

The role of ethylene on banana fruit ripening via sugar and starch metabolism

Abstract

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

Keywords: Postharvest, banana quality, 1-MCP, sugar metabolism, starch

Introduction

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

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

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

56 The use of ethylene inhibitors in banana can delay the maturation
57 process by increasing the postharvest life of the fruit. In the same vein, the
58 volatile ethylene nontoxic antagonists such as 1-methylcyclopropene (1-MCP)
59 has provided a useful tool for elucidating the role of ethylene in ripening
60 climacteric fruit (Dek *et al.*, 2018; Golding *et al.*, 1998; Mazorra *et al.*, 2013;
61 Nakatsuka *et al.*, 1997; Thongkum *et al.*, 2018). Recently, 1-MCP has been
62 extensively used on climacteric and non-climacteric fruits for delaying fruit
63 ripening and control fruit quality in tomato (Zou *et al.*, 2018), melon (Han *et al.*,
64 2015), plum (Martínez-Romero *et al.*, 2003), mango (Sakhale *et al.*, 2018;
65 Trindade *et al.*, 2015), strawberry (Jiang *et al.*, 2001), papaya (Mazorra *et al.*,
66 2013; Souza *et al.*, 2009), and bananas (Golding *et al.*, 1998; Jiang *et al.*,
67 1999). Although various studies have focused on the 1-MCP effect on the

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

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

80

81 **Material and Methods**

82

83 *Fruit material*

84

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

91

92 *Solution preparation and fruit treatment*

93

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

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

104

105 *Physical quality attributes*

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

113

114 *Determination of chemical quality parameters*

115

116 Around ten gram (10 g) of banana from each replicate were crushed and
117 homogenized with 100 ml boiled distilled water (previously adjusted to pH 8.3).
118 The pH was measured by using pH meter Crison MicropH 2001 (Crison
119 Instruments SA, Barcelona, Spain). The mixture was titrated with 0.10 M NaOH
120 to pH 8.3 and the result was expressed as mg malic acid per 100 g sample. The
121 total soluble sugar content (SSC) were determined according to Madamba
122 (1993) as previously described by (Bico *et al.*, 2009).

123

124 *Physiological parameters*

125

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

131

132 *Experimental design and statistical analyzes*

133

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

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

141

142 **Results**

143

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

145

146 As shown in Fig.S1, the 1-MCP could delay the ripening of banana fruit
147 and in complement, the physical quality parameters follows the same visual
148 response (Fig.1). Among the treatment, 50 $\mu\text{L.L}^{-1}$ 1-MCP treatment had the
149 similar effect with a normal ripening, which after 3 days there was not more
150 significantly difference in comparison with 0 $\mu\text{L.L}^{-1}$ 1-MCP (See in Fig.1 and
151 Supplemental table S.1). However, in higher concentrations the firmness in the
152 control fruit dropped sharply from of 30.59N on the 1st day to 4.03N on the 5th
153 day after remove from the refrigeration (Fig. 1A). Interestingly, the L value the 1-
154 MCP treatment has not shown drastically changes during fruit ripening, except
155 in 3th day where, the L value was reduced in 100, 150 and 200 $\mu\text{L.L}^{-1}$ 1-MCP
156 (60.97, 58.96 and 63.04 respectively, see in Fig.1B and supplemental table S1).
157 The change of the peel color was expressed in hue angle (h°) values. As is
158 shown in Fig. 1C, the peel color of banana fruit in all treatments turned more
159 yellow through the period of storage (Supplemental Fig.S1 and S2). Banana
160 treated with 1-MCP showed a significantly higher level of hue angle values
161 compared to that of other treatments. This effect was considered dose
162 response, the higher the concentration of 1-MCP, the greater the delay in the
163 peel color changes (Fig.1C and Supplemental Fig. S1). At the same time, the
164 Chroma (Fig.1D), a similar results from the previous observed with hue angle,
165 showing a higher color intensity (Chroma), mainly after 5th day of storage at
166 25°C with reduced values to 1-MCP treatments (Supplemental table S1).

167

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

169

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

189

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

191

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

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

220

221 *Multivariate analysis*

222

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

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

254
255

256 Discussion

257
258

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

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

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

296 The influences of 1-MCP treatment in respiration rate and ethylene
297 production is already well documented by Jiang et al. (1999) and (Harris et al.,
298 2000). Therefore, the reduction in respiration rate is possibly the result of
299 reduction in several essential respiratory steps such as, glycolysis and TCA, as
300 well as phosphorylating chain (OXPHOS), decreasing the ATP production and
301 starting the anaerobic pathway, resulting in lower SSC due to the slower
302 hydrolysis of carbohydrates (Starch to sugars) (Araujo et al., 2012; Krebs,
303 1937). Our results show that 1-MCP and low density polyethylene packaging

