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

5

4

1 2 3

6 Abstract

7 The temperature is one of the most used techniques to maintain fruit quality over 8 long time storage and consequently the fruit respiratory metabolism is directly 9 influenced. The combined use of CaCl₂ and hydrothermal treatment can be an 10 important alternative to improve banana fruit quality and influences the ratio 11 starch/sugar and consequently quality traits. Based on the hypothesis the calcium 12 and hydrothermal works synergistically modulating banana primary metabolism and 13 skin color changes. The starch content and chrome parameters were kept in higher 14 values at 2% e 3% (w/v) of CaCl₂. However, the fruit storage at control condition 15 have shown lower fresh weight loss (%), followed by total soluble solids and sugars 16 content. In addition, our study showed that, fruit firmness, titratable acidity, skin 17 brightness and hue angle were not significantly influenced by the treatments. In the 18 same vein, banana fruits when treated only by hydrothermal treatment maintained 19 better postharvest quality trait when compared to the fruits of the hydrothermal 20 treatment associated with calcium chloride. The hydrothermal treatment when 21 together with CaCl₂ cans influences in the banana fruit quality affecting skin color and 22 primary metabolism such as sugar and starch content.

23

24

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

25 26

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 (Lichtemberg & Lichtemberg, 2011). Additionally, according with Hadadinejad and Mohammadi (2018) one of the 33 most widely used techniques that has guaranteed effects on large scale conservation 34 is refrigeration. This effect is mainly due to the alteration in metabolic rates, affecting 35 the respiratory process, which in turn delays ripening in climacteric fruits, which leads 36 to an increase in the shelf life of these fruits (de Oliveira, Morales, Silva, da Cruz 37 Saraiva, Santana, dos Santos, et al., 2017). However, bananas fruit when stored at 38 temperatures below 13 °C, there is a classical disorders associated with chilling 39 injury, called chilling, causing the skin browning, affecting the visually quality-trait 40 which leads to lower acceptance by consumers (Nguyen, Ketsa, & van Doorn, 2003). 41 Furthermore, according (de Morais Cardoso, de Deus, de Barros Silva, de Andrade 42 Júnior, & Dessimoni-Pinto, 2012), the refrigeration techniques alone is not sufficient 43 to maintain fruit quality for long periods, and it is necessary to use other conservation 44 techniques such as edible coating (Hernández-Muñoz, Almenar, Valle, Velez, & 45 Gavara, 2008), hydrothermal treatment(Salvador-Figueroa, Aragón-Gómez, 46 Hernández-Ortiz, Vazquez-Ov, & de Lourdes Adriano-Anaya, 2011), chloride calcium 47 treatment (Hernández-Muñoz, Almenar, Ocio, & Gavara, 2006; Hernández-Muñoz, 48 Almenar, Valle, Velez, & Gavara, 2008).

49 The hydrothermal treatment is an increasingly used technique for the control of 50 pests and diseases in fruits (Moraes, Zambolim, Lima, Vale, & Salomão, 2006; 51 Ribeiro, Rodrigues, Fernandes, & Mizobutsi, 2018; Salvador-Figueroa, Aragón-52 Gómez, Hernández-Ortiz, Vazquez-Ov, & de Lourdes Adriano-Anaya, 2011) and as 53 consequence, provides as an additional advantage the maintenance of the quality 54 standard of the fruits. Moreover, in our knowledge, the postharvest characterization 55 of banana fruit cv. 'Prata Gorutuba' still remain poor understood, mainly associating 56 hydrothermal treatment associated with calcium chloride. The hydrothermal 57 treatment is well known to affect the fruit metabolism and increases thermo-tolerance 58 (Lurie, 1998). Remarkable, enabling store at a lower temperature than the one 59 normally recommended, thus providing greater fruit conservation (Schirra, Mulas, 60 Fadda, & Cauli, 2004). The positive effects is usually guaranteed by heat-shock 61 proteins (HSP), acting as molecular chaperones, maintaining the spatial structure of 62 other proteins under stresses such as an increase in temperature (Sabehat, Weiss, & 63 Lurie, 1998).

64 The calcium ions plays a crucial role in plant cell physiology. They are 65 important intracellular massagers and can act as a mediator to hormones. 66 Additionally, calcium plays an essential role in the membranes and cell wall structural 67 maintenance, mainly cross-link free carboxyl groups on adjacent poligalacturonase 68 chains present in the middle lamella of the plant cell wall contributing to cell-cell 69 adhesion and cohesion (David A. Brummell & Harpster, 2001). Postharvest treatment 70 with calcium salts have been effective in controlling several physiological disorders, 71 reducing the incidence of fungal pathogens and improving the fruit quality (Barman, 72 Sharma, & Siddiqui, 2018; Madani, Muda Mohamed, Biggs, Kadir, Awang, 73 Tayebimeigooni, et al., 2014; W. B. Silva, Silva, Silva, Waldman, & Oliveira, 2015)

74 The associated use between hydrothermal treatment and calcium chloride has 75 been investigated in different fruits such as pineapple (Annas comosus) (Youryon, 76 Supapvanich, Kongtrakool, & Wongs-Aree, 2018), Atemoya (Annona cherimola Mill × 77 A. squamosa L.) (Rasai, Kantharajah, & Dodd, 1994), fig fruit (Ficus carica) (Irfan, 78 Vanjakshi, Prakash, Ravi, & Kudachikar, 2013) and papaya (Ayón-Reyna, González-79 Robles, Rendón-Maldonado, Báez-Flores, López-López, & Vega-García, 2017; 80 Madani, Mirshekari, & Yahia, 2016; Madani, et al., 2014). Taken together, in our 81 understanding, the results are still poorly understood and so far to be completely 82 understood. Therefore, we truly believe that, news studies in banana could increase 83 knowledge and raise new questions for future research. Once, is already know that 84 hydrothermal treatment when investigated singly in banana fruit has provided better 85 physical, chemical and phytosanitary quality of fruits (Ribeiro, Rodrigues, Fernandes, 86 & Mizobutsi, 2018). Considering what was addressed, our research aimed to 87 evaluate the application of calcium chloride after hydrothermal treatment on 88 postharvest guality maintenance of banana fruit cv. 'Prata Gorutuba'.

