

2
3 **The role of ethylene on banana fruit ripening via sugar and starch**
4 **metabolism**

5
6
7 **Abstract**

8
9 Banana as a climacteric fruit has a relatively short shelf-life period and
10 thus technologies that decrease the metabolism and the triggering of the
11 maturation process are extremely necessary on its postharvest conservation.
12 However, the consequences of these technologies on quality attributes are
13 unknown. Therefore, we evaluate the effects of 1-MCP associated with low
14 density polyethylene bags on physical and chemical attributes in the
15 postharvest of banana fruits. Bananas were treated with different concentration
16 of 1-MCP as 0, 50, 100, 150 and 200 $\eta\text{L.L}^{-1}$ under refrigeration and harvest in
17 five different time of postharvest after low temperature storage. Further, fruits
18 treated with 50 $\eta\text{L.L}^{-1}$ showed a more advanced stage of ripening after the 25
19 days of storage. Altogether, our results suggest 1-MCP is an effective treatment
20 to control sugar and starch metabolism in banana and its efficiency is directly
21 dependent of storage temperature. Additionally we identify interestingly
22 correlation with skin color changes with sugars and starch content, which can
23 indicate its potentiality of the fitted equations for prediction of central
24 metabolism of bananas non-destructively using 'hue angle and chrome' value.

25
26 **Keywords:** Postharvest, banana quality, 1-MCP, sugar metabolism, starch
27

28 **Introduction**
29

30 Banana (*Musa* sp.) is an important commercial food crop exported to
31 many countries. Banana are considered as a climacteric fruits which is
32 characterized by two different of ethylene production are thought to operate,
33 exhibiting a short shelf-life (Pech *et al.*, 2008). According to Golding *et al.*

34 (1998), during the maturation, there is a low basal rate of ethylene production
35 termed System 1, followed by System 2 ethylene, which this second one is
36 characterized by autocatalytic climacteric rise in ethylene production. Under
37 normal condition, ethylene binds to receptor membrane proteins, triggering
38 responses associated with maturation (Dubois *et al.*, 2018; McAtee *et al.*,
39 2013). This phytohormone is directly associated with several changes during
40 the burst from autocatalytic stimulation (Yang and Hoffman, 1984). In this vein,
41 banana ripening, according to Medina-Suárez *et al.* (1997) and Ghosh *et al.*
42 (2016), is characterized by a significant up- and down-regulation of transcripts
43 that encode enzymes involved in ethylene biosynthesis, respiration, starch
44 metabolism as well as sugar metabolism and several other key metabolic
45 events on the primary and secondary metabolism, such as chlorophyll
46 breakdown and carotenoid accumulation (Katz *et al.*, 2004). Hence, it is
47 generally accepted that continued production and action of ethylene are
48 required for integration of these biochemical events (Golding *et al.*, 1998).

49 Notably, the quality of the fruit is related to the maintenance of the normal
50 characteristics of the product, such as texture, color, flavor and aroma, the way
51 the food is produced to the consumer for the longest possible time. However,
52 these characteristics are directly depending on metabolic activity which is
53 several factors-responsive. Thus, technologies that modulate the metabolism
54 and inhibit the senescence process at the same moment are needed.

55 The use of ethylene inhibitors in banana can delay the maturation
56 process by increasing the postharvest life of the fruit. In the same vein, the
57 volatile ethylene nontoxic antagonists such as 1-methylcyclopropene (1-MCP)
58 has provided a useful tool for elucidating the role of ethylene in ripening
59 climacteric fruit (Dek *et al.*, 2018; Golding *et al.*, 1998; Mazorra *et al.*, 2013;
60 Nakatsuka *et al.*, 1997; Thongkum *et al.*, 2018). Recently, 1-MCP has been
61 extensively used on climacteric and non-climacteric fruits for delaying fruit
62 ripening and control fruit quality in tomato (Zou *et al.*, 2018), melon (Han *et al.*,
63 2015), plum (Martínez-Romero *et al.*, 2003), mango (Sakhale *et al.*, 2018;
64 Trindade *et al.*, 2015), strawberry (Jiang *et al.*, 2001), papaya (Mazorra *et al.*,
65 2013; Souza *et al.*, 2009), and bananas (Golding *et al.*, 1998; Jiang *et al.*,
66 1999). Although various studies have focused on the 1-MCP effect on the

67 storage of banana fruit ((Jiang *et al.* (2004); Zewter *et al.*, 2012), still in our
68 knowledge, few works have been done associating the effect of 1-MCP
69 combined with refrigeration and packaging on metabolic adjustment as well as
70 changes in the color of the fruits. Furthermore, still remain unclear the
71 correlation pattern of these parameters when the ethylene action is inhibited
72 and how long they remain ethylene-dependent.

73 Hence, the objective of the present study was to evaluate the effect of 1-
74 methylcyclopropene associated with low density polyethylene packaging in the
75 preservation and maintenance of postharvest quality as well as its effects on the
76 metabolism and correlation pattern of chemical, physical and metabolic
77 attributes in banana 'Prata Gorutuba' (Musa spp. AAB 'Prata Anã' clone:
78 Gorutuba) stored under refrigeration.

79

80 **Material and Methods**

81

82 *Fruit material*

83

84 Bananas bunches (containing 4 individual fruits) cv. Prata Gorutuba
85 (*Musa* AAB 'Prata Anã') were provided by the Itapicuru Company, located in
86 Minas Gerais State, Southeastern Brazil. Mature fruit showing totally green skin
87 (maturity stage 2, see Supplementary fig.S1) were harvested, washed, carefully
88 selected, and transported in a refrigerated truck at 15°C during 4h to the
89 laboratory before performing analyzes.

90

91 *Solution preparation and fruit treatment*

92

93 The fruits were placed in hermetic plastic boxes (0.3 m³) and then
94 submitted to 0, 50, 100, 150 e 200 μ L.L⁻¹ of 1-methylcyclopropene (1-MCP)
95 (SmartFresh®) in the form of wettable-powder, containing 0,14% i.a. of 1-
96 methylcyclopropene. After complete dissolution, the banana's fruits were
97 exposed for 8 hours to the gas under room temperature (RT) 25±1°C. After the
98 treatment period, the fruits were packed in to Low Density Polyethylene (LDPE)
99 plastic bags (25 μ m), and then stored in cooling chamber at 14.5 ± 1 °C and
100 relative humidity air (RH) 95% ± 5 % during 25 days after treatment. Finally,

101 after this storage time, the banana's bunches were removed from the packages
102 and kept at RT for 5 days, and analyzes were taken daily until the last day.

103

104 *Physical quality attributes*

105 The firmness was performed using a penetrometer (Brookfield model
106 CT3) with 4mm diameter. The evaluation made in two equidistant regions, on
107 opposite sides, of the equatorial region of the fruits. The firmness was
108 measured as the maximum penetration force expressed in Newton (N). The
109 skin color of the fruit was performed using a Color Flex 45/0, stdzMode:45/0. To
110 determine the chromaticity values (L^* – lightness, and h° – hue angle) were
111 calculated using the chromaticity values a^* and b^* according to McGuire (1992).

112

113 *Determination of chemical quality parameters*

114

115 Around ten gram (10 g) of banana from each replicate were crushed and
116 homogenized with 100 ml boiled distilled water (previously adjusted to pH 8.3).
117 The pH was measured by using pH meter Crison MicropH 2001 (Crison
118 Instruments SA, Barcelona, Spain). The mixture was titrated with 0.10 M NaOH
119 to pH 8.3 and the result was expressed as mg malic acid per 100 g sample. The
120 total soluble sugar content (SSC) were determined according to Madamba
121 (1993) as previously described by (Bico *et al.*, 2009).

122

123 *Physiological parameters*

124

125 The soluble sugars were determined by the anthrone method Dubois *et*
126 *al.* (1956). The quantification of starch was carried out according to the method
127 described by Yemm and Willis (1954) and dosages were made by the anthrone
128 method Dubois *et al.* (1956). The starch was obtained by spectrophotometry,
129 with reading at 510 nm, according to the method described by Nelson (1944).

130

131 *Experimental design and statistical analyzes*

132

133 The experiment was performed in a completely randomized design
134 (CRD), with five replicates. Statistical analyzes were performed using the
135 GENES software (Cruz, 2006). The averages of the treatments were compared

136 by the Tukey's test ($P \leq 0.05$). Pearson's correlations were also calculated.
137 Analyzes were performed using Sigma Stat software v.2.0 (SPSS Inc., Chicago,
138 IL, USA) and GraphPad prism 6 (GraphPad Prism version 6 for Windows,
139 GraphPad Software, La Jolla, California, USA).

