and
215 increased up to 50N (15^{th} days after storage), followed by a drastic reduction at 30
216 days after storage (29.23 N) (Fig.4). In our understanding, this variation may be
217 explained due to unevenness in banana's fruit ripening. The firmness loss in fruits is
218 generally associated with the action of pectinolytic enzymes, which leads to
219 destabilization of the cell wall. According with (Santos *et al*., 2018), mean values of
220 firmness can be found in banana cv. 'Prata-Anã' ranged from 32.8 N ~ 40 N at 25
221 days of refrigerated storage under 13.5 °C. The fruit firmness is generally associated
222 with the integrity of the cell wall, the middle lamella and the cellular turgor, which both
223 are directly dependent of water potential (Brummell, 2006 and Brummell et al., 2001).
224 Therefore, losses in mass due to dehydration and respiration, very common during

storage, decreases turgidity, affecting fruit firmness. According to Brummell (2006), the loss of firmness of the fruit is an unavoidable characteristic in the ripening process, which is caused by the progressive cell wall solubilization.

2.10. The role of hydrothermal treatment and $CaCl_2$ in the chemical attributes in banana fruits.

The SSC were significantly (P<0.05) affected by ripening process as well as to $CaCl_2$ treatment associated with hydrothermal pretreatment (Fig.5). The SSC increased linearly during ripening process when stored until 30 days at 13.5ºC (Fig.5). Additionally, SSC increased proportionally with the concentrations of $CaCl_2$ (Fig. 5). The lowest SSC were observed in control (0% $CaCl_2$, 13.64ºBrix) and in opposite, 4% of $CaCl_2$ increased the SSC (13.31ºBrix). These 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 increased to a maximum of 27% in the ripening process, with a small decrease when the fruit is very ripe/senescence stage (Bleinroth, 1995). Remarkably, Coelho *et al*., (2001) showed that banana fruit cv. 'Prata' hydrothermal treatment at 50°C, 3' and 8' storage at room temperature (25ºC) increased SSC around 23 ~ 23.5 °Brix, receptivity.

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) Nunes-Nesi *et al.,* (2013). 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 degradation by respiration process. According with Bleinroth (1995), banana fruit in green stage are characterized by low acidity which can increase during fruit ripening reaching maximum values in senescence.

The pH variable showed a significate 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 Da Costa Gomes *et al*., (2007) in banana cv. 'Prata-Anã'. The reduction in pH values over ripening is partially explained by increase in sugar contents with decreasing in TA/SSC ratio (Braga *et al.,*2008).

The starch content was significantly affected by 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 0.5123%. After 30 days of storage, Control (0% $CaCl_2$) 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 increased under higher $CaCl_2$ concentrations, likely due to the lower respiratory rate and higher stabilization of the pectin connections promoted by calcium (Silva *et al*., 2015). According with Ali *et al*., (2004) 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 (Mbéguié et al 2009;Shiga et al., 2011). In banana, several genes are involved in starch-to-sugars conversions during ripening process, this has been reported, including the amylases such as *MAmy*, *Ma-bms*, *Maisa* and *MaDEBs* (Bierhals et a., 2004; Do Nascimento et al., 2006; Jurda et al., 2016 and Junior-Nascimento and Lajolo, 2006). During banana ripening, is one of the most notable changes during the conversion of starch to simple sugars such as glucose and fructose (8-10%) and sucrose (10-20%) (Viviani and Leal, 2007).

In order to better understand the role of hydrothermal treatment associated with $CaCl_2$ 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 $CaCl_2$. 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 (Wu *et al*., 2005). The reducing sugar and non-reducing sugar as total sugar were significantly affected by ripening process over storage time as well as by $CaCl_2$ treatment (Fig.8 C and D). Both sugars has their concentration increased during storage, with increase in 0.7738% and 0.204% to reducing and non-reducing sugars respectively for each day of evaluation. According with previously observed by (Jesus *et al*., 2004), 23.6% of reducing sugars and 1.3% of non-reducing sugar.

2.11. 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 $CaCl_2$ concentration (Fig.9). The PME activity started increasing sharply after hydrothermal and $CaCl_2$ 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 $CaCl_2$ 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 preciously observed in fruit firmness does not provide a 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 (Hultin and Levine, 1965). 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 (Ortiz *et al*., 2011), sweet pepper (*Capsicum annum* L.) (Rao *et al*., 2011). According with Almeida and Huber (1999), 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 $CaCl_2$ after hydrothermal treatment.