89

90 2. MATERIAL AND METHODS

91

92 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

99 lined and covered with papers and transported to the laboratory. The banana fruits 100 were selected according to their visual appearance, discarding damaged fruits as 101 well as with mechanical lesions. After selection, were kept 4 banana fruit per banana 102 bunch and then sterilized and washed. The fruits were treated with fungicide 103 Imazalil (Magnate®) according to the manufacturer's recommendations. The 104 statistical experimental design was completely randomized involving two main factors 105 such as 5 chlorite calcium concentration (0, 1, 2, 3 and 4% w) under 7 times under 106 treatment with 4 repetition which, was considered 4 fruits per repetition.

107

108 2.2. Postharvest treatment

109

110 The hydrothermal treatment was performed in a water bath at 54°C, during 5 111 min under immersion (time defined in preliminary experiments). The fruits were then 112 cooled in room temperature water (± 25°C) for min 5 min. After this period, the fruits 113 were treated with calcium chloride by immersion during more 5 min in concentrations 114 of 0 (Control), 1, 2, 3 and 4% CaCl₂ (w/v). The fruits were dried on benches in room 115 temperature and then stored in low density polyethylene packages of 16 μ m at 13.5 ± 1°C and relative humidity (RH) 85±5 % during 30 days and samples were collected 116 117 every 5 days during 35 days (7 time-points).

118

119 2.3. Fruit fresh weigh loss (%)

The banana fruit fresh weigh loss was determined as previously described by Hernández-Muñoz, Almenar, Valle, Velez, and Gavara (2008) and Batista Silva, Cosme Silva, Santana, Salvador, Medeiros, Belghith, 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.

126

127 2.4. Physical quality attributes

128

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 ($^{\circ}h^{*}$) 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).

- 138
- 139

2.5. Determination of chemical quality parameters

140 After physical measurement, in order to understand the effects hydrothermal 141 treatment associated with CaCl₂, we performed the soluble solids concentration 142 (SSC) and titratable acidity (TA), pH, starch and sugars content. The SSC was used 143 a hand refractometer (Model N-3000E, Atago, Japan), calibrated with distilled water 144 prior the readings. The titratable acidity (TA) analyzed according to the method of (Ranganna, 1986). The results of TA were according malic acid content per 100 g⁻¹ of 145 146 banana pulp. The pH was measured by using pH meter Crison MicropH 2001 (Crison 147 Instruments SA, Barcelona, Spain).

148

149 2.6. Physiological parameters

150

The soluble sugars were determined by the anthrone method Dubois, Gilles, Hamilton, Rebers, and Smith (1956). The quantification of starch was carried out according to the method described by Yemm and Willis (1954) and dosages were made by the anthrone method Dubois, Gilles, Hamilton, Rebers, and Smith (1956). The starch was obtained by spectrophotometry, with reading at 510 nm, according to the method described by Nelson (1944).

- 157
- 158 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 as according (H. Hultin, Sun, & Bulger, 1966). The PME activity unit was according with the enzyme capacity to catalyze the pectin demethylation by 163 1nM of NaOH consumption by fresh weight fruit during 1 minute of reaction.

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

170

171 3. RESULTS AND DISCUSSION

172

3.1. The role of hydrothermal treatment and CaCl₂ in the physical attributes in
banana fruits.

175

176 The ripening are a complex process genetically programmed, culminating in a 177 dramatic changes, mainly in color and fruit texture (Osorio, Scossa, & Fernie, 2013). 178 In order to characterize better the role of hydrothermal treatment and CaCl₂ in the 179 physical attributes in banana fruits we measured some physical attributes. The fresh 180 weight loss (FW) were significantly affected (p < 0.05) by days after storage and also 181 by CaCl₂ concentration. The FW loss was increased during fruit ripening over storage 182 time (Fig. 1A). The FW loss average at 30 days after storage was around 1.23% 183 independently of treatment concentration. The increases in FW partially explained by 184 respiration and fruit transpiration and seems to be the major determinant of storage 185 life and quality of banana (Hailu, Workneh, & Belew, 2013). Furthermore, according 186 with (Kader, 2008), the FW loss range admissible is around 5 and 10%. Our results 187 in the meantime did not reach 5%, which did not compromise the final fruit quality at 188 30 days of storage. As the CaCl₂ concentration increased, the FW loss increased 189 proportionally (Fig. 1B), which presented 0.45% and 0.85% of FW loss to 0 and 4% (w/v) CaCl₂, respectively. Interestingly, control treatment (0%) and 1% showed 190 191 significantly smaller averaged of FW loss. Similar results was observed in guavas cv. 192 Cortibel by (Terra Werner, Ganassali de Oliveira Junio, Bona, Cavati, & Hermogenes 193 Gomes, 2009). The effect of the salt present on banana's fruit skin surface can cause 194 dehydration, increasing the FW loss during the fruit storage (Azzolini, Jacomino, & 195 Bron, 2004).