140

141 **Results**

142

143 *Effect of 1-MCP in physical quality parameters during banana ripening*

144

145 As shown in Fig.S1, the 1-MCP could delay the ripening of banana fruit
146 and in complement, the physical quality parameters follows the same visual
147 response (Fig.1). Among the treatment, $50\eta\text{L.L}^{-1}$ 1-MCP treatment had the
148 similar effect with a normal ripening, which after 3 days there was not more
149 significantly difference in comparison with $0\eta\text{L.L}^{-1}$ 1-MCP (See in Fig.1 and
150 Supplemental table S.1). However, in higher concentrations the firmness in the
151 control fruit dropped sharply from of 30.59N on the 1st day to 4.03N on the 5th
152 day after remove from the refrigeration (Fig. 1A). Interestingly, the L value the 1-
153 MCP treatment has not shown drastically changes during fruit ripening, except
154 in 3th day where, the L value was reduced in 100, 150 and $200\eta\text{L.L}^{-1}$ 1-MCP
155 (60.97, 58.96 and 63.04 respectively, see in Fig.1B and supplemental table S1).
156 The change of the peel color was expressed in hue angle (h°) values. As is
157 shown in Fig. 1C, the peel color of banana fruit in all treatments turned more
158 yellow through the period of storage (Supplemental Fig.S1 and S2). Banana
159 treated with 1-MCP showed a significantly higher level of hue angle values
160 compared to that of other treatments. This effect was considered dose
161 response, the higher the concentration of 1-MCP, the greater the delay in the
162 peel color changes (Fig.1C and Supplemental Fig. S1). At the same time, the
163 Chroma (Fig.1D), a similar results from the previous observed with hue angle,
164 showing a higher color intensity (Chroma), mainly after 5th day of storage at
165 25°C with reduced values to 1-MCP treatments (Supplemental table S1).

166

167 *Effect of 1-MCP in chemical quality parameters during banana ripening*

168

169 As shown in Fig. 2A, the pH values had significant reduced during fruit
170 ripening and these effects were retard by with increased concentrations of 1-
171 MCP. The lowest values on the 4th day after treatment and storage (4.42, 4.43,
172 4.46 and 4.57) were detected in the control and fruits submitted to the lowest
173 concentrations of 1-MCP (0, 50, 100 and 150 η L. L-1, respectively). These
174 effects are in a close relationship with malic acids content in the (Fig.2B).
175 During fruit ripening fruit, the 1-MCP significantly has affected the malic acid,
176 which has kept enhanced the values in fruits treated with 100, 150 and 200 η L.
177 L-1 (Fig.2B and Supplemental table S2). As shown in Fig.2C, the SSC ($^{\circ}$ brix)
178 increased during fruit ripening in all treatments. A significant extension in
179 ripening time was obtained for all concentrations of 1-MCP with the increase in
180 time to ripen over untreated fruit. The 1-MCP treatment has suppressed
181 significantly the SSC content over fruit ripening as compared with control
182 (Fig.2C). The rate of sugar content evolution is drastically affected in 5th day
183 after treatment and storage, which 200 η L.L⁻¹ 1-MCP has suppressed ~50% of
184 SSC in the fruit juice. The malic content changes has influenced the brix/acid
185 ratio (Fig.2D), showing to be due mainly to a higher TA in 1-MCP treated fruit
186 rather than a lower level of SSC, which malic acid degradation over ripening is
187 reduced in 1-MCP treatment.

188

189 *Effect of 1-MCP in physiological quality parameters during banana ripening*

190

191 The ripening are a complex process genetically programmed, culminating
192 in a dramatic changes, mainly in color, texture, flavor and soluble solids and
193 volatile aroma (Osorio *et al.*, 2013). In order to characterize better the 1-MCP
194 after refrigerating storage on physiological traits, we measured the sugars,
195 starch contents as well as the ratio sugar/starch (Fig.3). As shown in Fig.3 the
196 non-reducing sugar has been found in larger quantities and lower concentration
197 to reducing-sugars (Fig.3A and B). However, both have increased in quantity
198 during fruit ripening, reaching the higher levels at 5th in control fruits.
199 Additionally, the sugar content has increased during ripening fruit, concomitantly
200 with the evolution of SSC. The 1-MCP treatment has delayed the accumulation
201 of sugars and starch degradation (Fig.3). As shown on Fig.3C, fruits treated
202 with 200 η .L.L⁻¹ 1-MCP have had reductions in the total sugar content of 25% in

203 the 5th day after treatment and lower temperature storage in comparison with
204 untreated fruits at the same time. Therefore, the 1-MCP combined with lower
205 temperature storage can significant influence on the ripening process of
206 bananas mainly controlling the sugar content in banana fruits. As shown in
207 Fig.3D, the starch content is decreased during fruit ripening, as expected,
208 however the 1-MCP has a directly influence in starch catabolism during ripening
209 process, suggesting the connection directly with enzymes involved with
210 degradation of this compound. The treatment with 200 η .L.L⁻¹ has reduced 2-
211 fold when compared with control (untreated fruits). Additionally, the variation in
212 the sugars and starch content correlated strongly with some physical properties
213 of the fruit, such as SSC, skin color parameter and the malic acids (Fig.5).
214 Interestingly, starch has negatively correlated with SSC (-0.92), the ratio
215 SSC/TA (-0.89), L^* (-0.81) and Chrome (-0.90) (Fig.5). This expected negative
216 correlation shows the importance of the degradation of the reserves (starch) for
217 increasing the sugars content of sugars and increase in the content of soluble
218 solids of the fruits.

219

220 *Multivariate analysis*

221

222 All measured variables were used to perform the analysis of the principal
223 components (PCA). Furthermore, the PCA was performed to explore more
224 deeply the contribution of changes 1-MCP treatment followed by lower
225 temperature storage in the metabolite composition as well as physical
226 parameters across fruit developmental stages by score plot and loading plot
227 (Fig.4). Through the PCA, this fingerprint analyzes showed that indeed the
228 dominant source of variation in the combined dataset is the differential
229 contribution of the metabolite composition across fruits ripening in 1-MCP
230 treatment. The first component (PC1) explained 89.6% of the variation and the
231 second component (PC2) only 9.7%, which showed no distinguish between the
232 1-MCP treatment and time after storage. Therefore, our attention was turned to
233 the PC1 (Fig.4A). Our results were separated in three groups whose were also
234 confirmed by Euclidean distances. In the group I, include the time over fruit
235 ripening, which low concentrations of 1-MCP such as 0 and 50 in ripe fruit (T4

236 and T5) characterized by low influences of 1-MCP and fruit complete ripe (See
237 Fig. 4A – orange circle) while the group II, include the blue circle group and it is
238 composed by intermediate fruit ripe and mixed with higher 1-MCP such as 100
239 and 150nL.L⁻¹, showing a mix of effect by 1-MCP treatment and time of storage.
240 Finally, the group III composed by unripe fruit under storage independently of 1-
241 MCP treatment and 1-MCP, mainly by higher 1-MCP concentrations influences
242 (Fig.4A). In a complementary manner, we also performed the loading plot,
243 intending to analyze the variables that contributed to the separation of the
244 groups. Ripe fruits (Group I) was separated mainly by non-reducing sugars
245 (Fructose and Glucose), total sugars content as well as the ratio SSC/TA, which
246 are associated with fruit quality (Fig.4B). This results shows that 50nL.L⁻¹ in T5
247 (5 days after storage) does not have any influences in avoid starch catabolism
248 and control of fruit ripening, showing results similar with control. The group II
249 was mainly separated by color and soluble solids contents and by reducing-
250 sugars. In the last case, the group III interestingly was separated by starch,
251 firmness and Angle hue. Both of these variables are directly controlled by 1-
252 MCP concentrations and stage of fruit ripening process (See in Fig.4B).

253

254

255 Discussion

256

257 The 1-MCP as an inhibitor of ethylene perception has been investigated
258 in a large number of researches as an agent maintaining the quality as well as
259 to investigate the role of ethylene in ripening and senescence of many fruits and
260 vegetables (Dek et al., 2018), including bananas (Harris *et al.*, 2000; Zhu *et al.*,
261 2015). These responses are also investigated alone, or in association with
262 different agents such as chitosan (Qiuping and Wenshui, 2007), and hormones
263 (Zhu et al., 2015) as well as atmospheric modification (Vilas-Boas and Kader,
264 2006). However, according with Blankenship and Dole (2003) the 1-MCP
265 treatment depend on numerous factors and are dependent on plant material. In
266 banana, with at least 5 and 50 nL.L⁻¹ had no effect on unripe bananas, 500
267 nL.L⁻¹ delayed ripening (Harris et al., 2000). However, few works has
268 associated the 1-MCP treatment associated with low density polyethylene
269 packaging in the preservation and maintenance of postharvest quality as well as

270 its effects on the metabolism of sugars and banana, once 1-MCP has a strong
271 complete ability with ethylene receptors and suppress the respiration and
272 climacteric peak in fruit (Blankenship and Dole, 2003; Harris et al., 2000; Lima
273 *et al.*, 2010).

