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Gibberellic acid in the postharvest quality of 'Nanicão' banana

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Abstract

The article aimed to verify the effect of doses of gibberellic acid on the extension of postharvest life on 'Nanicão' bananas. The fruits were harvested in color stage 1 (completely green peel). A completely randomized design was used, in an arrangement of subdivided plots, with doses of GA_3 allocated in the plots (0; 50; 100; 150 and 200 mg L^{-1}) and evaluation times allocated in the subplots (0; 3; 6; 9, 12, 15, 19, 23, 27 and 31 days of storage), with four replications, with a bouquet of three fruits per sample unit. The bunches were stored in a refrigeration chamber of 22 ± 1 °C and relative humidity of 91%. The study assessed fresh weight loss, peel color parameters, peel chlorophyll content, pulp firmness, soluble solids, titratable acidity, and CO_2 production. The loss of fresh matter was lower in fruits treated with 200 mg L^{-1}. Fruits treated with 200 mg L^{-1} had the highest Hue angle value (89.52). For the chlorophyll content, the highest doses favorably influenced the maintenance of the green color of the peel, delaying degradation. After 31 days of storage, the fruits treated with 200 mg L^{-1} of GA_3 were firmer. The soluble solids content increased from 5.5 °Brix to 9.1 °Brix, from day zero until the 15th day of storage for all treatments. There was an increase in the titratable acidity content with the advance of ripening. The fruits of all treatments showed a typical respiratory pattern, regardless of the dose of GA_3. After 31 days of storage, it was observed that the dose of 200 mg L^{-1} of GA_3 was favorable to the postharvest conservation of the banana' Nanicão.'

Keywords: gibereline, *Musa* sp., fruit quality

Introduction

Banana is a climacteric fruit with a high respiratory rate and ethylene production which triggers rapid ripening, characterized by changes in physical and chemical attributes, such as the conversion of starch into sugar, pulp softening, changes in aroma, flavor, and yellow pigmentation of the peel (Alkarkhi et al., 2011; Sembok et al., 2016). That is, after ripening, the banana has a relatively short shelf life, about 6 to 8 days at room temperature (Huang et al., 2014), causing losses, and making it difficult to sell the fruit to domestic and foreign markets.

The maintenance of banana quality is related to the minimization of the deterioration rate, to keep the fruits attractive to the consumer as long as possible. For this, it is necessary to search for new and simple technologies, which hinder the metabolism and delay the ripening process of the banana, aiming to extend shelf life, to serve consumers increasingly demanding for fruits with fresh aspect and that meet nutritional and sensory needs.

The cold chain can extend the shelf life of this fruit. However, the beneficial effects are limited to temperatures below 13 °C and an increase in storage time, due to the 'chilling' that causes the peel to darken and makes it difficult to ripen (Oliveira et al., 2016). Darkening has a significant impact on the color change of the peel and results in a poor quality of the banana, depreciating the product, impairing commercialization.

Another option is the use of gibberellic acid (GA_3), which maintains the quality and extends the postharvest life of the fruit, preventing the action of chlorophyllases and inhibiting the production of ethylene, reducing the speed of color transition of the fruits of the green to yellow (Taiz et al., 2017). Besides, GA_3 delays pulp softening and carotenoid accumulation, preventing excessive losses in marketing (Archana & Sivachandiran, 2015; Sembok et

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al., 2016).

Recent scientific results of research on plant growth regulators are reported in the scientific literature, especially gibberellic acid. Beneficial results of GA_3, applied in pre-harvest (Dagar et al., 2012; Weksler et al., 2012) and in postharvest (Al-Qurashi & Awad, 2019; Aquino et al., 2016a; Archana & Sivachandiran, 2015; Huang et al., 2014), have been reported to improve postharvest quality and increase shelf life of peach, nectarine, and banana. However, few studies assess the use of GA_3 in extending the postharvest life of bananas. Developing research using this technology could contribute to increasing storage and transport times, especially for cultivars that, due to the lack of adequate postharvest technology, do not support transport and storage conditions over long distances. In this perspective, the objective was to identify the effect of doses of gibberellic acid on the extension of the postharvest life of banana 'Nanicão'.

Material and Methods

Bunches of banana 'Nanicão' (*Musa* spp. AAA) were harvested from plants spaced 3.5 m x 2.5 m, in a seven-year-old experimental orchard at UFV (Federal University of Viçosa), in Viçosa, Minas Gerais, with an altitude of 648 m. The bunches were harvested in the pre-climacteric phase, in the color 1 stage (completely green bark) (Dadzie & Orchard, 1997). Of these, the second, third, and fourth bunches were selected, from which damaged, diseased and malformed fruits were eliminated.

After harvesting, the bunches were decomposed in bouquets with three fruits each, which were washed with water and 0.2% neutral detergent, for 5 min, for latex coagulation and surface cleaning. Subsequently, they were dried in the air until no more drops of water were observed on the surface of the fruits. The fruits that made up each bouquet were selected for uniformity in weight and dimensions. Subsequently, they were immersed in a Procloraz fungicide solution (0.22 g L^{-1}) for five minutes and air-dried.

After drying, the bouquets were randomly divided into five groups to receive the treatments, which consisted of the following concentrations of GA_3 (Sigma-Aldrich, USA): A) only water (control); B) 50 mg L^{-1}; C) 100 mg L^{-1}; D) 150 mg L^{-1}; E) 200 mg L^{-1}. The bouquets were immersed in the solutions for 10 minutes at room temperature. Then, the bouquets were entirely dried in the air, packed in plastic boxes, and stored in a refrigeration chamber at 22 ± 1 °C and relative humidity of 91% until complete ripening.