According with skin brighthless (L^*), as an important fruit/vegetable postharvest parameter once is possible to identify the visual appearence. The L * values range

198 from 0 for fully black samples to 100 for totally white samples, the lower values 199 indicate opaque shell (No brighthless) and higher values indicate brighter fruits 200 (Lancaster, Lister, Reay, & Triggs, 1997). Our results have showed significantly 201 effects (P<0.05) of storage times under banana's fruit L^* . During fruit ripening, the L^* 202 was increased (45.82) at 0 day after treatment to 58.47 at 30 days after treatment 203 storage at 13.5°C (Fig. 2A), which are indicating increases in fruit brighthless over 204 fruit ripening which are significantly correlated to skin pigments (Chlorophyll and 205 Carotenoids) changes (Song, Deng, Beaudry, & Armstrong, 1997) which can be 206 influenced by ethylene (Kajuna, Bilanski, & Mittal, 1998). Our results is in agreement 207 with previously observed by Castricini, Santos, Deliza, Coelho, and RODRIGUES 208 (2015). As to L^* , h^* was significantly affected by fruit ripening without changes by 209 treatment (Fig. 2B). However, no effect was observed by hydrothermal treatment and 210 neither by calcium concentration. As expected, h^* decreased during fruit ripening and 211 remained within the angular range of the green color until around the 15th day of 212 storage with 93.76°, which shows that there was delay in the ripening process. The 213 changes of coloration occurring during the ripening of the fruits are related to the 214 degradation and / or biosynthesis of pigments(Gross, 2012). In the banana, 215 chlorophyll degradation (green color) is intense during maturation, showing the pre-216 existence of carotenoid pigments (yellow to orange color), while the synthesis of 217 other pigments is performed at relatively low levels (C. d. S. Silva, Lima, Santos, 218 Camili, Vieira, Martin, et al., 2006). Chromaticity is an objective specification of the 219 quality of a color regardless of its luminance (Hunt, 1977). Chroma values around (0) 220 zero represent neutral colors, while values close to 60 express intense colors and it 221 means ripe fruits (Mendonça, Jacomino, Melhem, & Kluge, 2003). Chroma values 222 slightly increased during storage period and was also significantly influenced by 223 CaCl₂ treatments (Fig.3). Although a linear increase of Chroma was observed during 224 fruit ripening over storage time, fruits treated with 2 and 3% of CaCl₂ had a lower 225 color intensity of the skin color with average of Chroma of 37.96 and 37.75, 226 respectively (Figure 3B).

The fruit firmness as expected was influenced significantly (P<0.05) by fruit ripening over storage period (Fig. 4). However, the CaCl₂ 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 days after storage), followed by a drastic reduction at 30 231 232 days after storage (29.23 N) (Fig.4). In our understanding, this variation may be 233 explained due an unevenness in banana's fruit ripening. The firmness loss in fruits is 234 generally associated with the action of pectinolytic enzymes which leads to 235 destabilization of the cell wall. According with (Santos, Aguiar, Rodrigues, Mizobutsi, 236 & Pinheiro, 2018), mean values of firmness can be found in banana cv. 'Prata-Anã' 237 ranged from 32.8 N ~ 40 N at 25 days of refrigerated storage under 13.5 °C. The fruit 238 firmness is generally associated with the integrity of the cell wall, the middle lamella 239 and the cellular turgor, which both are directly dependent of water potential (David A 240 Brummell, 2006; David A. Brummell & Harpster, 2001). Therefore, losses in mass 241 due to dehydration and respiration, very common during storage, decreases turgidity, affecting fruit firmness. According to David A Brummell (2006), the loss of firmness of 242 243 the fruit is an unavoidable characteristic in the ripening process, which is caused by 244 the progressive cell wall solubilization.

245

3.2. The role of hydrothermal treatment and CaCl₂ in the chemical attributes in
banana fruits.

248

The SSC were significantly (P<0.05) affected by ripening process as well as to 249 250 CaCl2 treatment associated with hydrothermal pretreatment (Fig.5). The SSC 251 increased linearly during ripening process when stored until 30 days at 13.5°C 252 (Fig.5). Additionally, SSC increased proportionally with the concentrations of $CaCl_2$ 253 (Fig. 5). The lowest SSC were observed in control (0% CaCl₂, 13.64°Brix) and in 254 opposite, 4% of CaCl₂ increased the SSC (13.31°Brix). This results suggest that, 255 hydrothermal (control) treatment delayed the conversion of the starch to sugars and 256 that the hydrothermal treatments associated to the higher concentrations of calcium 257 chloride had higher soluble solids contents, therefore they were in a more mature 258 maturation stage. The banana's SSC increases to a maximum of 27% in the ripening 259 process, with a small decrease when the fruit is very ripe/senescence stage 260 (Bleinroth, 1995). Remarkable, (Coelho, Werner, Poncio, Ferreira, & Nóbrega, 2011) 261 showed that banana fruit cv. 'Prata' hydrothermal treatment at 50°C, 3' and 8' 262 storage at room temperature (25°C) increased SSC around 23 ~ 23.5 °Brix, 263 receptivity.

264 There is no significantly difference in TA under CaCl₂ associated with 265 hydrothermal treatment. However, the ripening process affected significantly the TA 266 concentration over fruit storage (Fig.6). The acidity can be used as a point of 267 reference for fruit ripening, which is attributed mainly to organic acids. Organic acids 268 are used as a substrate during fruit respiration, leading ATP production in the 269 mitochondrial electron transport chain (mETC) (Nunes-Nesi, Araújo, Obata, & 270 Fernie, 2013). Our results showed an increased concentration during ripening 271 process which can be partially explained by organic acid production by mainly TCA 272 cycle pathway in the mitochondria in comparison with com a degradation by 273 respiration process. According with Bleinroth (1995), banana fruit in green stage are 274 characterized by low acidity which can increase during fruit ripening reaching 275 maximum values in senescence.

276 The pH variable showed a significate interaction (P<0.05) between days after 277 storage and CaCl₂ concentration. During the storage time, the banana pulp pH was 278 reduced in all treatment (Fig. 7). Complementary, 30 days after storage all pH were 279 similar for all treatment with pH average 4.05, 4.05, 4.05, 4.04 and 4.04 in the 0, 1, 280 2, 3 and 4% CaCl₂ (w/v). Our results were in close agreement with previously 281 observed by (da Costa Gomes, Pio Viana, Gonçalves de Oliveira, Gonzaga Pereira, 282 Menezes Goncalves, & Fortes Ferreira, 2007) in banana cv. 'Prata-Anã'. The 283 reduction in pH values over ripening is partially explained by increases in sugar 284 contents with decreasing in TA/SSC ratio (Braga do Nascimento Junior, Pecanha 285 Ozorio, Moraes Rezende, Gomes Soares, & de Oliveira Fonseca, 2008).