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

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

337 according with Zewter et al. (2012), bananas treated with 1-MCP kept in a
338 package of non-perforated polyethylene, showed an increase in total sugars,
339 provided by conversion of starch to sugar, as with the advancement of ripening
340 a decline occurs after reaching a certain peak when the fruits enter the
341 senescence phase. In summary, the treatment 100, 150 and 200 $\eta\text{L.L}^{-1}$ de 1-
342 MCP in bananas 'Prata Gorutuba' for 8 hours delay the ripening in
343 approximately 25 days, when packed in PEDB and stored under refrigeration
344 (14.5°C), without changes in their physical and chemical characteristics. In
345 complement, our results suggest 1-MCP treatment effectively prolongs the
346 quality attributes not compromising the normal ripening after removal of
347 package and kept in room temperature (25°C), accumulating sugars by starch
348 degradation. Furthermore, the correlation pattern of physical and chemical
349 attributes demonstrates thus the dependence of ethylene action and of the
350 interplay between these events despite mechanism to be distinct and act in
351 different area in the fruit.

352

353 **Conclusion**

354

355 As conclusion, our results suggest that, 1-MCP is an effective treatment to
356 control sugar and starch metabolism in banana and its efficiency is directly
357 dependent of storage temperature. In addition, we identified a straight
358 correlation with skin color changes and carbohydrates content, which can
359 indicate its potentiality of the fitted equations for prediction of central
360 metabolism of bananas non-destructively using 'hue angle and chrome' value

361

362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408

REFERENCES

Ali ZM, Chin, LH, Lazan H. A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Sci.* 2004; 167: 317-327.

Araujo, WL, Nunes-Nesi A, Nikoloski Z, Sweetlove LJ, Fernie, AR. Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues. *Plant Cell Environ.* 2012; 35: 1-21.

Bagnato N, Barrett R, Sedgley M, Klieber A. The effects on the quality of Cavendish bananas, which have been treated with ethylene, of exposure to 1-methylcyclopropene. *International j food sci tech.* 2003; 38: 745-750.

Bico SLS, Raposo MFJ, Morais RMSC, Morais AMMB. Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana. *Food Con.* 2009; 20: 508-514.

Bierhals JD, Lajolo FM, Cordenunsi BR, Oliveira do Nascimento JR. Activity, cloning, and expression of an isoamylase-type starch-debranching enzyme from banana fruit. *J Agric Food Chem.* 2004; 52: 7412-7418.

Blankenship SM, Dole, JM. 1-Methylcyclopropene: a review. *Postharvest Biol Technol.* 2003; 28: 1-25.

Cruz CD. Programa Genes: estatística experimental e matrizes. UFV. 2006.

Dek MSP, Padmanabhan P, Subramanian J, Paliyath G. Inhibition of tomato fruit ripening by 1-MCP, wortmannin and hexanal is associated with a decrease in transcript levels of phospholipase D and other ripening related genes. *Postharvest Biol Tech.* 2018; 140: 50-59.

do Nascimento JRO, Júnior AV, Bassinello PZ, Cordenunsi BR, Mainardi JA, Purgatto E and Lajolo FM. Beta-amylase expression and starch degradation during banana ripening. *Postharvest Biol Tech.* 2006; 40: 41-47.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. *Analytical chem.* 1956; 28; 350-356.

Dubois M, Van den Broeck L, Inzé D. The pivotal role of ethylene in plant growth. *Trends Plant Sci.* 2018; 23(4):311-323

Ghosh, A., Ganapathi, T., Bapat, V., 2016. Molecular Analysis of Fruit Ripening in Banana, *Banana: Genomics and Transgenic Approaches for Genetic Improvement.* 2016: 93-105.