274 This search becomes pertinent, since it is known that one of the factors
275 that most affects the responses to 1-MCP is the active concentration and
276 treatment duration and the relation between concentrations exposing time are
277 directly interdependent (Bagnato *et al.*, 2003). In our study, the 1-MCP
278 treatment associated low density polyethylene packaging in the preservation
279 can effectively delay ripening via significantly changes in physical (See in Fig.1
280 and Supplemental table S1), chemical (See in Fig.2 and Supplemental table S2)
281 and physiological traits (See in Fig.3 and Supplemental table S3), delaying fruit
282 firmness, skin color and controlling the cellular pH by increasing of malic acid
283 which are directly associated with ripening rate. Consequently, the ratio
284 SSC/TA is altered which is separated by PCA analysis in three independent
285 group according fruit age and 1-MCP treatment (Fig.4). Additionally, the sugar
286 content both, non-reducing and reducing-sugar are suppressed by 1-MCP
287 treatment, which can be explained partially by starch degradation (Fig. 3D).
288 Starch degradation is delayed over fruit ripening by 1-MCP treatment driving a
289 few alterations in sugars and soluble solids contents. Therefore, reducing in
290 sugar/starch ration in treated fruit was reduced in comparison with untreated
291 fruits (Fig.3E). Overall, our data indicate that the conversion of starch to sugars
292 is in good agreement with fruit softening and ethylene production during banana
293 ripening.

294 The influence of 1-MCP treatment in respiration and ethylene production
295 is already well documented by Jiang et al. (1999) and (Harris et al., 2000).
296 Therefore, the reduction in respiration rate is possibly the result of reduction in
297 several essential respiratory steps such as, glycolysis and TCA, as well as
298 phosphorylating chain (OXPHOS), decreasing the ATP production and starting
299 the anaerobic pathway, resulting in lower SSC due to the slower hydrolysis of
300 carbohydrates (Starch to sugars) (Araujo *et al.*, 2012; Krebs, 1937). Our results
301 show that 1-MCP and low density polyethylene packaging might be a viable
302 alternative to extend the post-harvest life of banana and function as a

303 controlling agent of the nutritional levels. In agreement with those found by
304 (Purgatto *et al.*, 2002) which banana fruit ripening is characterized by textural
305 softening, sugar content, acidity and color changes. In addition, Ziliotto *et al.*
306 (2008) have shown in transcriptome profiling of ripening nectarine treated with
307 1-MCP has in comparison with untreated fruit after 24h, 106 targets differentially
308 genes were expressed and 30% of their targets correspond to gene involved in
309 primary metabolism related with ethylene and other phytohormones as well as
310 some gene involved in softening, skin color development and sugar
311 metabolism. Interestingly, in our results, angle hue ($^{\circ}h$) is an important skin-
312 color parameters to identify the sugar content in banana fruit, hence they shows
313 a higher and positive correlation with starch content and been an important
314 variable that has contributed to separate groups in PCA analysis (See in Fig.4A
315 and Fig.5).

316 Faced to physical variables changes, such as Firmness, L^* , h° and chrome, the
317 firmness reduced during maturation for all treatments, however the fruit
318 softening was reduced following the increase in the 1-MCP concentrations,
319 resulting in a smaller loss of firmness after removal of the refrigerated chamber.
320 According with Ali *et al.* (2004), the fruit softening occurs due to deterioration of
321 structural and non-structural carbohydrates such as, cell wall and-or starch
322 oxidation, resulting in an increase in the sugars content. In banana fruit
323 softening were reported by a coordinated degradation of pectic, hemicellulosic
324 polysaccharides in the cell wall and starch (Shiga *et al.*, 2011). In banana,
325 several gene is are involved in starch-to-sugars conversions during ripening
326 process has been reported, including the amylases such as *MAmy*, *Ma-bms*,
327 *Maisa* and *MaDEBs* (Bierhals *et al.*, 2004; do Nascimento *et al.*, 2006; Jourda
328 *et al.*, 2016; Junior *et al.*, 2006). Recently, Xiao *et al.* (2018) has shown a
329 complex actions of numerous enzymes related to starch breakdown at
330 transcriptional and translational levels and proved that MabHLH6 may act as a
331 positive regulator of this process via activation of a series of starch degradation-
332 related genes. Taken together, our study suggests that 1-MCP treatment can be
333 involved regulating somehow the starch-degradation gene and acting mediating
334 partially transcription factors responsive to ethylene. In the same way,
335 according with Zewter *et al.* (2012), bananas treated with 1-MCP kept in a

336 package of non-perforated polyethylene, showed an increase in total sugars,
337 provided by conversion of starch to sugar, as with the advancement of ripening
338 a decline occurs after reaching a certain peak when the fruits enter the
339 senescence phase. In summary, the treatment 100, 150 and 200 $\mu\text{L.L}^{-1}$ de 1-
340 MCP in bananas 'Prata Gorutuba' for 8 hours delay the ripening in
341 approximately 25 days, when packed in PEDB and stored under refrigeration
342 (14.5°C), without changes in their physical and chemical characteristics. In
343 complement, our results suggest 1-MCP treatment effectively prolongs the
344 quality attributes not compromising the normal ripening after removal of
345 package and kept in room temperature (25°C), accumulating sugars by starch
346 degradation. Furthermore, the correlation pattern of physical and chemical
347 attributes demonstrates thus the dependence of ethylene action and of the
348 interplay between these events despite mechanism to be distinct and act in
349 different area in the fruit.

350

351

UNDER PEER REVIEW

352 **REFERENCES**

353

- 354 Ali, Z.M., Chin, L.-H., Lazan, H., 2004. A comparative study on wall degrading
355 enzymes, pectin modifications and softening during ripening of selected tropical
356 fruits. *Plant Science* 167, 317-327.
- 357 Araujo, W.L., Nunes-Nesi, A., Nikoloski, Z., Sweetlove, L.J., Fernie, A.R., 2012.
358 Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic
359 and heterotrophic plant tissues. *Plant, Cell & Environment* 35, 1-21.
- 360 Bagnato, N., Barrett, R., Sedgley, M., Klieber, A., 2003. The effects on the
361 quality of Cavendish bananas, which have been treated with ethylene, of
362 exposure to 1-methylcyclopropene. *International journal of food science &
363 technology* 38, 745-750.
- 364 Bico, S.L.S., Raposo, M.F.J., Morais, R.M.S.C., Morais, A.M.M.B., 2009.
365 Combined effects of chemical dip and/or carrageenan coating and/or controlled
366 atmosphere on quality of fresh-cut banana. *Food Control* 20, 508-514.
- 367 Bierhals, J.D., Lajolo, F.M., Cordenunsi, B.R., Oliveira do Nascimento, J.R.,
368 2004. Activity, cloning, and expression of an isoamylase-type starch-
369 debranching enzyme from banana fruit. *Journal of agricultural and food
370 chemistry* 52, 7412-7418.
- 371 Blankenship, S.M., Dole, J.M., 2003. 1-Methylcyclopropene: a review.
372 *Postharvest biology and technology* 28, 1-25.
- 373 Cruz, C.D., 2006. Programa Genes: estatística experimental e matrizes. UFV.
- 374 Dek, M.S.P., Padmanabhan, P., Subramanian, J., Paliyath, G., 2018. Inhibition
375 of tomato fruit ripening by 1-MCP, wortmannin and hexanal is associated with a
376 decrease in transcript levels of phospholipase D and other ripening related
377 genes. *Postharvest Biology and Technology* 140, 50-59.
- 378 do Nascimento, J.R.O., Júnior, A.V., Bassinello, P.Z., Cordenunsi, B.R.,
379 Mainardi, J.A., Purgatto, E., Lajolo, F.M., 2006. Beta-amylase expression and
380 starch degradation during banana ripening. *Postharvest Biology and
381 Technology* 40, 41-47.
- 382 Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.t., Smith, F., 1956.
383 Colorimetric method for determination of sugars and related substances.
384 *Analytical chemistry* 28, 350-356.
- 385 Dubois, M., Van den Broeck, L., Inzé, D., 2018. The pivotal role of ethylene in
386 plant growth. *Trends in plant science*.
- 387 Ghosh, A., Ganapathi, T., Bapat, V., 2016. Molecular Analysis of Fruit Ripening
388 in Banana, *Banana: Genomics and Transgenic Approaches for Genetic
389 Improvement*. Springer, pp. 93-105.
- 390 Golding, J.B., Shearer, D., Wyllie, S.G., McGlasson, W.B., 1998. Application of
391 1-MCP and propylene to identify ethylene-dependent ripening processes in
392 mature banana fruit. *Postharvest Biology and Technology* 14, 87-98.
- 393 Han, C., Zuo, J., Wang, Q., Xu, L., Wang, Z., Dong, H., Gao, L., 2015. Effects of
394 1-MCP on postharvest physiology and quality of bitter melon (*Momordica
395 charantia* L.). *Scientia horticulturae* 182, 86-91.
- 396 Harris, D., Seberry, J., Wills, R., Spohr, L., 2000. Effect of fruit maturity on
397 efficiency of 1-methylcyclopropene to delay the ripening of bananas.
398 *Postharvest Biology and Technology* 20, 303-308.
- 399 Jiang, Y., Joyce, D.C., Jiang, W., Lu, W., 2004. Effects of chilling temperatures
400 on ethylene binding by banana fruit. *Plant Growth Regulation* 43, 109-115.