The starch content was significantly affected the ripening process as well by 286 287 CaCl₂ associated with hydrothermal treatment (Fig.8). As expected, the starch 288 content decreased during ripening process independently of the treatment (Fig. 8A). 289 Interestingly, each single day of storage, the starch content were decreased around 290 1.217% and the 1% of CaCl₂ treatment 0.5123%. After 30 days of storage, Control 291 (0% CaCl₂) showed starch content around 2% while, when treated with 4% CaCl₂, 292 the starch content was around 4.05%, indicating an efficient process to reduce 293 degradation of starch and reducing the starch conversion to sugars. The starch 294 content was higher in the higher concentrations of calcium chloride, probably due to 295 the lower respiratory rate and higher stabilization of the pectic connections promoted 296 by calcium(W. B. Silva, Silva, Silva, Waldman, & Oliveira, 2015). According with (Ali,

297 Chin, & Lazan, 2004), the fruit softening occurs due to deterioration of structural and 298 non-structural carbohydrates such as, cell wall and or starch oxidation, resulting in an 299 increase in the sugars content. In banana fruit softening were reported by a 300 coordinated degradation of pectic, hemicellulosic polysaccharides in the cell wall and 301 starch (Mbéguié-A-Mbéguié, Hubert, Baurens, Matsumoto, Chillet, Fils-Lycaon, et al., 302 2009; Shiga, Soares, Nascimento, Purgatto, Lajolo, & Cordenunsi, 2011). In banana, 303 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 304 305 MaDEBs (Bierhals, Lajolo, Cordenunsi, & Oliveira do Nascimento, 2004; do 306 Nascimento, Júnior, Bassinello, Cordenunsi, Mainardi, Purgatto, et al., 2006; Jourda, 307 Cardi, Gibert, Giraldo Toro, Ricci, & Yahiaoui, 2016; Junior, Nascimento, & Lajolo, 308 2006). During banana ripening, No amadurecimento da banana one of the most 309 notable changes is the conversion of starch to simple sugars such as glucose and 310 fructose (8-10%) and sucrose (10-20%) (Viviani & Leal, 2007).

311 In order to better understand the role of hydrothermal treatment associated 312 with CaCl₂ in sugar content, we evaluated the reducing, non-reducing and total sugar 313 accumulated during fruit ripening (Fig.8B, C and D). The total sugar (Fig.8B) 314 increased significantly during ripening process over storage time with a daily increase 315 of 0.998%. Interestingly, the highest total sugar levels observed were those when 316 treated with increasing CaCl₂. The sugar content determines the degree of 317 sweetness of the banana and together with the acidity, is a measure more directly 318 correlated with the taste quality (Wu, Quilot, Génard, Kervella, & Li, 2005). The 319 reducing sugar and non-reducing sugar as total sugar were significantly affected by 320 ripening process over storage time as well as by CaCl₂ treatment (Fig.8 C and D). 321 Both sugar has their concentration increased during storage with increases in 322 0.7738% and 0.204% to reducing and non-reducing sugars respectively for each day 323 of evaluation. According with previously observed by (Jesus, Matsuura, Cardoso, & 324 Folegatti, 2004), 23.6% of reducing sugars and 1.3% of non-reducing sugar.

325

326 **3.3**. The PME activity

327

To further understand the effect of chloride calcium on fruit firmness we have measured the pectinmethylesterase on banana's fruit previously treated with

330 hydrothermal with subsequence different CaCl₂ concentration (Fig.9). The PME 331 activity started increasing sharply after hydrothermal and CaCl₂ treatment until 10 332 days after storage, reaching up to 2-fold in comparison with the initial value for all the 333 treatments (Fig.9). Surprisingly, no difference was observed in its activity 334 independently of CaCl₂ concentration (Fig.9). PME activity started decreasing 335 gradually after day and by day 20 was as low as on day 0. Therefore, in can suggest 336 that the treatment as preciously observed in fruit firmness does not provide a 337 beneficial effect on cell wall degradation in banana fruit cv. 'Prata Gorutuba'. A 338 similar pattern was observed during banana ripening with increases followed by 339 decreasing on enzyme activity in banana pulp over ripening stage(H. O. Hultin & 340 Levine, 1965). Differently as we observed in our study, a common answer is an 341 inhibition on PME activity under calcium application as previously showed in different 342 fruits such as in apples (Ortiz, Graell, & Lara, 2011), sweet pepper (Capsicum annum 343 L.) (Rao, Gol, & Shah, 2011). According with Almeida and Huber (1999), changes in 344 the cell wall-related enzymes such as PME, during fruit ripening is dependent of pH 345 apoplast-variation. We suggest that with a few changes in banana pH as reported in 346 Fig.7, the PME activity has not affected by CaCl₂ after hydrothermal treatment.

347

348 CONCLUSION

349

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 green coloration of the fruits until fifteen days of storage, without compromising the flavor.