409 Golding, J.B., Shearer, D., Wyllie, S.G., McGlasson, W.B. Application of 1-MCP
410 and propylene to identify ethylene-dependent ripening processes in mature
411 banana fruit. *Postharvest Biol Tech.* 1998;(1);14: 87-98.
412
413 Han C, Zuo J, Wang Q, Xu L, Wang Z, Dong H, Gao L. Effects of 1-MCP on
414 postharvest physiology and quality of bitter melon (*Momordica charantia* L.). *Sci*
415 *hort.* 2015;(182): 86-91.
416
417 Harris D, Seberry J, Wills R, Spohr L. Effect of fruit maturity on efficiency of 1-
418 methylcyclopropene to delay the ripening of bananas. *Postharvest Biol Tech.*
419 2000; 20(3): 303-308.
420
421 Jiang Y, Joyce DC, Jiang W, Lu W. Effects of chilling temperatures on ethylene
422 binding by banana fruit. *Plant Growth Regul.* 2004 43;(2): 109-115.
423
424 Jiang Y, Joyce, D.C., Macnish, A.J., 1999. Extension of the shelf life of banana
425 fruit by 1-methylcyclopropene in combination with polyethylene bags.
426 *Postharvest Biol Tech.* 1999; 16; (2): 187-193.
427
428 Jiang Y, Joyce DC, Terry LA. 1-Methylcyclopropene treatment affects
429 strawberry fruit decay. *Postharvest Biol Tech.* 2001; 23;(3): 227-232.
430
431 Jourda C, Cardi C, Gibert O, Giraldo TA, Ricci J, Yahiaoui N. Lineage-specific
432 evolutionary histories and regulation of major starch metabolism genes during
433 banana ripening. *Front Plant Sci.* 2016; 7:1-21.
434
435 Junior, A.V., Nascimento, J.R.O.d., Lajolo, F.M., 2006. Molecular cloning and
436 characterization of an α -amylase occurring in the pulp of ripening bananas and
437 its expression in *Pichia pastoris*. *J agri food chem.* 2006; 54;(21): 8222-8228.
438
439 Krebs, HA. The intermediate metabolism of carbohydrates.
440 *Lancet.* 1937;233:736-738
441
442 Lima MACd, Alves RE, Filgueiras HAC. Respiratory behavior and softening of
443 soursop fruit (*Annona muricata* L.) after postharvest treatments with wax and 1-
444 methylcyclopropene. *Ciencia Agrotec.* 2010; 34;(1): 155-162.
445
446 Madamba L. Technical analysis I. Foods and feeds. Laguna, Philippines:
447 Institute of Chemistry, University of the Philippines Los Banos. 1993.
448
449 Martínez-Romero D, Dupille E, Guillén F, Valverde JM, Serrano M, Valero D. 1-
450 Methylcyclopropene increases storability and shelf life in climacteric and
451 nonclimacteric plums. *J agric food chem.* 2003; 51;(13): 4680-4686.
452
453 Mazorra LM, Oliveira MG, Souza AF, Silva WB, Santos GM, Silva LRA, Silva,
454 MG, Bartoli CG, Oliveira JG. 2013. *Int Theoretical and Experimental Plant*
455 *Physiology* 25; (3): 203-212.
456

457 McAtee P, Karim S, Schaffer RJ, David K. A dynamic interplay between
458 phytohormones is required for fruit development, maturation, and ripening.
459 *Front Plant Sci.* 2013; 4:1-7.

460

461 McGuire R.G. Reporting of objective color measurements. *Hort. Science* . 1992;
462 27: 1254-1255.

463

464 Medina-Suárez R, Manning K, Fletcher J, Aked J, Bird CR, Seymour GB. Gene
465 expression in the pulp of ripening bananas. two-dimensional sodium dodecyl
466 sulfate-polyacrylamide gel electrophoresis of in vitro translation products and
467 cDNA cloning of 25 different ripening-related mRNAs. *Plant Physiol.* 1997;
468 115;(2): 453-461

469

470 Nakatsuka A, Shiomi S, Kubo Y, Inaba A. Expression and internal feedback
471 regulation of ACC synthase and ACC oxidase genes in ripening tomato fruit.
472 *Plant Cell Physiol.* 1997; 38; (10): 1103-1110.

473

474 Nelson N. A photometric adaptation of the Somogyi method for the
475 determination of glucose. *J. biol. Chem.* 1994; 153: 375-380.

476

477 Osorio S, Scossa F, Fernie A. Molecular regulation of fruit ripening. *Front Plant*
478 *Sci.* 2013; 4:1-8.

479

480 Pech JC, Bouzayen M, Latché A. Climacteric fruit ripening: ethylene-dependent
481 and independent regulation of ripening pathways in melon fruit. *Plant Sci.* 2008;
482 175: 114-120.

483

484 Purgatto E, do Nascimento JRO, Lajolo FM, Cordenunsi BR. The onset of
485 starch degradation during banana ripening is concomitant to changes in the
486 content of free and conjugated forms of indole-3-acetic acid. *J Plant Phys.*
487 2002; 159: 1105-1111.

488

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

492

493 Sakhale B, Gaikwad S, Chavan R. Application of 1-methylcyclopropene on
494 mango fruit (Cv. Kesar): potential for shelf life enhancement and retention of
495 quality. *J food Sci. tech.* 2018; 55: 776-781.

496

497 Shiga TM, Soares CA, Nascimento JR, Purgatto E, Lajolo FM, Cordenunsi BR.
498 Ripening-associated changes in the amounts of starch and non-starch
499 polysaccharides and their contributions to fruit softening in three banana
500 cultivars. *J Sci. Food Agric.* 2011; 91: 1511-1516.

501

502 Souza MS, Azevedo, I.G.d., Corrêa, S.F., Silva, M.G.d., Pereira, M.G., Oliveira,
503 J.G.d. Responses of 1-MCP applications in 'Golden' papaya fruits on different
504 maturation stages. *Rev Brasileira Frutic.* 2009; 31: 693-700.

505

506 Thongkum M, Imsabai W, Burns P, McAtee PA, Schaffer RJ, Allan AC, Ketsa S.
507 The effect of 1-methylcyclopropene (1-MCP) on expression of ethylene receptor
508 genes in durian pulp during ripening. *Plant Physiol Biochem.* 2018; 125: 232-
509 238.

510
511 Trindade DCG, Lima MAC, Assis JS. 1-methylcyclopropene action on
512 postharvest conservation of 'Palmer' mango fruit at different maturity stages.
513 *Pesquisa Agropecuária Brasileira.* 2015; 50: 753-762.