CONCLUSION

The hydrothermal treatment presents a better post-harvest quality of the banana 'Prata Gorutuba' when associated with calcium chloride. The hydrothermal treatment and immersion in calcium chloride maintained the skin green color of the fruits until fifteen days of storage.

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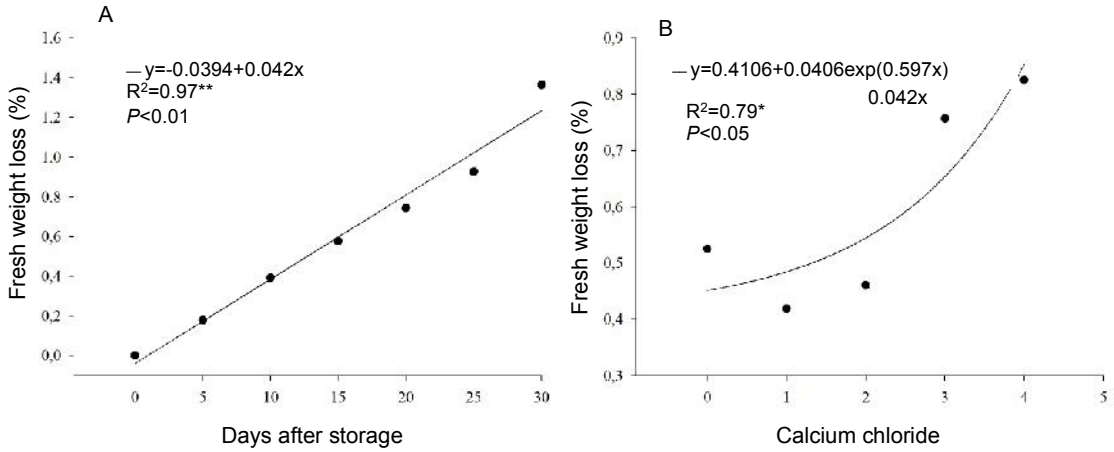
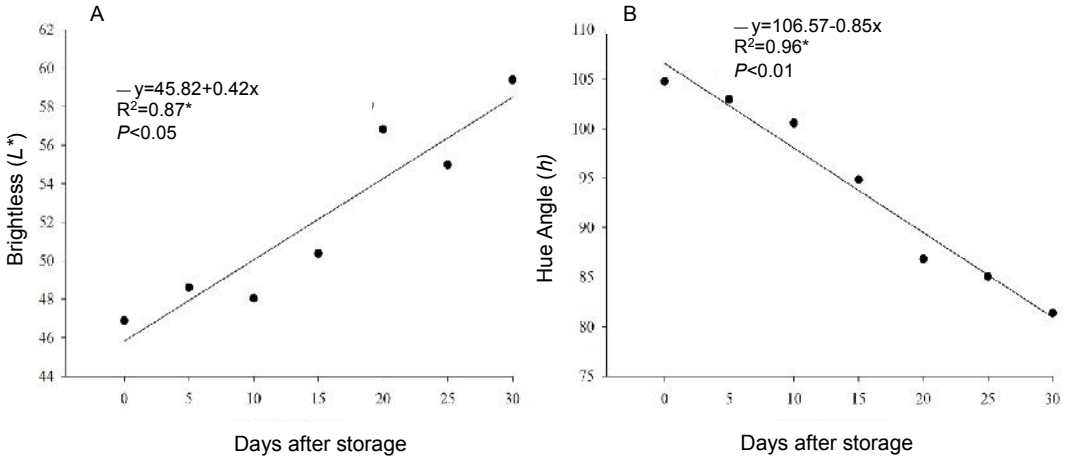
582 Figures



583
584 **Fig. 1**. The effect of hydrothermal treatment and $CaCl_2$ on fresh weight loss during 30
585 days after treatment in banana fruit cv. 'Prata Gorutuba'. A) Fresh weight loss (%)
586 over days after storage and b) Fresh weight loss response under different $CaCl_2$
587 concentration