355 **REFERENCES**

- 356
- Ali, Z. M., Chin, L.-H., & Lazan, H. (2004). A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science*, *167*(2), 317-327.
- Almeida, D. P. F., & Huber, D. J. (1999). Apoplastic pH and inorganic ion levels in
 tomato fruit: A potential means for regulation of cell wall metabolism during
 ripening. *Physiologia plantarum*, *105*(3), 506-512.
- Ayón-Reyna, L. E., González-Robles, A., Rendón-Maldonado, J. G., Báez-Flores, M.
 E., López-López, M. E., & Vega-García, M. O. (2017). Application of a
 hydrothermal-calcium chloride treatment to inhibit postharvest anthracnose
 development in papaya. *Postharvest Biology and Technology, 124*, 85-90.
- Azzolini, M., Jacomino, A. P., & Bron, I. U. (2004). Índices para avaliar qualidade
 pós-colheita de goiabas em diferentes estádios de maturação. *Pesquisa Agropecuária Brasileira, 39*(2), 139-145.
- Barman, K., Sharma, S., & Siddiqui, M. W. (2018). Calcium: An Indispensable
 Element Affecting Postharvest Life of Fruits and Vegetables. In *Emerging Postharvest Treatment of Fruits and Vegetables*, (pp. 85-110): Apple
 Academic Press.
- Batista Silva, W., Cosme Silva, G. M., Santana, D. B., Salvador, A. R., Medeiros, D.
 B., Belghith, I., da Silva, N. M., Cordeiro, M. H. M., & Misobutsi, G. P. (2018).
 Chitosan delays ripening and ROS production in guava (Psidium guajava L.)
 fruit. *Food Chemistry*, 242, 232-238.
- Bierhals, J. D., Lajolo, F. M., Cordenunsi, B. R., & Oliveira do Nascimento, J. R.
 (2004). Activity, cloning, and expression of an isoamylase-type starchdebranching enzyme from banana fruit. *Journal of agricultural and food chemistry*, *52*(24), 7412-7418.
- Bleinroth, E. (1995). Banana: cultura, matéria-prima, processamento e aspectos
 econômicos. rev. e ampl. *Campinas: Instituto de Tecnologia de Alimentos- ITAL*.
- Braga do Nascimento Junior, B., Peçanha Ozorio, L., Moraes Rezende, C., Gomes
 Soares, A., & de Oliveira Fonseca, M. J. (2008). Diferenças entre bananas de
 cultivares Prata e Nanicão ao longo do amadurecimento: características
 físico-químicas e compostos voláteis. *Ciência e Tecnologia de Alimentos,*28(3).
- Brummell, D. A. (2006). Cell wall disassembly in ripening fruit. *Functional Plant Biology*, 33(2), 103-119.
- Brummell, D. A., & Harpster, M. H. (2001). Cell wall metabolism in fruit softening and
 quality and its manipulation in transgenic plants. *Plant molecular biology*,
 47(1), 311-339.
- Buescher, R., & Furmanski, R. (1978). Role of pectinesterase and polygalacturonase
 in the formation of woolliness in peaches. *Journal of Food Science, 43*(1),
 264-266.
- Castricini, A., Santos, L. O., Deliza, R., Coelho, E. F., & RODRIGUES, M. G. V.
 (2015). Postharvest and sensory characterization of banana genotypes type
 Prata. *Revista Brasileira de Fruticultura*, *37*(1), 27-37.
- 401Coelho, S. R., Werner, S. S., Poncio, A. P., Ferreira, L., & Nóbrega, L. H. (2011).402Performanceduringpost-harveststorageofbanana

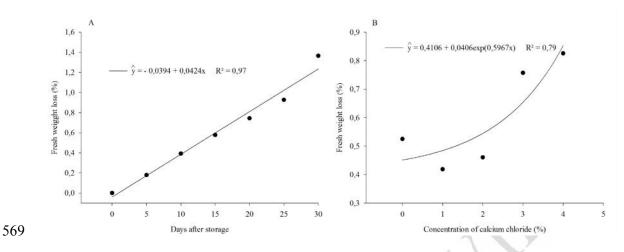
- 403cv.'prata','maçã'and'nanica'exposed to physical and chemical treatments.404Engenharia Agrícola, 31(4), 727-734.
- da Costa Gomes, M., Pio Viana, A., Gonçalves de Oliveira, J., Gonzaga Pereira, M.,
 Menezes Gonçalves, G., & Fortes Ferreira, C. (2007). Avaliação de
 germoplasma elite de bananeira. *Revista Ceres*, *54*(312).
- de Morais Cardoso, L., de Deus, V. A., de Barros Silva, E., de Andrade Júnior, V. C.,
 & Dessimoni-Pinto, N. A. V. (2012). Qualidade pós-colheita de morangos
 cv.'diamante'tratados com cloreto de cálcio associado a hipoclorito de sódio.
 Brazilian Journal of Food & Nutrition/Alimentos e Nutrição, 23(4).
- de Oliveira, J. G., Morales, L. M. M., Silva, G. M. C., da Cruz Saraiva, K. D., Santana,
 D. B., dos Santos, C. P., Oliveira, M. G., & Costa, J. H. (2017). Procedures of
 Mitochondria Purification and Gene Expression to Study Alternative
 Respiratory and Uncoupling Pathways in Fruits. In *Plant Respiration and Internal Oxygen*, (pp. 143-165): Springer.
- do Nascimento, J. R. O., Júnior, A. V., Bassinello, P. Z., Cordenunsi, B. R., Mainardi,
 J. A., Purgatto, E., & Lajolo, F. M. (2006). Beta-amylase expression and starch
 degradation during banana ripening. *Postharvest Biology and Technology*,
 420 40(1), 41-47.
- 421 Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t., & Smith, F. (1956).
 422 Colorimetric method for determination of sugars and related substances.
 423 Analytical chemistry, 28(3), 350-356.
- 424 Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. *Ciencia e* 425 *Agrotecnologia, 35*(6), 1039-1042.
- 426 Gross, J. (2012). *Pigments in vegetables: chlorophylls and carotenoids*: Springer 427 Science & Business Media.
- Hadadinejad, M., & Mohammadi, A. A. (2018). Effect of Storage Temperature and
 Packaging Material on Shelf Life of Thornless Blackberry. *International Journal of Horticultural Science and Technology*, *5*(1), 93-103.
- Hailu, M., Workneh, T. S., & Belew, D. (2013). Review on postharvest technology of
 banana fruit. *African Journal of Biotechnology*, *12*(7).
- Hernández-Muñoz, P., Almenar, E., Ocio, M. J., & Gavara, R. (2006). Effect of
 calcium dips and chitosan coatings on postharvest life of strawberries
 (Fragaria x ananassa). *Postharvest Biology and Technology*, *39*(3), 247-253.
- Hernández-Muñoz, P., Almenar, E., Valle, V. D., Velez, D., & Gavara, R. (2008).
 Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragaria×ananassa) quality during refrigerated storage. *Food Chemistry*, *110*(2), 428-435.
- Hultin, H., Sun, B., & Bulger, J. (1966). Pectin methyl esterases of the banana.
 Purification and properties. *Journal of Food Science*, *31*(3), 320-327.
- Hultin, H. O., & Levine, A. S. (1965). Pectin Methyl Esterase in the Ripening Banana.
 Journal of Food Science, 30(6), 917-921.
- Hunt, R. (1977). The specification of colour appearance. I. Concepts and terms.
 Color Research & Application, 2(2), 55-68.
- Irfan, P. K., Vanjakshi, V., Prakash, M. N. K., Ravi, R., & Kudachikar, V. B. (2013).
 Calcium chloride extends the keeping quality of fig fruit (Ficus carica L.) during storage and shelf-life. *Postharvest Biology and Technology*, *82*, 70-75.
- Jesus, S. C. d., Matsuura, F. C. A. U., Cardoso, R. L., & Folegatti, M. I. d. S. (2004).
 Caracterização física e química de frutos de diferentes genótipos de bananeira.