401 Jiang, Y., Joyce, D.C., Macnish, A.J., 1999. Extension of the shelf life of banana
402 fruit by 1-methylcyclopropene in combination with polyethylene bags.
403 *Postharvest Biology and Technology* 16, 187-193.

404 Jiang, Y., Joyce, D.C., Terry, L.A., 2001. 1-Methylcyclopropene treatment
405 affects strawberry fruit decay. *Postharvest Biology and Technology* 23, 227-
406 232.

407 Jourda, C., Cardi, C., Gibert, O., Giraldo Toro, A., Ricci, J., Yahiaoui, N., 2016.
408 Lineage-specific evolutionary histories and regulation of major starch
409 metabolism genes during banana ripening. *Frontiers in plant science* 7, 1778.

410 Junior, A.V., Nascimento, J.R.O.d., Lajolo, F.M., 2006. Molecular cloning and
411 characterization of an α -amylase occurring in the pulp of ripening bananas and
412 its expression in *Pichia pastoris*. *Journal of agricultural and food chemistry* 54,
413 8222-8228.

414 Krebs, H.A., 1937. The citric acid cycle. *Science, Technology and Management*
415 5.

416 Lima, M.A.C.d., Alves, R.E., Filgueiras, H.A.C., 2010. Respiratory behavior and
417 softening of soursop fruit (*Annona muricata* L.) after postharvest treatments with
418 wax and 1-methylcyclopropene. *Ciencia e Agrotecnologia* 34, 155-162.

419 Madamba, L., 1993. Technical analysis I. Foods and feeds. Laguna,
420 Philippines: Institute of Chemistry, University of the Philippines Los Banos.

421 Martínez-Romero, D., Dupille, E., Guillén, F., Valverde, J.M., Serrano, M.,
422 Valero, D., 2003. 1-Methylcyclopropene increases storability and shelf life in
423 climacteric and nonclimacteric plums. *Journal of agricultural and food chemistry*
424 51, 4680-4686.

425 Mazorra, L.M., Oliveira, M.G., Souza, A.F., Silva, W.B.d., Santos, G.M.d., Silva,
426 L.R.A.d., Silva, M.G.d., Bartoli, C.G., Oliveira, J.G.d., 2013. Involvement of
427 brassinosteroids and ethylene in the control of mitochondrial electron transport
428 chain in postharvest papaya fruit. *Theoretical and Experimental Plant*
429 *Physiology* 25, 203-212.

430 McAtee, P., Karim, S., Schaffer, R.J., David, K., 2013. A dynamic interplay
431 between phytohormones is required for fruit development, maturation, and
432 ripening. *Frontiers in Plant Science* 4, 79.

433 McGuire, R.G., 1992. Reporting of objective color measurements. *HortScience*
434 27, 1254-1255.

435 Medina-Suárez, R., Manning, K., Fletcher, J., Aked, J., Bird, C.R., Seymour,
436 G.B., 1997. Gene expression in the pulp of ripening bananas (two-dimensional
437 sodium dodecyl sulfate-polyacrylamide gel electrophoresis of in vitro translation
438 products and cDNA cloning of 25 different ripening-related mRNAs). *Plant*
439 *Physiology* 115, 453-461.

440 Nakatsuka, A., Shiomi, S., Kubo, Y., Inaba, A., 1997. Expression and internal
441 feedback regulation of ACC synthase and ACC oxidase genes in ripening
442 tomato fruit. *Plant and Cell Physiology* 38, 1103-1110.

443 Nelson, N., 1944. A photometric adaptation of the Somogyi method for the
444 determination of glucose. *J. biol. Chem* 153, 375-380.

445 Osorio, S., Scossa, F., Fernie, A., 2013. Molecular regulation of fruit ripening.
446 *Frontiers in Plant Science* 4, 198.

447 Pech, J.-C., Bouzayen, M., Latché, A., 2008. Climacteric fruit ripening: ethylene-
448 dependent and independent regulation of ripening pathways in melon fruit. *Plant*
449 *Science* 175, 114-120.

450 Purgatto, E., do Nascimento, J.R.O., Lajolo, F.M., Cordenunsi, B.R., 2002. The
451 onset of starch degradation during banana ripening is concomitant to changes
452 in the content of free and conjugated forms of indole-3-acetic acid. *Journal of*
453 *Plant Physiology* 159, 1105-1111.

454 Qiuping, Z., Wenshui, X., 2007. Effect of 1-methylcyclopropene and/or chitosan
455 coating treatments on storage life and quality maintenance of Indian jujube fruit.
456 *LWT-Food Science and Technology* 40, 404-411.

457 Sakhale, B., Gaikwad, S., Chavan, R., 2018. Application of 1-
458 methylcyclopropene on mango fruit (Cv. Kesar): potential for shelf life
459 enhancement and retention of quality. *Journal of food science and technology*
460 55, 776-781.

461 Shiga, T.M., Soares, C.A., Nascimento, J.R., Purgatto, E., Lajolo, F.M.,
462 Cordenunsi, B.R., 2011. Ripening-associated changes in the amounts of starch
463 and non-starch polysaccharides and their contributions to fruit softening in three
464 banana cultivars. *Journal of the Science of Food and Agriculture* 91, 1511-1516.

465 Souza, M.S.d., Azevedo, I.G.d., Corrêa, S.F., Silva, M.G.d., Pereira, M.G.,
466 Oliveira, J.G.d., 2009. Responses of 1-MCP applications in 'Golden' papaya fruits
467 on different maturation stages. *Revista Brasileira de Fruticultura* 31, 693-700.

468 Thongkum, M., Imsabai, W., Burns, P., McAtee, P.A., Schaffer, R.J., Allan,
469 A.C., Ketsa, S., 2018. The effect of 1-methylcyclopropene (1-MCP) on
470 expression of ethylene receptor genes in durian pulp during ripening. *Plant*
471 *Physiology and Biochemistry* 125, 232-238.

472 Trindade, D.C.G.d., Lima, M.A.C.d., Assis, J.S.d., 2015. 1-methylcyclopropene
473 action on postharvest conservation of Palmer mango fruit at different maturity
474 stages. *Pesquisa Agropecuária Brasileira* 50, 753-762.

475 Vilas-Boas, E.V.d.B., Kader, A.A., 2006. Effect of atmospheric modification, 1-
476 MCP and chemicals on quality of fresh-cut banana. *Postharvest Biology and*
477 *Technology* 39, 155-162.

478 Xiao, Y.y., Kuang, J.f., Qi, X.n., Ye, Y.j., Wu, Z.X., Chen, J.y., Lu, W.j., 2018. A
479 comprehensive investigation of starch degradation process and identification of
480 a transcriptional activator MabHLH6 during banana fruit ripening. *Plant*
481 *Biotechnology Journal* 16, 151-164.

482 Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in
483 higher plants. *Annual review of plant physiology* 35, 155-189.

484 Yemm, E., Willis, A., 1954. The estimation of carbohydrates in plant extracts by
485 anthrone. *Biochemical journal* 57, 508.

486 Zewter, A., Woldetsadik, K., Workneh, T., 2012. Effect of 1-methylcyclopropene,
487 potassium permanganate and packaging on quality of banana. *African Journal*
488 *of Agricultural Research* 7, 2425-2437.

489 Zhu, X., Shen, L., Fu, D., Si, Z., Wu, B., Chen, W., Li, X., 2015. Effects of the
490 combination treatment of 1-MCP and ethylene on the ripening of harvested
491 banana fruit. *Postharvest Biology and Technology* 107, 23-32.