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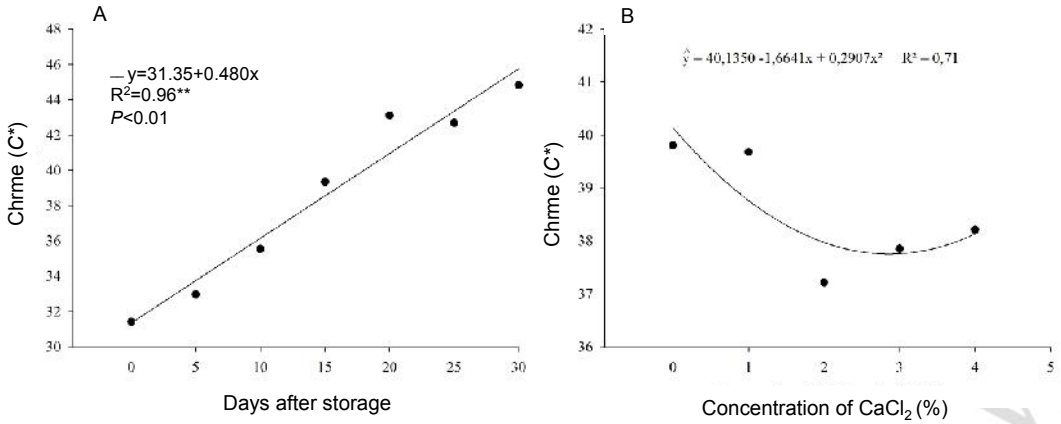
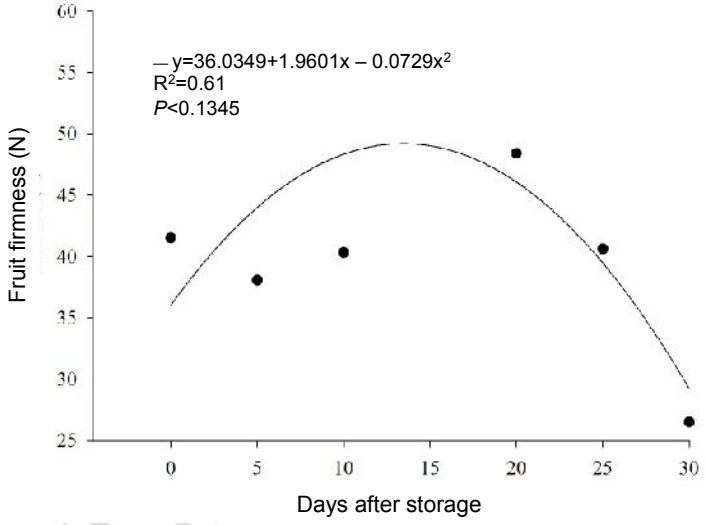
590 **Fig. 2 –** The effect of hydrothermal treatment and CaCl2 on skin color during 30
591 days after treatment in banana fruit cv. 'Prata Gorutuba'.A)Brightless and B) Hue
592 angle.



593

594 **Fig. 3**. The effect of hydrothermal treatment and $CaCl_2$ on Chroma (*C**) during 30
595 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata
596 Gorutuba'.



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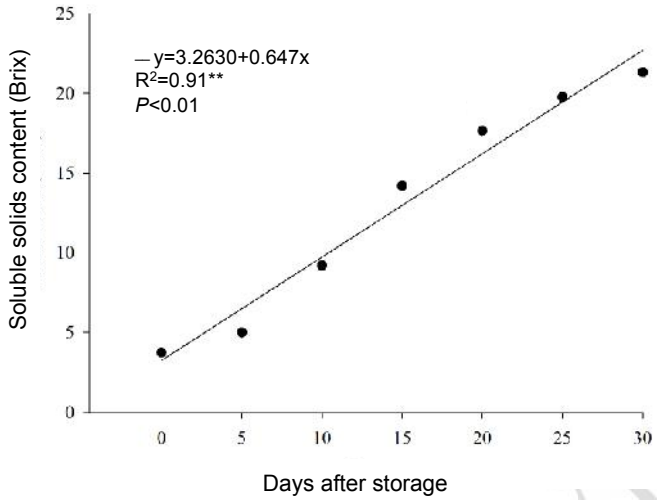
598 **Fig. 4**. The effect of hydrothermal treatment and $CaCl_2$ on fruit firmness during 30
599 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata
600 Gorutuba'.