- Jourda, C., Cardi, C., Gibert, O., Giraldo Toro, A., Ricci, J., & Yahiaoui, N. (2016).
 Lineage-specific evolutionary histories and regulation of major starch metabolism genes during banana ripening. *Frontiers in plant science*, *7*, 1778.
- Junior, A. V., Nascimento, J. R. O. d., & Lajolo, F. M. (2006). Molecular cloning and
 characterization of an α-amylase occuring in the pulp of ripening bananas and
 its expression in Pichia pastoris. *Journal of agricultural and food chemistry*,
 54(21), 8222-8228.
- Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture, 88*(11), 1863-1868.
- Kajuna, S., Bilanski, W., & Mittal, G. (1998). Color changes in bananas and plantains
 during storage. *Journal of Food Processing and preservation*, 22(1), 27-40.
- Lancaster, J. E., Lister, C. E., Reay, P. F., & Triggs, C. M. (1997). Influence of
 pigment composition on skin color in a wide range of fruit and vegetables. *Journal of the American Society for Horticultural Science, 122*(4), 594-598.
- 466 Lichtemberg, L. A., & Lichtemberg, P. d. S. (2011). Avanços na bananicultura 467 brasileira. *Revista Brasileira de Fruticultura, 33*(1).
- 468 Lurie, S. (1998). Postharvest heat treatments. *Postharvest Biology and Technology*,
 469 14(3), 257-269.
- Madani, B., Mirshekari, A., & Yahia, E. (2016). Effect of calcium chloride treatments
 on calcium content, anthracnose severity and antioxidant activity in papaya
 fruit during ambient storage. *Journal of the Science of Food and Agriculture*,
 96(9), 2963-2968.
- Madani, B., Muda Mohamed, M. T., Biggs, A. R., Kadir, J., Awang, Y.,
 Tayebimeigooni, A., & Shojaei, T. R. (2014). Effect of pre-harvest calcium
 chloride applications on fruit calcium level and post-harvest anthracnose
 disease of papaya. *Crop Protection*, *55*, 55-60.
- Mbéguié-A-Mbéguié, D., Hubert, O., Baurens, F., Matsumoto, T., Chillet, M., FilsLycaon, B., & Sidibé-Bocs, S. (2009). Expression patterns of cell wallmodifying genes from banana during fruit ripening and in relationship with
 finger drop. *Journal of experimental botany*, *60*(7), 2021-2034.
- 482 Mendonça, K., Jacomino, A., Melhem, T., & Kluge, R. (2003). Concentração de
 483 etileno e tempo de exposição para desverdecimento de limão "siciliano".
 484 Brazilian Journal of Food Technology, 6(2), 179-183.
- 485 Moraes, W. d. S., Zambolim, L., Lima, J. D., Vale, F. X. R. d., & Salomão, L. C.
 486 (2006). Termoterapia e quimioterapia de banana'Prata-Anã'associadas à
 487 temperatura de armazenamento no controle de podridões em pós-colheita.
 488 *Fitopatologia Brasileira*, 17-22.
- 489 Nelson, N. (1944). A photometric adaptation of the Somogyi method for the
 490 determination of glucose. *J. biol. Chem*, *153*(2), 375-380.
- Nguyen, T. B. T., Ketsa, S., & van Doorn, W. G. (2003). Relationship between
 browning and the activities of polyphenoloxidase and phenylalanine ammonia
 lyase in banana peel during low temperature storage. *Postharvest Biology and Technology*, *30*(2), 187-193.
- 495 Nunes-Nesi, A., Araújo, W. L., Obata, T., & Fernie, A. R. (2013). Regulation of the
 496 mitochondrial tricarboxylic acid cycle. *Current opinion in plant biology, 16*(3),
 497 335-343.
- 498 Ortiz, A., Graell, J., & Lara, I. (2011). Preharvest calcium applications inhibit some 499 cell wall-modifying enzyme activities and delay cell wall disassembly at