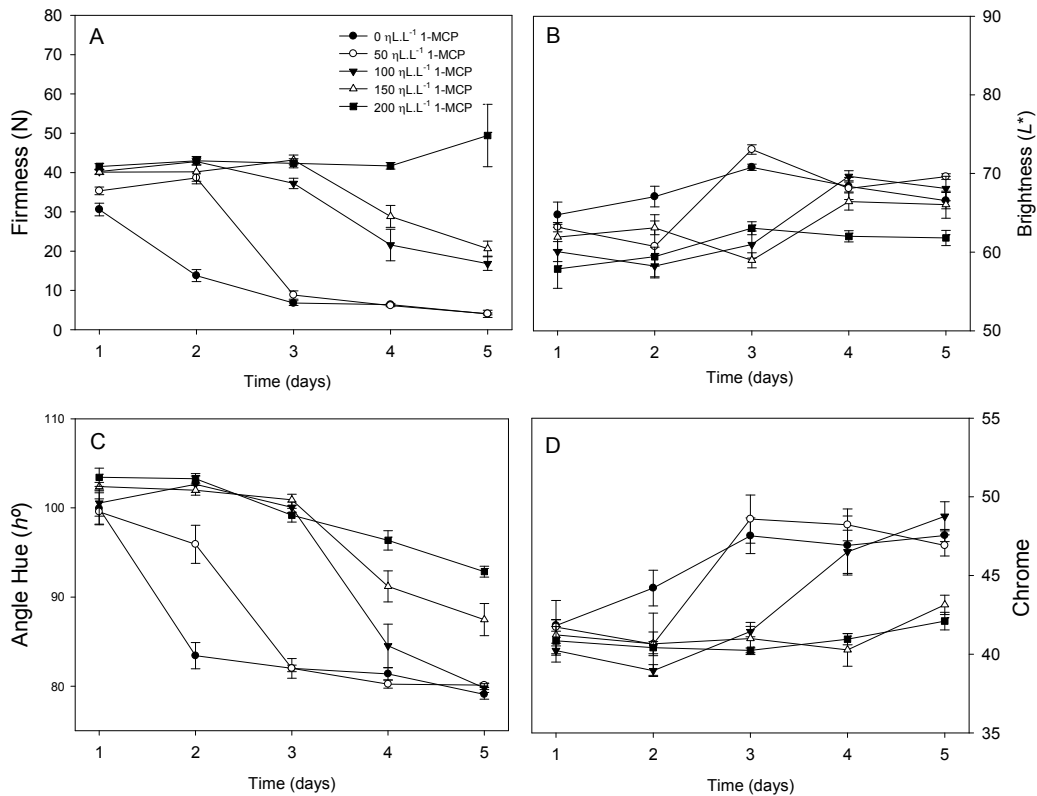
492 Ziliotto, F., Begheldo, M., Rasori, A., Bonghi, C., Tonutti, P., 2008.
493 Transcriptome profiling of ripening nectarine (*Prunus persica* L. Batsch) fruit
494 treated with 1-MCP. *Journal of experimental botany* 59, 2781-2791.

495 Zou, J., Chen, J., Tang, N., Gao, Y., Hong, M., Wei, W., Cao, H., Jian, W., Li,
496 N., Deng, W., 2018. Transcriptome analysis of aroma volatile metabolism
497 change in tomato (*Solanum lycopersicum*) fruit under different storage
498 temperatures and 1-MCP treatment. *Postharvest Biology and Technology* 135,
499 57-67.

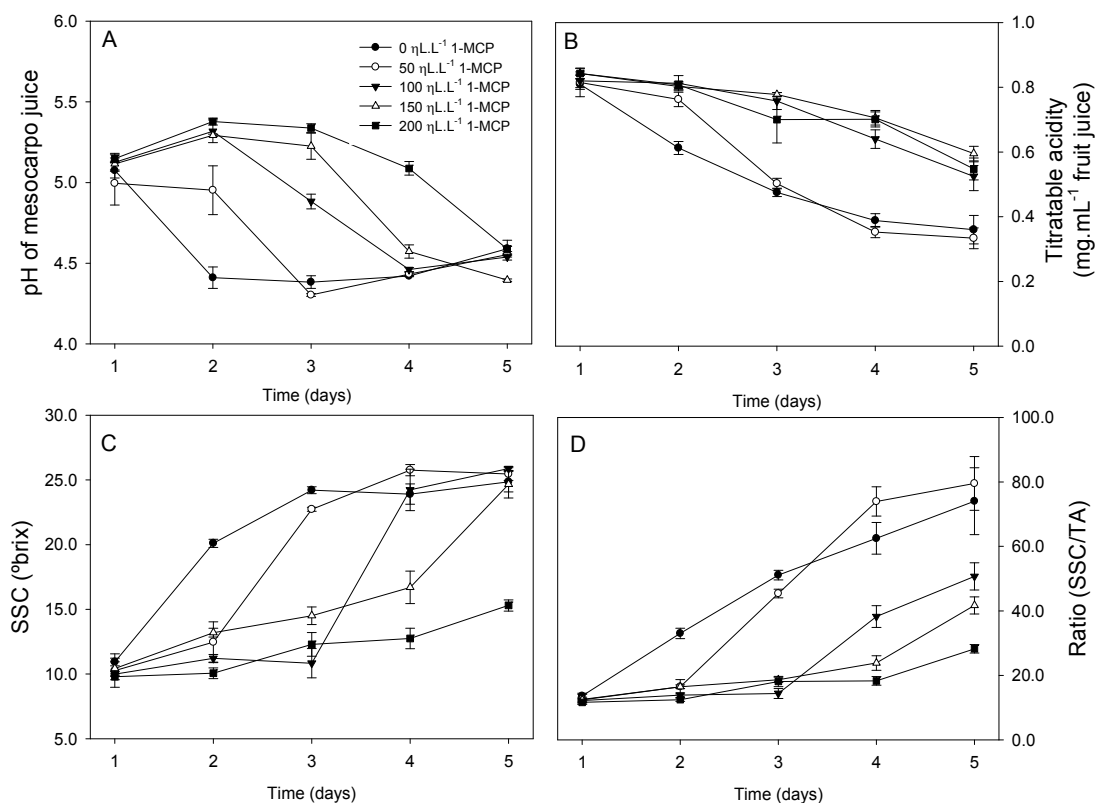
501 **Highlights**

- 502 • 1-MCP is an effective treatment to control sugar and starch metabolism
503 in banana.
- 504 • Skin color changes is an important predictive variable to identify changes
505 in the central metabolism in banana fruits.
- 506 • Ethylene action is an interplay of physical and chemical changes in
507 banana fruit.