514
515 Vilas-Boas EVB, Kader AA. Effect of atmospheric modification, 1-MCP and
516 chemicals on quality of fresh-cut banana. *Postharvest Biol Tech.* 2006; 39: 155-
517 162.

518
519 Xiao Y, Kuang Jf, Qi X, Ye Y, Wu ZX, Chen J, Lu W. A comprehensive
520 investigation of starch degradation process and identification of a transcriptional
521 activator MabHLH6 during banana fruit ripening. *Plant Biotech J.* 2018; 16: 151-
522 164.

523
524 Yang SF, Hoffman NE. Ethylene biosynthesis and its regulation in higher plants.
525 *Annu Rev Plant Physiol.* 1984; 35: 155-189.

526
527 Yemm, E., Willis, A., 1954. The estimation of carbohydrates in plant extracts by
528 anthrone. *Biochemical journal* 57, 508.

529
530 Zewter A, Woldetsadik K, Workneh T. Effect of 1-methylcyclopropene,
531 potassium permanganate and packaging on quality of banana. *African Journal*
532 *of Agricultural Research.* 2012; 7: 2425-2437.

533
534 Zhu X, Shen L, Fu D, Si Z, Wu B, Chen W, Li X. Effects of the combination
535 treatment of 1-MCP and ethylene on the ripening of harvested banana fruit.
536 *Postharvest Biol Tech.* 2015; 107: 23-32.

537
538 Ziliotto F, Begheldo M, Rasori A, Bonghi C, Tonutti P. Transcriptome profiling of
539 ripening nectarine (*Prunus persica* L. Batsch) fruit treated with 1-MCP. *J Exp*
540 *Bot.* 2008; 59: 2781-2791.

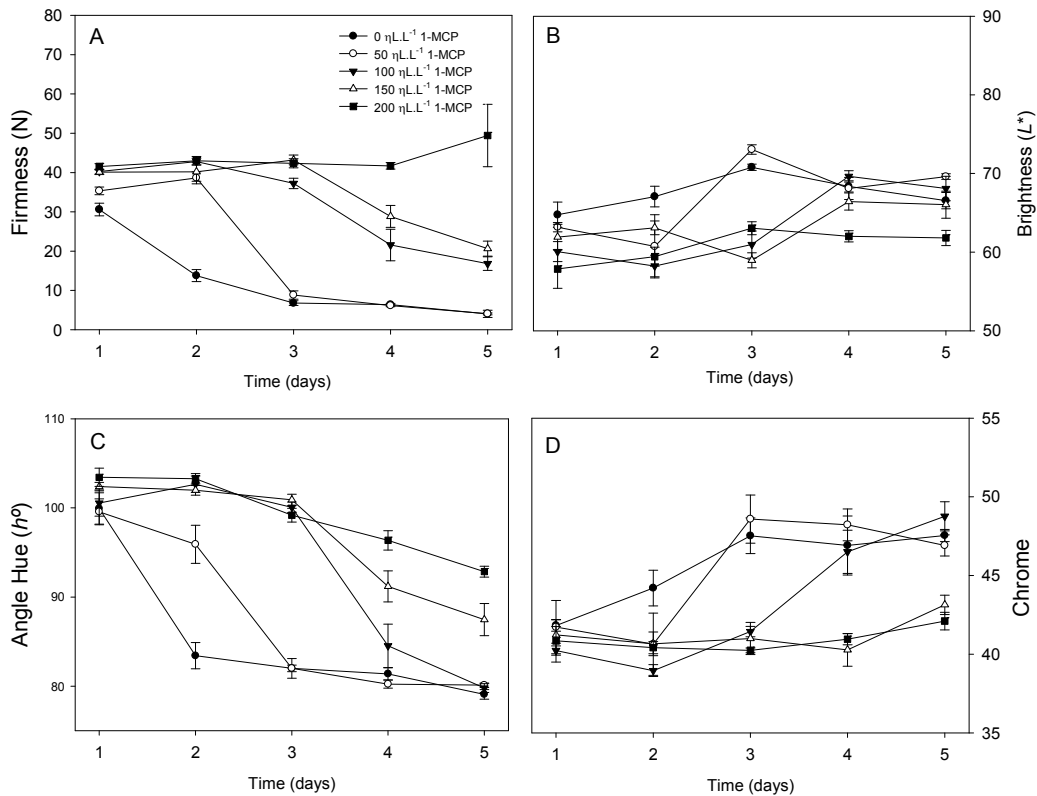
541
542 Zou J, Chen J, Tang N, Gao Y, Hong M, Wei W, Cao H, Jian W, Li N, Deng W.
543 Transcriptome analysis of aroma volatile metabolism change in tomato
544 (*Solanum lycopersicum*) fruit under different storage temperatures and 1-MCP
545 treatment. *Postharvest Biol Tech.* 2018; 135: 57-67.