- 500 commercial harvest of 'Fuji Kiku-8' apples. *Postharvest Biology and* 501 *Technology*, 62(2), 161-167.
- 502 Osorio, S., Scossa, F., & Fernie, A. (2013). Molecular regulation of fruit ripening. 503 *Frontiers in plant science, 4*, 198.
- PBMH, & PIF. (2006). Programa brasileiro para a modernização da horticultura &
 produção integrada de frutas. Normas de Classificação de Banana.,
 Documento 29, CEAGESP, São Paulo-SP.
- Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable
 products: Tata McGraw-Hill Education.
- Rao, T. V. R., Gol, N. B., & Shah, K. K. (2011). Effect of postharvest treatments and
 storage temperatures on the quality and shelf life of sweet pepper (Capsicum
 annum L.). Scientia horticulturae, 132, 18-26.
- Rasai, S., Kantharajah, A., & Dodd, W. (1994). The Effect of Growth-Regulators,
 Source of Explants and Irradiance on <I>in vitro</I> Regeneration of Atemoya.
 Australian Journal of Botany, 42(3), 333-340.
- Ribeiro, R. C. F., Rodrigues, M. L. M., Fernandes, M. B., & Mizobutsi, E. H. (2018).
 Hydrothermal treatment in the management of anthracnose in 'Prata-Anã'banana produced in the semiarid region of Minas Gerais, Brazil. *Revista Brasileira de Fruticultura, 40*(2).
- 519 Sabehat, A., Weiss, D., & Lurie, S. (1998). Heat-shock proteins and cross-tolerance 520 in plants. *Physiologia plantarum, 103*(3), 437-441.
- Salvador-Figueroa, M., Aragón-Gómez, W. I., Hernández-Ortiz, E., Vazquez-Ov, J.
 A., & de Lourdes Adriano-Anaya, M. (2011). Effect of chitosan coating on
 some characteristics of mango (Mangifera indica L.) Ataulfo subjected to
 hydrothermal process. *African Journal of Agricultural Research*, 6(27), 5800 5807.
- Santos, T. C., Aguiar, F. S., Rodrigues, M. L. M., Mizobutsi, G. P., & Pinheiro, J. M.
 d. S. (2018). Quality of bananas harvested at different development stages and subjected to cold storage. *Pesquisa Agropecuária Tropical*, *48*(2), 90-97.
- Schirra, M., Mulas, M., Fadda, A., & Cauli, E. (2004). Cold quarantine responses of
 blood oranges to postharvest hot water and hot air treatments. *Postharvest Biology and Technology*, *31*(2), 191-200.
- 532 Shiga, T. M., Soares, C. A., Nascimento, J. R., Purgatto, E., Lajolo, F. M., & 533 Cordenunsi, B. R. (2011). Ripening-associated changes in the amounts of
- 534 starch and non-starch polysaccharides and their contributions to fruit softening 535 in three banana cultivars. *Journal of the Science of Food and Agriculture*, 536 91(8), 1511-1516.
- Silva, C. d. S., Lima, L. C., Santos, H. S., Camili, E. C., Vieira, C. R. Y. I., Martin, C.
 d. S., & Vieites, R. L. (2006). Amadurecimento da banana-prata climatizada em diferentes dias após a colheita. *Ciencia e Agrotecnologia*, 103-111.
- Silva, W. B., Silva, G. M. C., Silva, L. R. d., Waldman, W. R., & Oliveira, J. G. d.
 (2015). Treatment with calcium chloride at postharvest delays the degreening and loss of firmness of papaya Uenf/Caliman01. *Revista Brasileira de Fruticultura, 37*(3), 588-599.
- 544 Song, J., Deng, W., Beaudry, R. M., & Armstrong, P. R. (1997). Changes in 545 chlorophyll fluorescence of apple fruit during maturation, ripening, and 546 senescence. *HortScience*, *32*(5), 891-896.

- 547 Terra Werner, E., Ganassali de Oliveira Junio, L. F., Bona, A. P. d., Cavati, B., &
 548 Hermogenes Gomes, T. D. U. (2009). Efeito do cloreto de cálcio na pós549 colheita de goiaba Cortibel. *Bragantia*, 68(2).
- Vilas-Boas, E. V. d. B., & Kader, A. A. (2006). Effect of atmospheric modification, 1 MCP and chemicals on quality of fresh-cut banana. *Postharvest Biology and Technology*, 39(2), 155-162.
- 553 Viviani, L., & Leal, P. M. (2007). Qualidade pós-colheita de banana Prata Anã 554 armazenada sob diferentes condições. *Revista Brasileira de Fruticultura*, 555 29(3), 465-470.
- Wu, B. H., Quilot, B., Génard, M., Kervella, J., & Li, S. H. (2005). Changes in sugar
 and organic acid concentrations during fruit maturation in peaches, P.
 davidiana and hybrids as analyzed by principal component analysis. *Scientia horticulturae*, *103*(4), 429-439.
- 560 Yemm, E., & Willis, A. (1954). The estimation of carbohydrates in plant extracts by 561 anthrone. *Biochemical journal, 57*(3), 508.
- Youryon, P., Supapvanich, S., Kongtrakool, P., & Wongs-Aree, C. (2018). Calcium
 chloride and calcium gluconate peduncle infiltrations alleviate the internal
 browning of Queen pineapple in refrigerated storage. *Horticulture, Environment, and Biotechnology,* 59(2), 205-213.
- 566





570 **Fig. 1**. The effect of hydrothermal treatment and $CaCl_2$ on fresh weight loss during 30 571 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata

572 Gorutuba'. Values are presented as means ± SE (n=4).

573

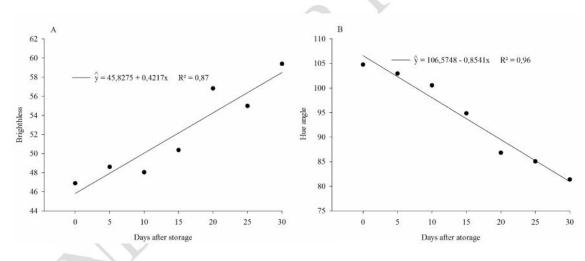


Fig. 2. The effect of hydrothermal treatment and CaCl₂ on Brighthless (A) and Hue Angle (B) during 30 days after treatment at 13.5 ± 1 °C and RH85 $\pm 5\%$ in banana fruit cv. 'Prata Gorutuba'. Values are presented as means \pm SE (n=4).