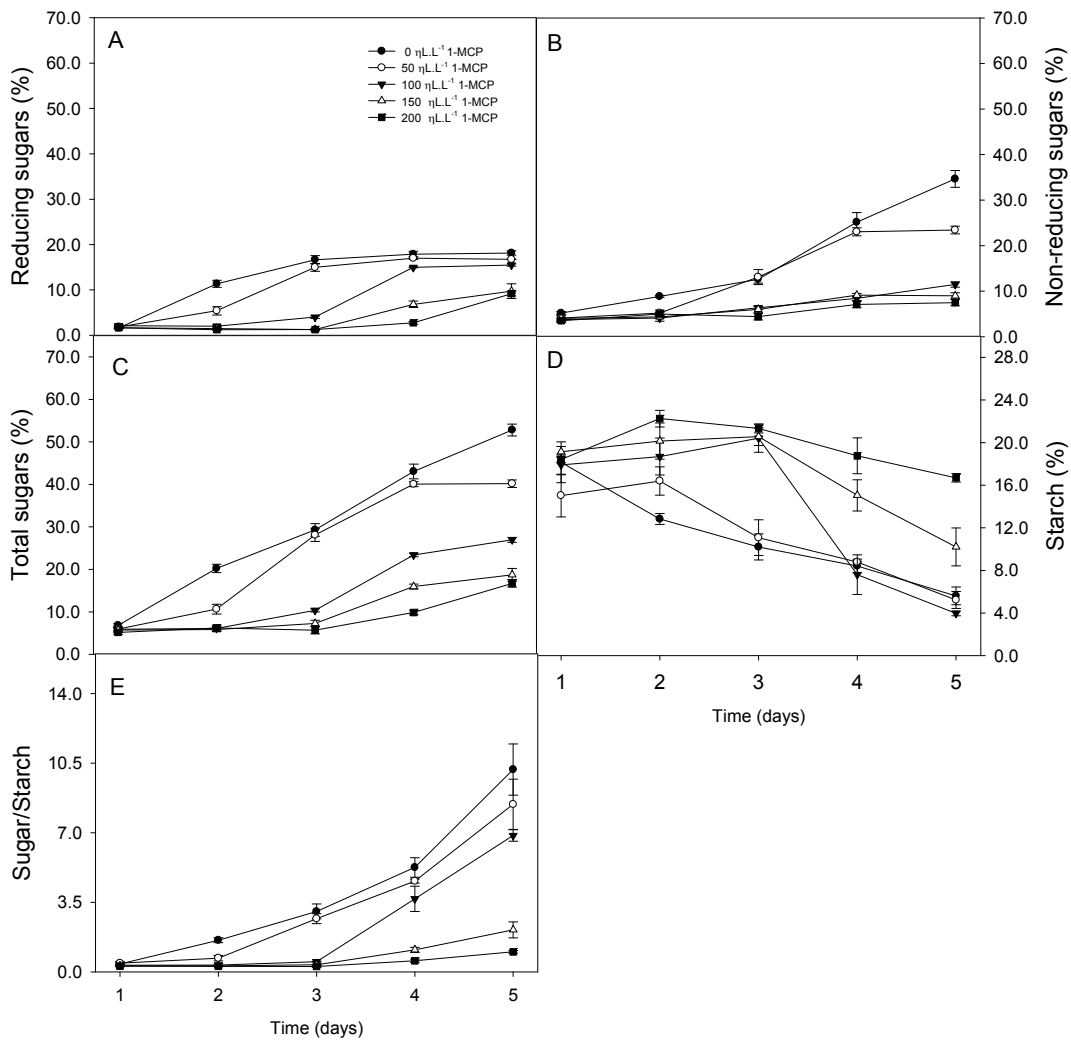
508 **Figures**



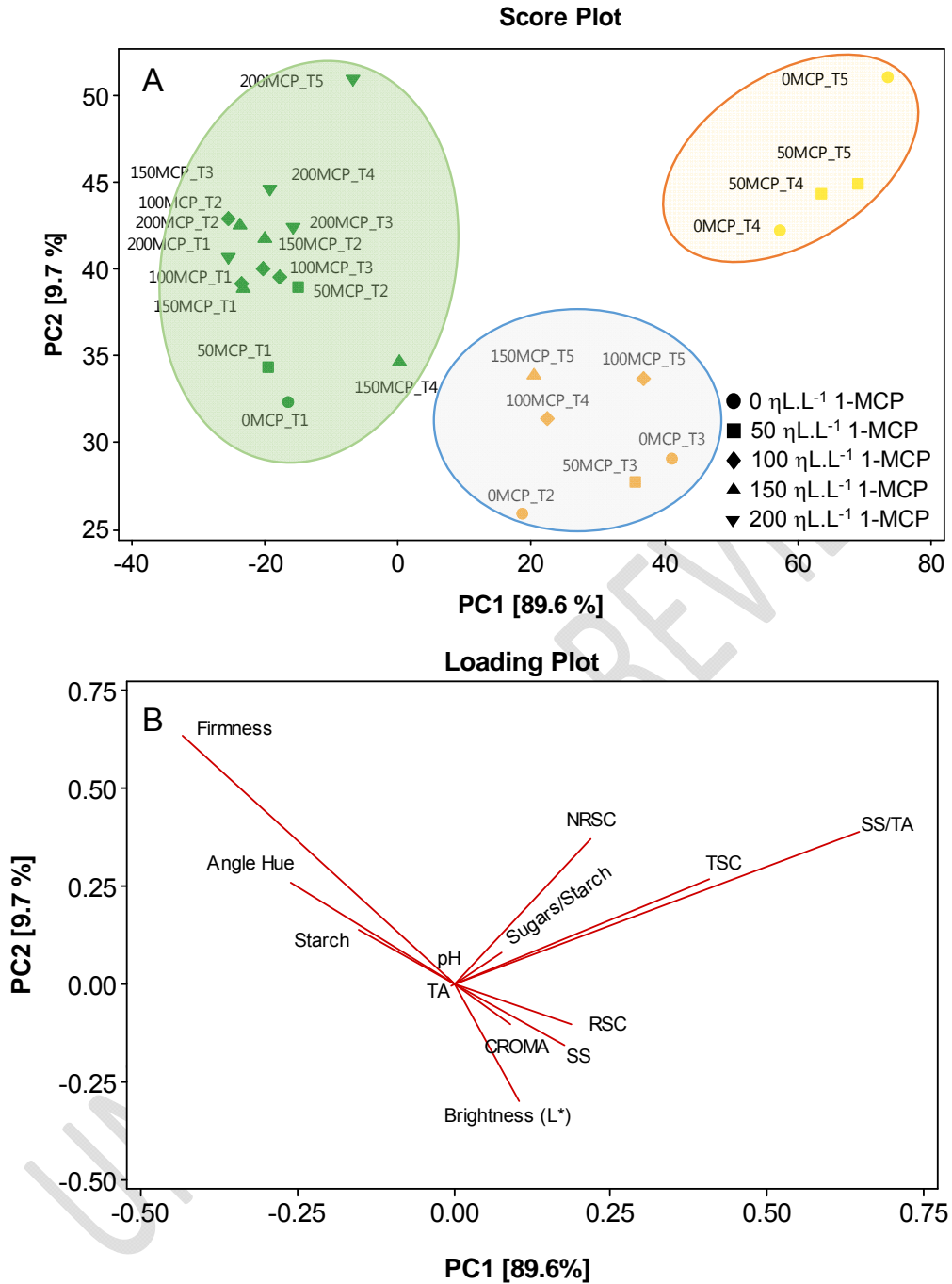
509 Fig. 1 – Physical quality attributes in banana cv. Prata Gorutuba (*Musa AAB* ‘Prata
510 Anã’) under different concentrations of 1-MCP during 5 times of storage in room
511 temperature. **A:** Firmness, **B:** Brightness, **C:** Angle hue and **D:** Chrome. Each value is
512 the mean for four replicates, and vertical bars indicate the standard errors (n = 5).
513
514



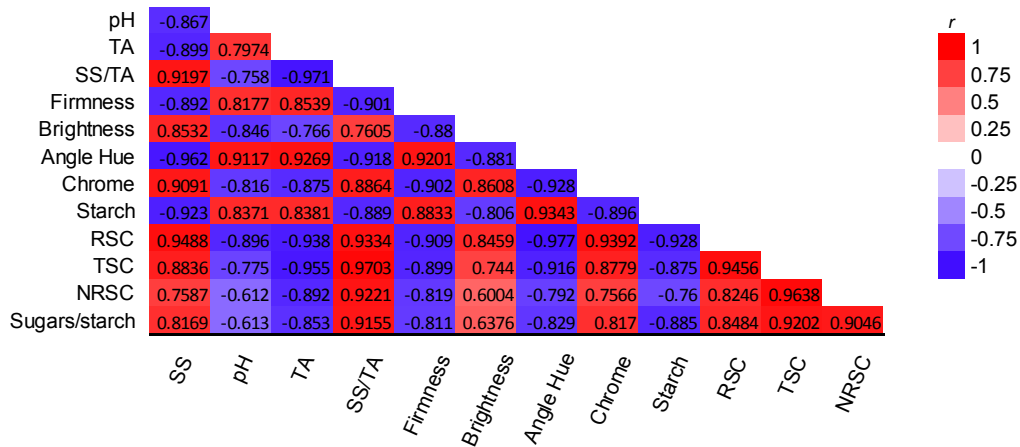
516
 517 Fig. 2 – Chemical traits of quality in banana cv. Prata Gorutuba (*Musa* AAB 'Prata
 518 Anã') under different concentrations of 1-MCP during 5 times of storage in room
 519 temperature. **A:** pH of mesocarp juice, **B:** Titratable acidity, **C:** SSC and **D:** SSC/TA.
 520 Each value is the mean for four replicates, and vertical bars indicate the standard
 521 errors (n = 5).
 522



523
 524 Fig.3 - Physiological traits of quality in banana cv. Prata Gorutuba (*Musa* AAB 'Prata
 525 Anã') under different concentrations of 1-MCP during 5 times of storage in room
 526 temperature. **A:** Reducing sugars content, **B:** Non-reducing sugars content, **C:** Total
 527 sugar content **D:** Starch content and **E:** Sugar/Starch ratio. Each value is the mean for
 528 four replicates, and vertical bars indicate the standard errors (n = 5).



529
 530 Fig.4 - Principal component analysis. (A) Score plot derived data of 1-MCP treatment
 531 under different days after treatment [0 days (T1), 1 day (T1), 2 days (T2), 3 days (T3),
 532 4 days (T4) and 5 days (T5). The large circles represent the three clusters formed by
 533 the Euclidean distance method. (B) In loading plot the direction and length of the lines
 534 are directly proportional to variables importance in separating groups. PC1, principal
 535 component 1; PC2, principal component 2. Abbreviations: Hydrogenionic potential (pH),
 536 NRSC (Non-reducing sugar content), Total sugar content (TSC), Reducing sugar
 537 content (RSC), Soluble solids content/Titratable acidity ratio (SS/TA), Soluble solids
 538 content (SS), Titratable acidity (TA).



539
 540 Fig. 5. Correlation matrix based on Pearson coefficients derived from physical,
 541 chemical and physiological traits data from banana cv. Prata Gorutuba (*Musa*
 542 AAB 'Prata Anã') under different concentrations of 1-MCP during 5 times of
 543 storage in room temperature. Correlation coefficients are presented in colors,
 544 and the significant ones are indicated in bold (P) based on p-value corrected by
 545 FDR correction (Bonferroni-Hochberg). Abbreviations: Hydrogenionic potential
 546 (pH), NRSC (Non-reducing sugar content), Total sugar content (TSC),
 547 Reducing sugar content (RSC), Soluble solids content/Titratable acidity ratio
 548 (SS/TA), Soluble solids content (SS), Titratable acidity (TA).