546

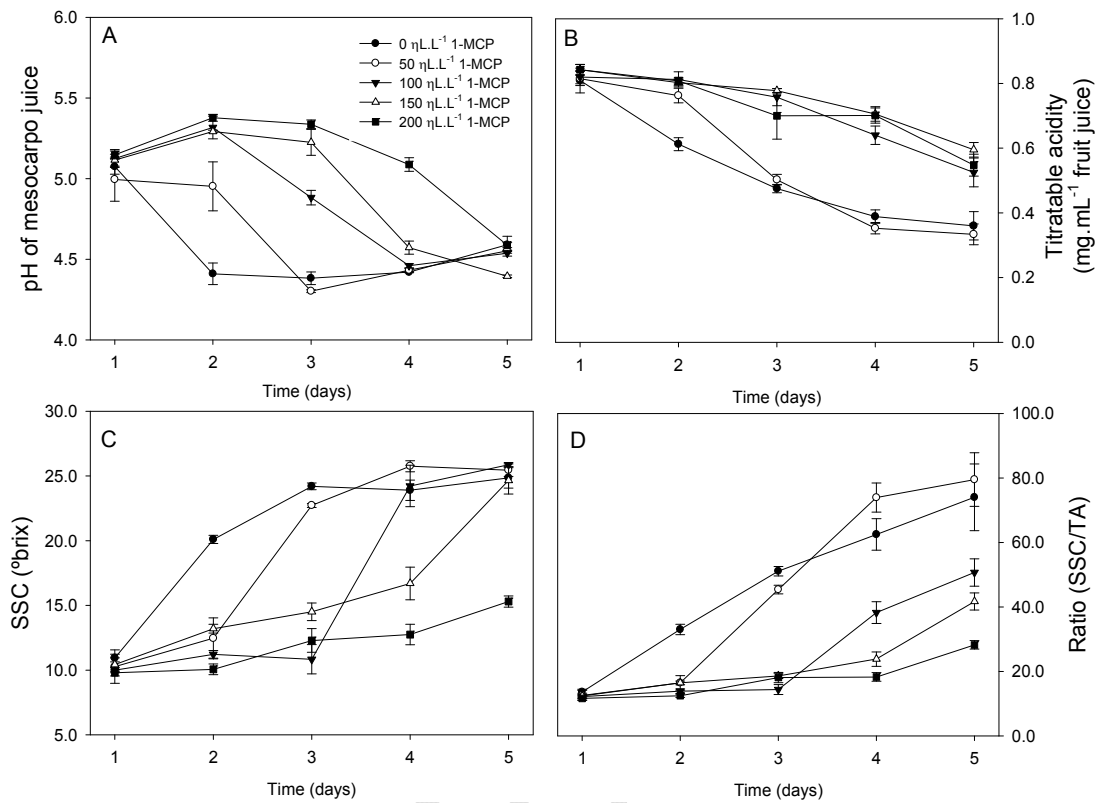
547 **Highlights**

- 548 • 1-MCP is an effective treatment to control sugar and starch metabolism
549 in banana.
550 • Skin color changes is an important predictive variable to identify changes
551 in the central metabolism in banana fruits.
552 • Ethylene action is an interplay of physical and chemical changes in
553 banana fruit.