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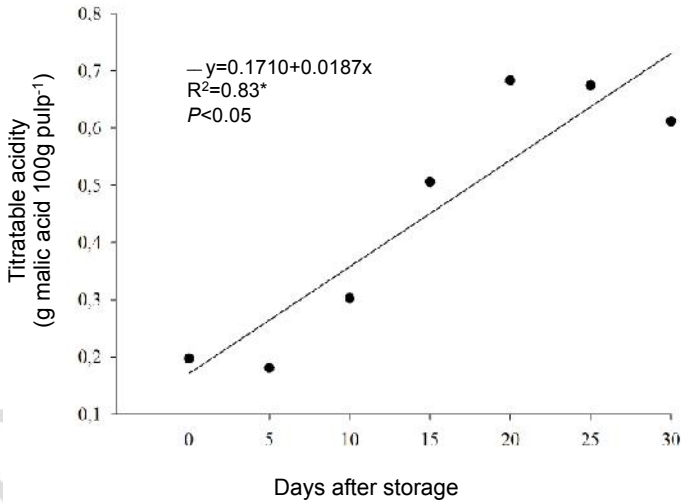
602 **Fig. 5.** The effect of hydrothermal treatment and $CaCl_2$ on SSC during 30 days after
603 treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'.



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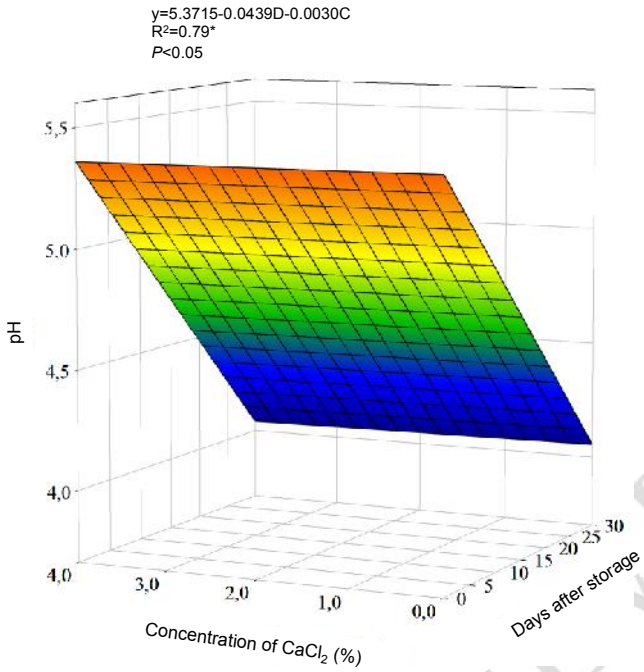
605 **Fig. 6**. The effect of hydrothermal treatment and $CaCl_2$ on TA during 30 days after
606 treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'



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609 **Fig. 7**. The effect of hydrothermal treatment and $CaCl_2$ on pH during 30 days after
610 treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values
611 are presented as means ± SE (n=4).