549
 550

551
552
553

Supplemental Figure

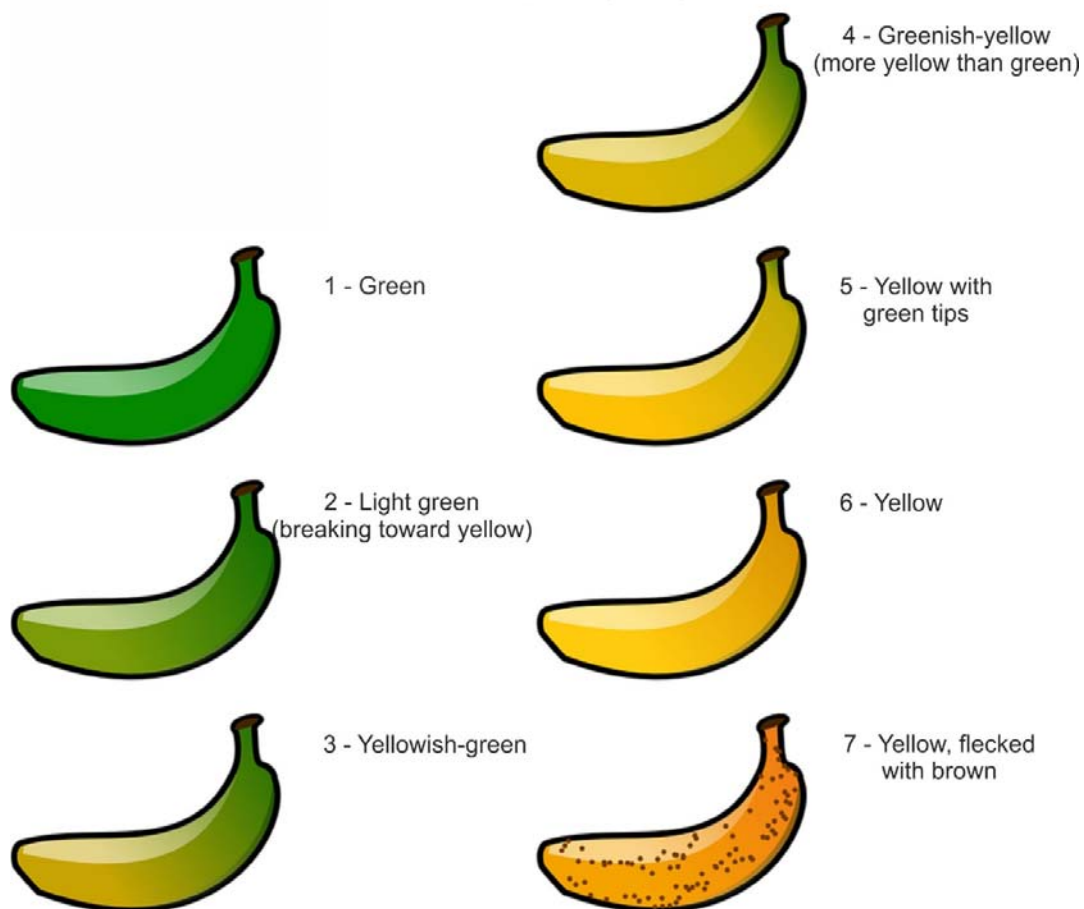


554
555
556
557

Supplemental Fig.S 1 – Representative figure of banana ripening under different 1-MCP concentration in 4 replicates after 25 days stored at 14 °C.

UNDER PEER REVIEW

Von Loesecke's classification of ripening stages of bananas



558

559 Supplemental Fig. S2. Color index scheme number of banana ripening.
560 Banana's ripening pattern during the final development of fruits. Scale marking
561 of color changes of the skin color 1 (green), 2 (light green), 3 (yellowish-green),
562 4 (greenish-yellow), 5 (yellow with green tips), 6 (yellow) and finally 7 (yellow,
563 flecked with brown). Adapted from CQH/CEAGESP. 2003. São Paulo
564 (CEAGESP, 2009. Doc.33).

Supplemental table S1. Effect of 1-MCP on banana fruits stored at 14.5 °C on physical quality parameters during 5 days at shelf life. Each value is the mean for four replicates \pm standard error (n = 5).

Treatment η L.L ⁻¹ of 1-MCP	Time after treatment				
	1	2	3	4	5
Firmness (N)					
0	30.59 \pm 1.61 bA	13.78 \pm 1.52 bB	6.78 \pm 0.57 bC	6.41 \pm 0.30 cC	4.03 \pm 0.23 cD
50	35.33 \pm 0.99 abA	38.65 \pm 1.54 aA	8.84 \pm 1.06 bB	6.16 \pm 0.31 cB	4.06 \pm 0.90 cC
100	40.26 \pm 0.62 abA	42.81 \pm 0.84 aA	37.23 \pm 0.32 aA	21.58 \pm 4.01 bB	16.83 \pm 1.72 bB
150	40.10 \pm 0.60 abA	40.19 \pm 2.43 aA	43.22 \pm 1.23 aA	28.84 \pm 1.79 bB	20.63 \pm 1.91 bB
200	41.54 \pm 0.71 aA	43.00 \pm 1.09 aA	42.37 \pm 1.19 aA	41.71 \pm 0.82 aA	49.42 \pm 7.94 aA
Brightness (L*)					
0	64.75 \pm 1.61 aA	67.07 \pm 1.31 aA	70.80 \pm 0.40 aA	68.36 \pm 0.72 abA	66.56 \pm 1.04 abA
50	63.18 \pm 0.59 abBC	60.74 \pm 4.02 abC	73.05 \pm 0.60 aA	68.10 \pm 1.41 abAB	69.61 \pm 0.36 aAB
100	60.07 \pm 1.27 abB	58.24 \pm 1.37 bB	60.97 \pm 1.72 bB	69.64 \pm 0.73 aA	68.09 \pm 1.55 abA
150	61.91 \pm 1.62 abAB	63.09 \pm 0.88 abAB	58.96 \pm 0.96 bB	66.43 \pm 1.09 abA	66.04 \pm 1.72 abA
200	57.86 \pm 2.46 bA	59.42 \pm 1.22 bA	63.04 \pm 0.83 bA	62.01 \pm 0.72 bA	61.81 \pm 0.96 bA
Angle hue (h)					
0	94.56 \pm 1.67 aA	83.42 \pm 1.47 cB	81.99 \pm 1.10 bB	81.38 \pm 0.67 bB	79.08 \pm 0.54 cC
50	99.57 \pm 1.46 aA	95.92 \pm 2.13 bA	82.00 \pm 0.37 bB	80.23 \pm 0.45 bC	80.11 \pm 0.24 cC
100	100.55 \pm 1.47 aA	102.65 \pm 0.92 aA	100.07 \pm 0.58 aA	84.53 \pm 2.43 bB	79.79 \pm 0.49 cC
150	102.39 \pm 0.47 aA	101.98 \pm 0.53 aA	100.90 \pm 0.63 aA	91.20 \pm 1.74 aB	87.46 \pm 1.81 bB
200	103.43 \pm 1.02 aA	103.28 \pm 0.57 aA	99.19 \pm 0.77 aAB	96.36 \pm 1.09 aBC	92.86 \pm 0.61 aC
Chrome (C)					
0	41.83 \pm 0.36 aB	44.20 \pm 1.13 aAB	47.52 \pm 1.13 aA	46.91 \pm 1.88 aA	47.54 \pm 0.38 abA
50	41.72 \pm 1.69 aB	40.63 \pm 1.97 abB	48.59 \pm 1.53 aA	48.22 \pm 1.01 aA	46.91 \pm 0.67 abA
100	40.23 \pm 0.29 aB	38.96 \pm 0.37 bB	41.44 \pm 0.33 bB	46.51 \pm 1.37 aA	48.76 \pm 0.92 aA
150	39.23 \pm 0.53 aA	40.66 \pm 0.75 abA	41.00 \pm 1.02 bA	40.27 \pm 1.04 bA	43.14 \pm 0.61 bcA
200	40.85 \pm 1.35 aA	40.41 \pm 0.38 abA	40.24 \pm 0.15 bA	40.95 \pm 0.35 bA	42.10 \pm 0.56 cA

*Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly by the Tukey test ($P < 0.05$).

Supplemental table S2. Effect of 1-MCP on banana fruits stored at 14.5 °C on chemical quality parameters during 5 days at shelf life. Each value is the mean for four replicates \pm standard error (n = 5).