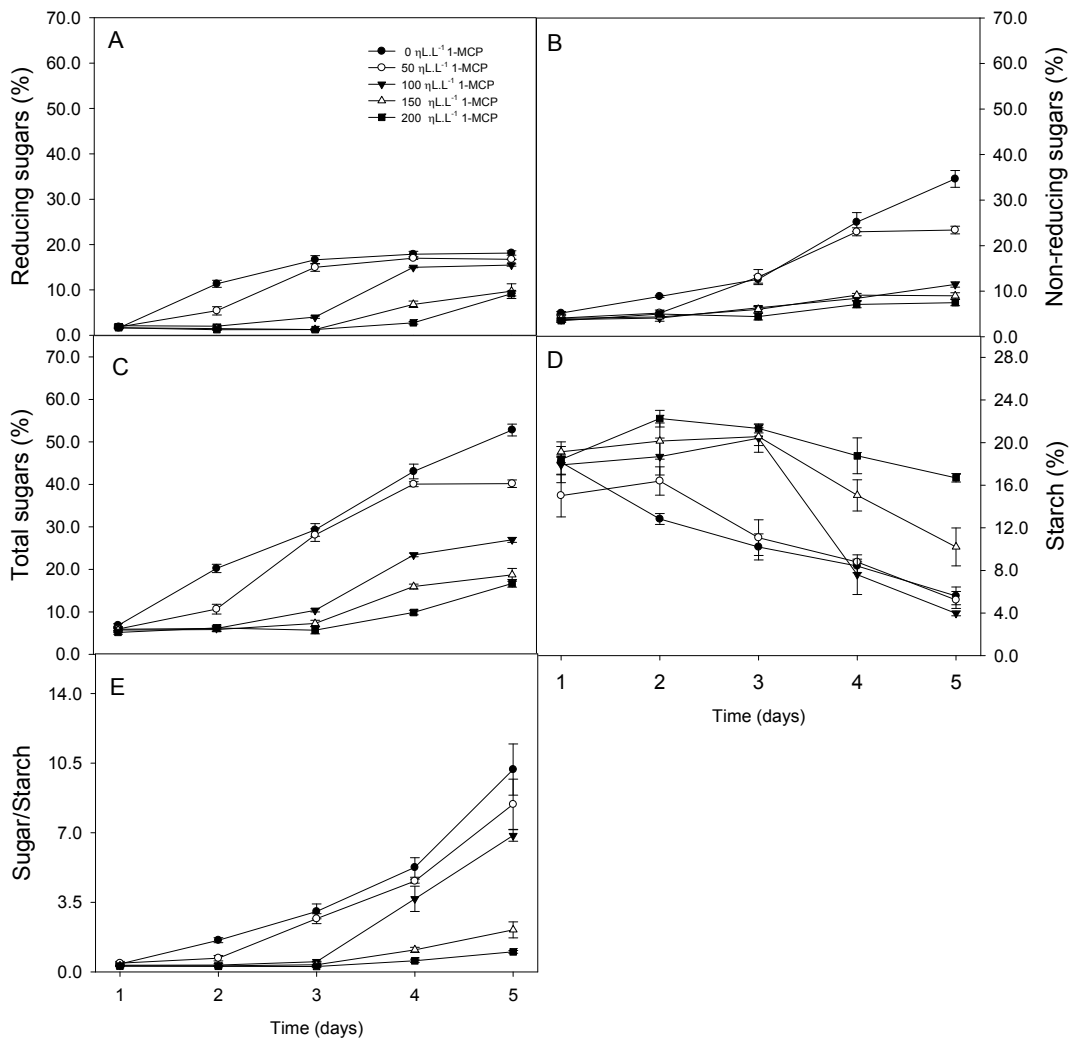
554 **Figures**



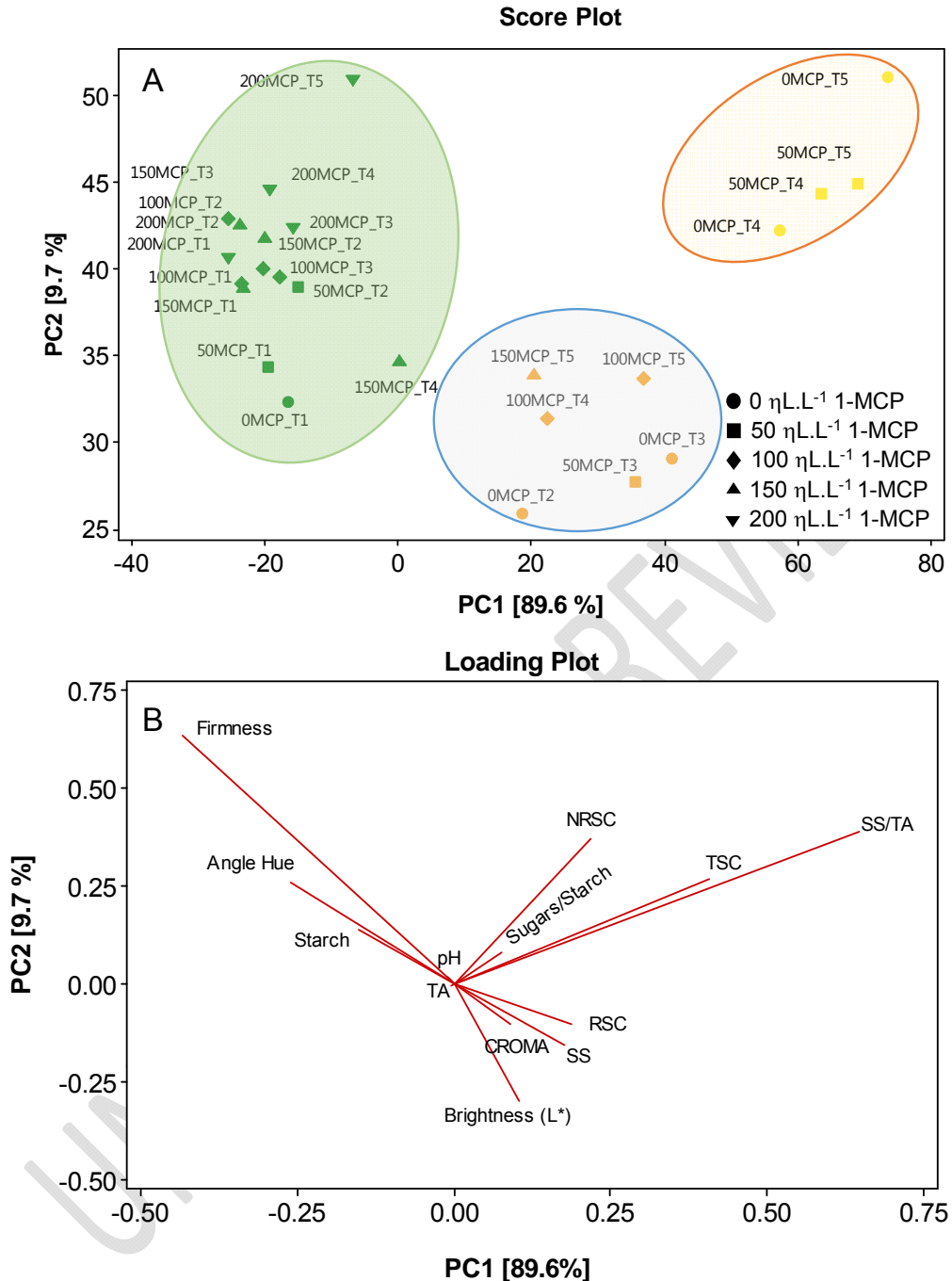
555 Fig. 1 – Physical quality attributes in banana cv. Prata Gorutuba (*Musa* AAB
556 ‘Prata Anã’) under different concentrations of 1-MCP during 5 times of storage
557 in room temperature. **A:** Firmness, **B:** Brightness, **C:** Angle hue and **D:** Chroma.
558 Each value is the mean for four replicates, and vertical bars indicate the
559 standard errors (n = 5).
560
561