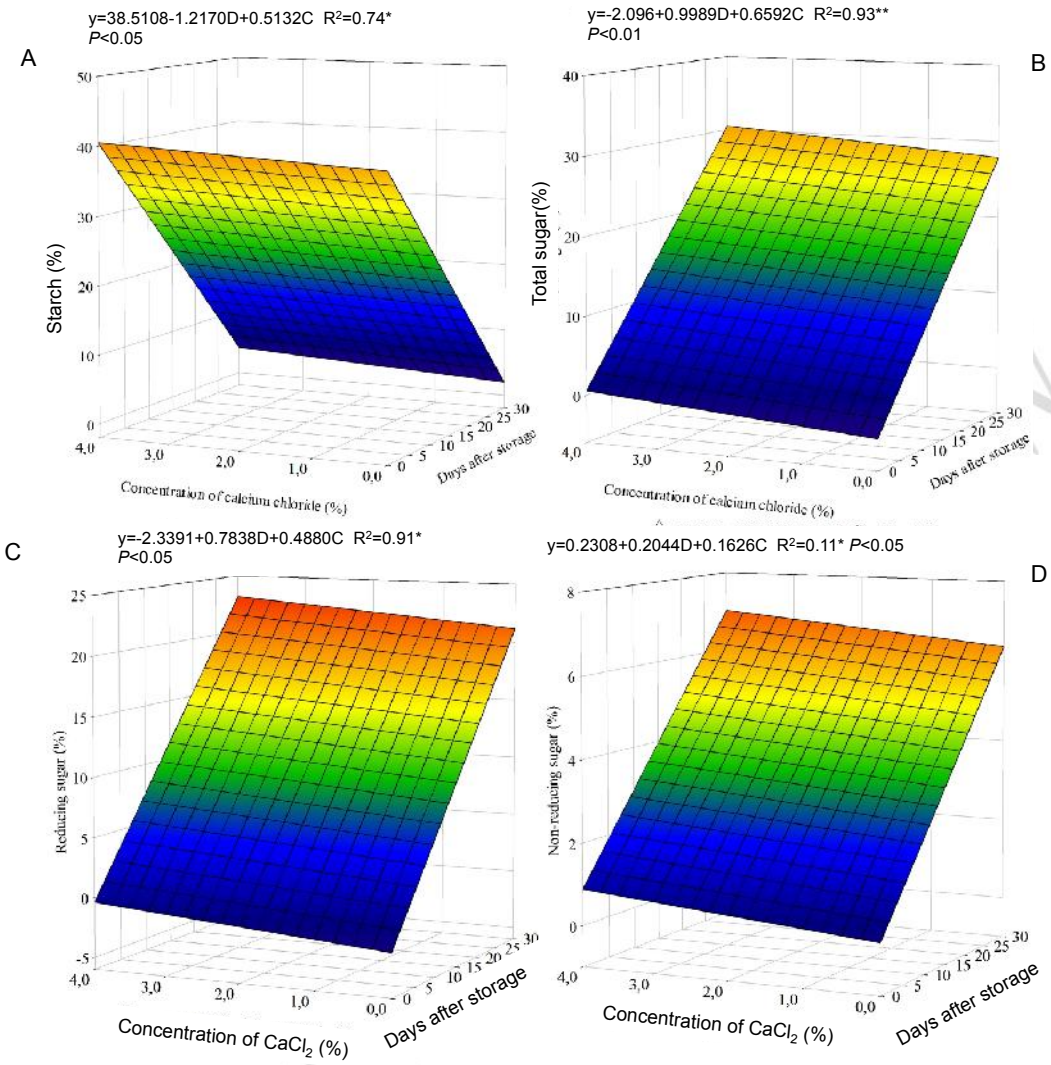

Fig. 8. The effect of hydrothermal treatment and $CaCl_2$ on Starch (A), Total Sugar (B), Reducing sugar (C) and Non-reducing sugar (D) during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'.

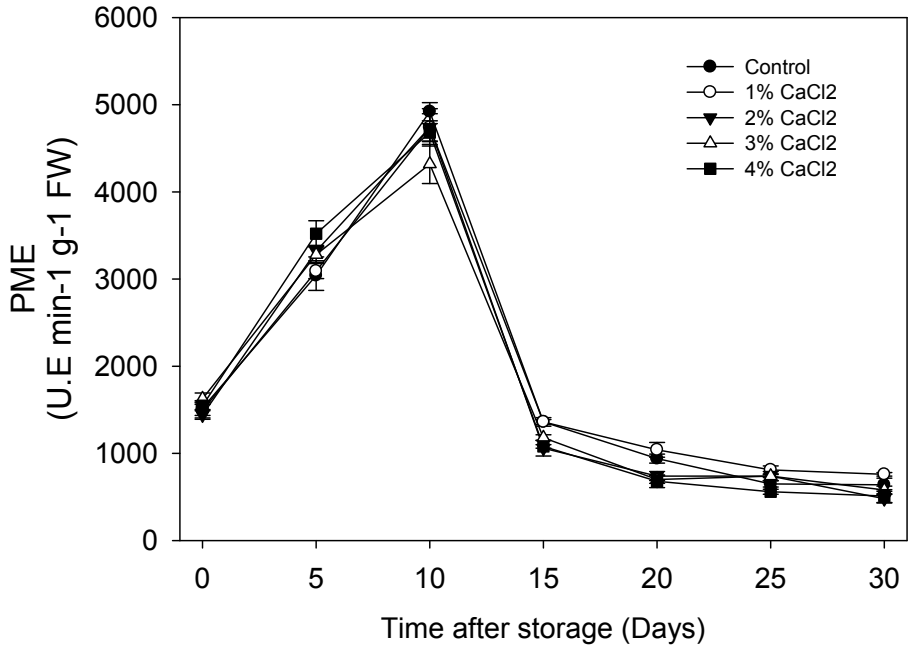

Fig 9. The effect of different calcium chloride concentration on pectinmethylesterase (PME) activity during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values are presented as means ± SE (n=4).