578

574

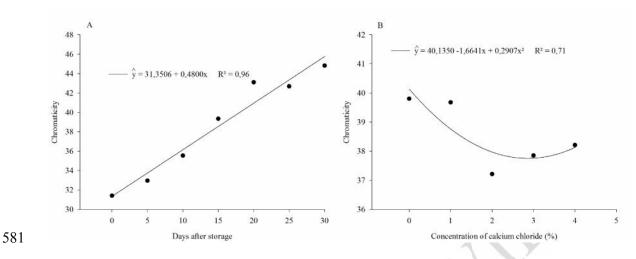
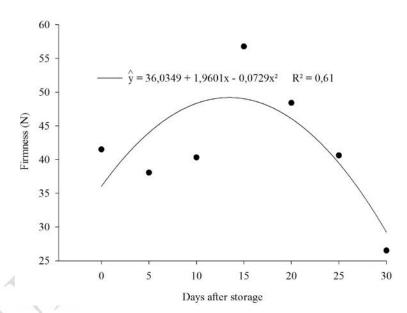
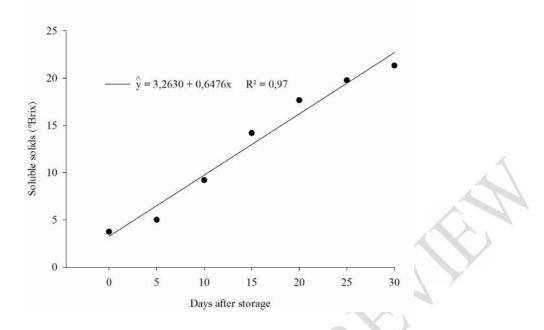


Fig. 3. The effect of hydrothermal treatment and CaCl₂ on Chroma (C^*) during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values are presented as means ± SE (n=4).



580

Fig. 4. The effect of hydrothermal treatment and CaCl₂ on fruit firmness 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 \pm SE (n=4).



589

590 **Fig. 5.** The effect of hydrothermal treatment and $CaCl_2$ on SSC during 30 days after 591 treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values

592 are presented as means ± SE (n=4).

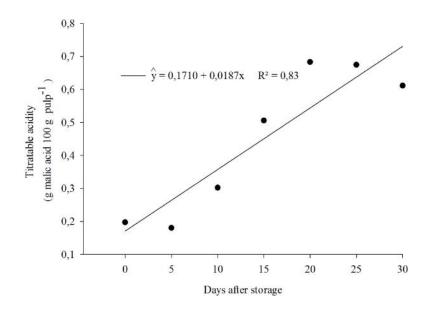
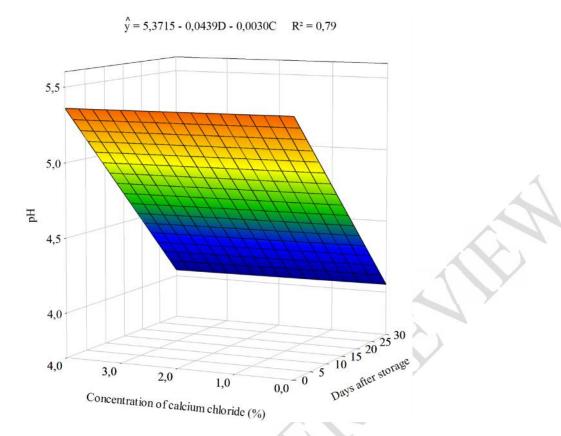




Fig. 6. The effect of hydrothermal treatment and $CaCl_2$ on TA during 30 days after treatment at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values are presented as means ± SE (n=4).



- 598
- 599 Fig. 7. The effect of hydrothermal treatment and CaCl₂ on pH during 30 days after
- treatment at 13.5 \pm 1 °C and RH85 \pm 5% in banana fruit cv. 'Prata Gorutuba'. Values
- 601 are presented as means ± SE (n=4).

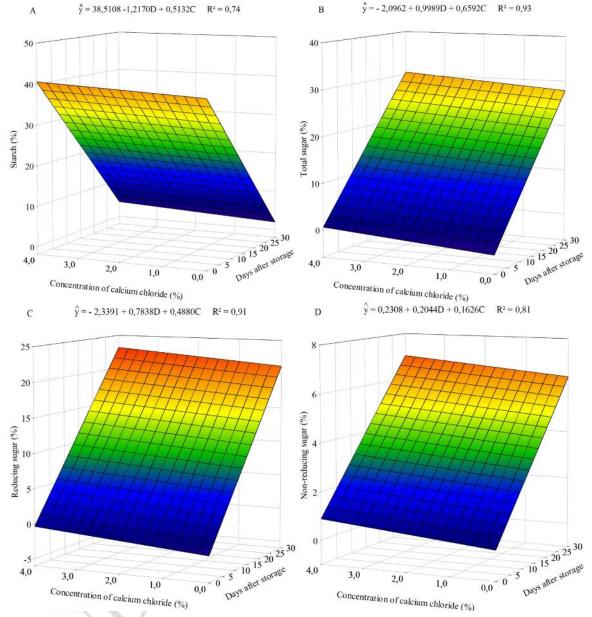
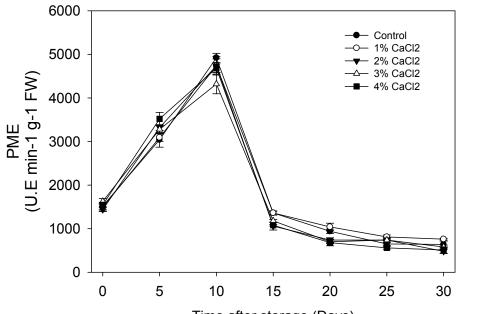


Fig. 8. The effect of hydrothermal treatment and $CaCl_2$ on Starch (A), Total Sugar 604 (B), Reducing sugar (C) and Non-reducing sugar (D) during 30 days after treatment 605 at 13.5 ± 1 °C and RH85 ± 5% in banana fruit cv. 'Prata Gorutuba'. Values are 606 presented as means ± SE (n=4).



Time after storage (Days)

611 **Fig 9**. The effect of different calcium chloride concentration on pectinmethylesterase 612 (PME) activity during 30 days after treatment at 13.5 ± 1 °C and RH85 $\pm 5\%$ in 613 banana fruit cv. 'Prata Gorutuba'. Values are presented as means \pm SE (n=4).

614