563 Fig. 2 – Chemical traits of quality in banana cv. Prata Gorutuba (*Musa* AAB
 564 'Prata Anã') under different concentrations of 1-MCP during 5 times of storage
 565 in room temperature. **A:** pH of mesocarp juice, **B:** Titratable acidity, **C:** SSC and
 566 **D:** SSC/TA. Each value is the mean for four replicates, and vertical bars
 567 indicate the standard errors (n = 5).
 568
 569

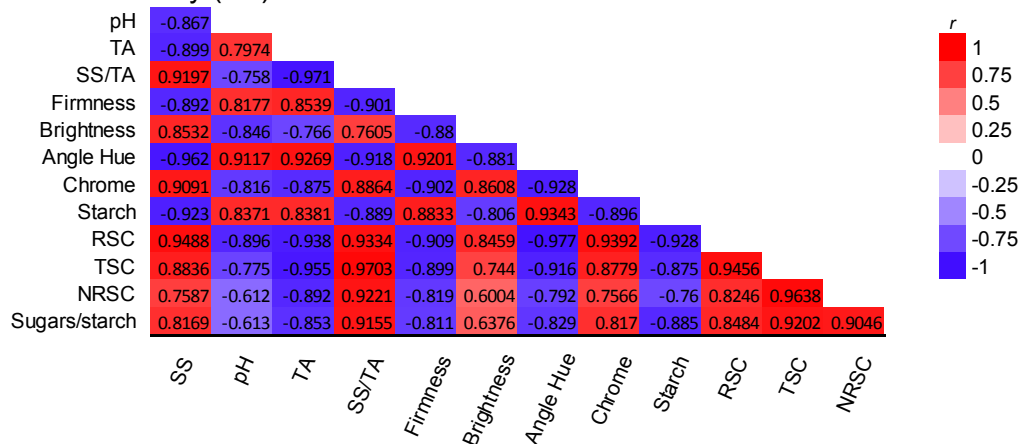


570
 571 Fig.3 - Physiological traits of quality in banana cv. Prata Gorutuba (*Musa* AAB
 572 'Prata Anã') under different concentrations of 1-MCP during 5 times of storage
 573 in room temperature. **A:** Reducing sugars content, **B:** Non-reducing sugars
 574 content, **C:** Total sugar content **D:** Starch content and **E:** Sugar/Starch ratio.
 575 Each value is the mean for four replicates, and vertical bars indicate the
 576 standard errors (n = 5).



577
 578 Fig.4 - Principal component analysis. (A) Score plot derived data of 1-MCP
 579 treatment under different days after treatment [0 days (T1), 1 day (T1), 2 days
 580 (T2), 3 days (T3), 4 days (T4) and 5 days (T5)]. The large circles represent the
 581 three clusters formed by the Euclidean distance method. (B) In loading plot the
 582 direction and length of the lines are directly proportional to variables importance
 583 in separating groups. PC1, principal component 1; PC2, principal component 2.
 584 Abbreviations: Hydrogenionic potential (pH), NRSC (Non-reducing sugar
 585 content), Total sugar content (TSC), Reducing sugar content (RSC), Soluble

586 solids content/Titratable acidity ratio (SS/TA), Soluble solids content (SS),
 587 Titratable acidity (TA).



588

589 Fig. 5. Correlation matrix based on Pearson coefficients derived from physical,
 590 chemical and physiological trails data from banana cv. Prata Gorutuba (*Musa*
 591 AAB 'Prata Anã') under different concentrations of 1-MCP during 5 times of
 592 storage in room temperature. Correlation coefficients are presented in colors,
 593 and the significant ones are indicated in bold (P) based on p-value corrected by
 594 FDR correction (Bonferroni-Hochberg). Abbreviations: Hydrogenionic potential
 595 (pH), NRSC (Non-reducing sugar content), Total sugar content (TSC),
 596 Reducing sugar content (RSC), Soluble solids content/Titratable acidity ratio
 597 (SS/TA), Soluble solids content (SS), Titratable acidity (TA).