Treatment ηL.L-1 of 1-MCP	Time after treatment				
	1	2	3	4	5
pH					
0	5.08 \pm 0.04 aA	4.47 \pm 0.02 cB	4.38 \pm 0.04 cB	4.42 \pm 0.01 bB	4.59 \pm 0.05 aB
50	4.99 \pm 0.13 aA	4.95 \pm 0.15 bA	4.30 \pm 0.01 cB	4.43 \pm 0.01 bB	4.55 \pm 0.00 aB
100	5.13 \pm 0.05 aAB	5.32 \pm 0.07 aA	4.88 \pm 0.05 bB	4.46 \pm 0.01 bC	4.54 \pm 0.02 aC
150	5.11 \pm 0.03 aA	5.29 \pm 0.01 aA	5.23 \pm 0.08 bB	4.57 \pm 0.04 bB	4.40 \pm 0.01 aB
200	5.14 \pm 0.02 aAB	5.38 \pm 0.02 aA	5.34 \pm 0.02 aAB	5.09 \pm 0.04 aB	4.59 \pm 0.02 aC
AT					
0	0.81 \pm 0.04 aA	0.61 \pm 0.02 bB	0.48 \pm 0.01 bC	0.39 \pm 0.02 bC	0.36 \pm 0.05 bC
50	0.82 \pm 0.02 aA	0.76 \pm 0.02 bA	0.50 \pm 0.02 bB	0.35 \pm 0.02 bC	0.33 \pm 0.03 bC
100	0.82 \pm 0.02 aA	0.81 \pm 0.02 aA	0.76 \pm 0.03 aAB	0.64 \pm 0.03 aBC	0.53 \pm 0.04 aC
150	0.84 \pm 0.02 aA	0.80 \pm 0.01 aAB	0.78 \pm 0.01 aAB	0.71 \pm 0.02 aBC	0.60 \pm 0.02 aC
200	0.84 \pm 0.02 aA	0.81 \pm 0.01 aAB	0.70 \pm 0.07 aB	0.70 \pm 0.02 aB	0.55 \pm 0.03 aC
SSC					
0	10.93 \pm 0.30 aC	20.10 \pm 0.32 aB	24.20 \pm 0.26 aA	23.90 \pm 0.79 aA	24.85 \pm 0.79 aA
50	10.27 \pm 1.29 aB	12.46 \pm 1.58 bB	22.73 \pm 0.15 aA	25.75 \pm 0.43 aA	25.45 \pm 0.48 aA
100	10.00 \pm 0.19 aB	11.22 \pm 0.29 bB	10.84 \pm 1.13 cB	24.23 \pm 1.59 aA	25.88 \pm 0.15 aA
150	10.43 \pm 0.51 aC	13.20 \pm 0.34 bBC	14.51 \pm 0.68 bB	16.70 \pm 1.26 bB	24.65 \pm 1.04 aA
200	9.79 \pm 0.23 aC	10.06 \pm 0.41 bBC	12.29 \pm 0.91 bcB	12.75 \pm 0.79 cB	15.30 \pm 0.43 bA
SSC/AT					
0	13.66 \pm 0.85 aD	33.03 \pm 1.63 aC	51.10 \pm 1.49 aB	62.50 \pm 4.92 aAB	74.02 \pm 10.36 aA
50	12.58 \pm 1.53 aC	16.46 \pm 2.25 bC	45.39 \pm 1.32 aB	73.94 \pm 4.53 aA	79.51 \pm 8.32 aA
100	12.22 \pm 0.37 aB	13.89 \pm 0.72 bB	14.40 \pm 1.55 bB	38.26 \pm 3.39 bA	50.70 \pm 4.23 bA
150	12.42 \pm 0.79 aB	16.47 \pm 0.57 bB	18.64 \pm 0.79 bB	23.83 \pm 2.25 bcB	41.72 \pm 2.66 bcA
200	11.66 \pm 0.49 aB	12.46 \pm 0.48 bB	18.11 \pm 1.61 bAB	18.29 \pm 1.36 cAB	28.24 \pm 1.32 cA

*Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly by the Tukey test ($P < 0.05$).

Supplemental table S3. Effect of 1-MCP on banana fruits stored at 14.5 °C on physiological quality parameters during 5 days at shelf life. Each value is the mean for four replicates \pm standard error (n = 5).

Treatment	Time after treatment				
η L.L-1 of 1-MCP	1	2	3	4	5
RSC					
0	1.64 \pm 0.203 aC	11.36 \pm 0.779 aB	16.66 \pm 0.904 aA	17.89 \pm 0.617 aA	18.15 \pm 0.473 aA
50	1.84 \pm 0.325 aC	5.42 \pm 0.921 bB	14.97 \pm 0.871 aA	17.00 \pm 0.271 abA	16.74 \pm 0.054 abA
100	2.12 \pm 0.266 aB	2.01 \pm 0.203 cB	4.01 \pm 0.284 bB	14.98 \pm 0.093 bA	15.49 \pm 0.262 bA
150	1.67 \pm 0.053 aC	1.51 \pm 0.090 cC	1.26 \pm 0.013 cC	6.83 \pm 0.704 cB	9.73 \pm 1.633 cB
200	1.66 \pm 0.186 aB	1.24 \pm 0.104 cB	1.27 \pm 0.197 cB	2.75 \pm 0.269 dB	9.20 \pm 0.531 cA
NRSC					
0	5.18 \pm 0.368 aD	8.85 \pm 0.230 aCD	12.62 \pm 0.869 aC	25.15 \pm 2.087 aB	34.65 \pm 1.857 aA
50	4.10 \pm 0.863 aC	5.20 \pm 0.649 abC	13.10 \pm 1.642 aB	23.04 \pm 0.866 aA	23.42 \pm 0.858 bA
100	3.77 \pm 0.301 aC	4.09 \pm 0.680 bC	6.35 \pm 0.359 bBC	8.42 \pm 0.461 bAB	11.47 \pm 0.569 cA
150	4.05 \pm 0.556 aB	4.34 \pm 0.334 bB	6.01 \pm 0.753 bAB	9.11 \pm 0.391 bA	8.97 \pm 0.680 cdA
200	3.50 \pm 0.327 aB	4.99 \pm 0.279 abAB	4.41 \pm 0.737 bAB	7.09 \pm 0.750 bAB	7.50 \pm 0.693 dA
TS					
0	6.82 \pm 0.408 aE	20.21 \pm 0.996 aD	29.28 \pm 1.492 aC	43.04 \pm 1.749 aB	52.80 \pm 1.389 aA
50	5.94 \pm 0.556 aD	10.62 \pm 1.147 bC	28.07 \pm 1.468 aB	40.04 \pm 0.596 aA	40.16 \pm 0.884 bA
100	5.89 \pm 0.191 aC	6.10 \pm 0.777 cC	10.36 \pm 0.360 bB	23.40 \pm 0.434 bA	26.96 \pm 0.583 cA
150	5.72 \pm 0.575 aB	5.84 \pm 0.382 cB	7.27 \pm 0.751 bcB	15.94 \pm 0.597 cA	18.69 \pm 1.516 dA
200	5.16 \pm 0.399 aC	6.23 \pm 0.247 cBC	5.68 \pm 0.884 cC	9.84 \pm 0.551 dB	16.70 \pm 0.912 dA
Starch					
0	18.15 \pm 1.919 aA	12.83 \pm 0.516 cAB	10.21 \pm 1.231 bBC	8.42 \pm 0.638 bBC	5.60 \pm 0.838 bcC
50	15.03 \pm 2.000 aA	16.39 \pm 1.326 bcA	11.08 \pm 1.677 bAB	8.78 \pm 0.081 bBC	5.23 \pm 0.799 bcC
100	17.92 \pm 0.949 aA	18.69 \pm 1.734 abA	20.41 \pm 1.321 aA	7.60 \pm 1.867 bB	3.97 \pm 0.229 cB
150	19.14 \pm 0.496 aA	20.15 \pm 1.700 abA	20.57 \pm 0.831 aA	15.05 \pm 1.469 aAB	10.21 \pm 1.782 bB
200	18.42 \pm 0.310 aA	22.26 \pm 0.777 aA	21.33 \pm 0.414 aA	18.76 \pm 1.687 aA	16.70 \pm 0.401 aA
Sugar/Starch					
0	0.40 \pm 0.053 aD	1.59 \pm 0.125 aCD	3.04 \pm 0.37 aC	5.25 \pm 0.49 aB	10.18 \pm 1.29 aA
50	0.43 \pm 0.088 aC	0.69 \pm 0.137 aC	2.67 \pm 0.24 aB	4.56 \pm 0.10 aB	8.43 \pm 1.26 abA
100	0.33 \pm 0.011 aC	0.34 \pm 0.058 bC	0.51 \pm 0.02 bC	3.67 \pm 0.64 aB	6.86 \pm 0.28 bA
150	0.30 \pm 0.032 aA	0.30 \pm 0.031 bA	0.35 \pm 0.02 bcA	1.11 \pm 0.12 bA	2.11 \pm 0.40 cA
200	0.28 \pm 0.025 aA	0.28 \pm 0.011 bA	0.27 \pm 0.04 cA	0.55 \pm 0.08 bA	1.01 \pm 0.07 cA

*Means followed by the same lower case letters in a column and capital letters on the lines do not differ significantly by the Tukey test ($P < 0.05$).