598

599

600
601
602

Supplemental Figure

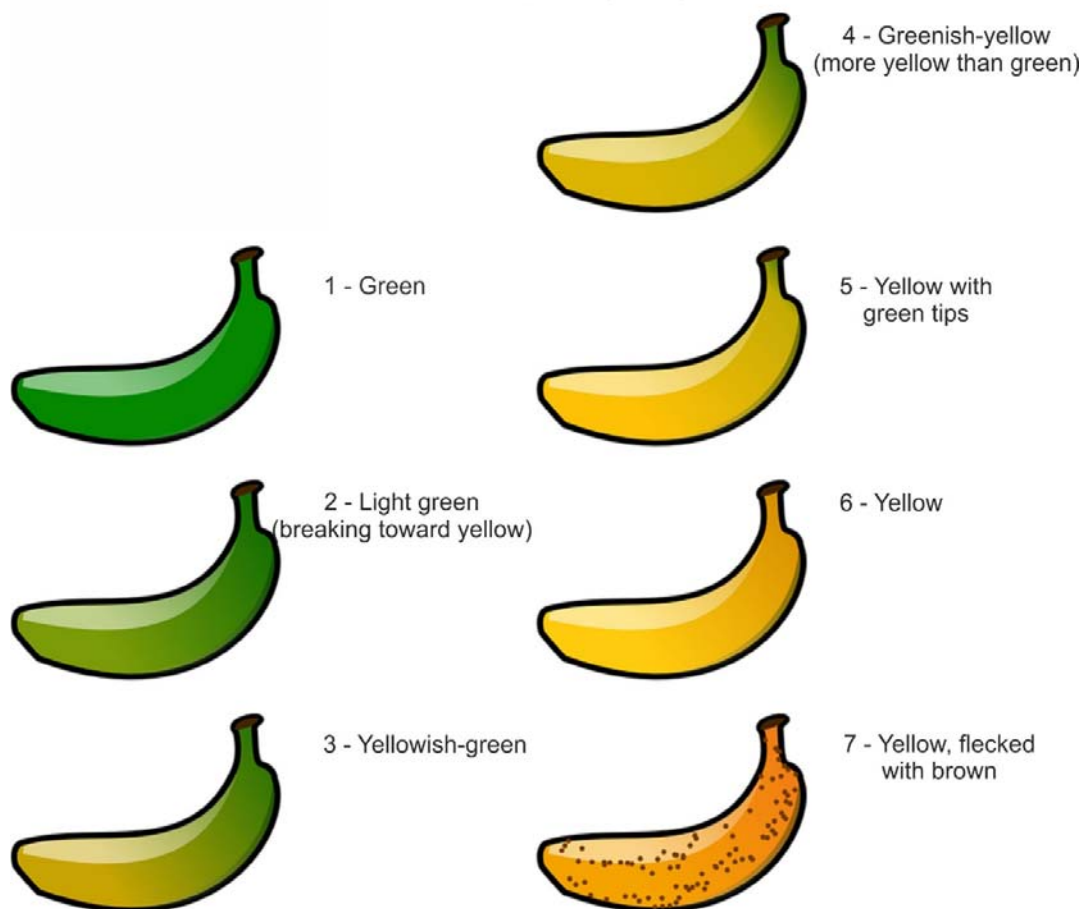


603
604
605
606

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



607

608 Supplemental Fig. S2. Color index scheme number of banana ripening.

609 Banana's ripening pattern during the final development of fruits. Scale marking

610 of color changes of the skin color 1 (green), 2 (light green), 3 (yellowish-green),

611 4 (greenish-yellow), 5 (yellow with green tips), 6 (yellow) and finally 7 (yellow,

612 flecked with brown). Adapted from CQH/CEAGESP. 2003. São Paulo

613 (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 □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 □L.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 aAB	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				
	1	2	3	4	5
□L.L-1 of 1-MCP					
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 ± 0.053 aD	1.59 ± 0.125 aCD	3.04 ± 0.37 aC	5.25 ± 0.49 aB	10.18 ± 1.29 aA
50	0.43 ± 0.088 aC	0.69 ± 0.137 aC	2.67 ± 0.24 aB	4.56 ± 0.10 aB	8.43 ± 1.26 abA
100	0.33 ± 0.011 aC	0.34 ± 0.058 bC	0.51 ± 0.02 bC	3.67 ± 0.64 aB	6.86 ± 0.28 bA
150	0.30 ± 0.032 aA	0.30 ± 0.031 bA	0.35 ± 0.02 bcA	1.11 ± 0.12 bA	2.11 ± 0.40 cA
200	0.28 ± 0.025 aA	0.28 ± 0.011 bA	0.27 ± 0.04 cA	0.55 ± 0.08 bA	1.01 ± 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 ($P < 0.05$).

UNDER PEER REVIEW