The evaluations were performed on the day of storage (day 0) and, subsequently, on days 3; 6; 9; 12; 15; 19; 23; 27, and 31 of storage. Each day, four bouquets were removed per treatment to assess the loss of fresh mass, pulp firmness, and peel color parameters. Then, these fruits were peeled and processed in a domestic blender, and a sample composed of the pulp was taken for analysis of soluble solids and titratable acidity. Bark samples were taken to determine the chlorophyll content. The production of CO_2 by the fruits was also evaluated.

As for the loss of fresh weight, a semi-analytical balance was used, the results being expressed as a percentage. The firmness of the pulp was measured by a SHIMPO penetrometer model DFG 100 (Digital Force Gauge) with an 8 mm diameter tip, in the middle of each fruit after removing a portion of the peel, the results being expressed in Newton. The Konica - Minolta colorimeter, Model CR 10, was used to supply the values of the Hue° angle of the shell.

The content of soluble solids was determined with the aid of a portable digital refractometer Atago model N1. For the titratable acidity, composite samples of 5.0 g were titrated with 0.05 N NaOH solution, previously standardized with potassium biftalate.

Chlorophyll content was determined by processing 2 g of bark samples in an Ultra Stirrer homogenizer (model D-500) together with 80% acetone for 2 min. The ketone extract was then vacuum filtered, and the absorbances read at 644 and 662 nm in a spectrophotometer. The chlorophyll contents was determined according to the equations of Smith & Benitez (1955).

The CO_2 production by the fruits was determined by gas chromatography. For that, the fruits were packed in airtight glass bottles with a volume of 3,280 mL. Thirty minutes after, the vials were closed, 1.0 mL aliquots of its atmosphere were removed with a syringe and injected in a GOW MAC gas chromatograph, Series 550, equipped with a thermal conductivity detector, and an aluminum column filled with Porapak Q. Working conditions form: 40 mL flow per minute of helium gas (carrier gas); electric current of 150 mA; column, detector and injector temperatures of 50; 70 and 80 °C, respectively; and room temperature from 20 to 23 °C. CO_2 quantification was done by comparing the sample's peaks in the chromatogram and those produced by the injection of a standard aliquot composed of 4.99 mol% CO_2 per mol of $CO_2 + N_2$ mixture. The results were expressed in mg CO_2 kg h^{-1}.

To evaluate the significance of the effects of the sources of variation by the F test, a completely randomized design was considered, in a split-plot scheme. Thus, five doses of GA_3 were allocated in the plots and the subplots the evaluation days, with four repetitions (bouquets) of three fruits.

Thirteen models of multiple regression were tested to adjust the qualitative characters of the fruits in the function of the doses of GA_3 and days of evaluation (Table 1). The Akaike information criteria checked the quality of the models' fit when the lowest estimates indicate the best-adjusted model (Azevedo et al., 2016). All statistical evaluations were performed using the R software, (R Development Core Team, 2013).

Table 1. Multiple regression models tested to describe quantitative characters in 'Nanicão' banana fruits as a function of GA_3 doses (xi) and postharvest days (y_i).

Models	Function
1	$z_i = a + bx_i + e_i$
2	$z_i = a + by_i + e_i$
3	$z_i = a + bx_i + cy_i + e_i$
4	$z_i = a + bx_i + cx_i^2 + dy_i + e_i$
5	$z_i = a + bx_i + cy_i + dy_i^2 + e_i$
6	$z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + e_i$
7	$z_i = a + bx_i + cy_i + dx_iy_i + e_i$
8	$z_i = a + bx_i + cx_i^2 + dy_i + fx_iy_i + e_i$
9	$z_i = a + bx_i + cy_i + dy_i^2 + fx_iy_i + e_i$
10	$z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_iy_i + e_i$
11	$z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_iy_i + hx_i^2y_i + e_i$
12	$z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_iy_i + hx_iy_i^2 + e_i$
13	$z_i = a + bx_i + cx_i^2 + dy_i + fy_i^2 + gx_iy_i + hx_i^2y_i + jx_iy_i^2 + e_i$

The *stats* package *aov* function verified the significance of variation sources effects by the F test. The least-squares method was used with the aid of the lm function of the *stats* package adjusted the multiple regression models. From the values predicted by the regressions, surface response graphs were obtained with the aid of the Sigma-Plot 11.0 software.

Results and Discussion

The F test at the level of 5% of significance verified that the doses of GA_3 had a significant effect on the loss of fresh matter, soluble solids, and the chlorophyll content. For the evaluation of the days, and the interaction between doses and evaluation days, there was a significant effect ($p <0.05$) for all evaluated characteristics (Table 2).

Based on the Akaike criteria, the best fit of model 11 was found for fresh weight loss (Table 3). For Hue angle, and chlorophyll content, the best fit was obtained by model 5, while for the firmness of the pulp and soluble solids, model 6 was the one with the best fit. This information is also important from the biological point of view, indicating that the doses of GA_3 have a linear effect for the characteristics in which the model 5 was better adjusted and a quadratic effect for the days of storage. The quadratic effect both for the doses of GA_3 and storage days enabled the better adjustment for model 6 (Table 1).

Table 2. P-values obtained by the F test of the sources of variation (SV) controlled in the study of quality characters in banana 'Nanicão' as a function of GA_3 doses and evaluation days.

SV	Fresh weight loss	Hue Angle	Chlorophyll content	Pulp firmness	Soluble solids	Titratable acidity
Doses	0.01	0.09	<0.01	0.07	0.02	0.35
Days	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Doses x Days	0.02	0.03	<0.01	0,04	<0.01	<0.01
VC_1 (%)	27.23	6.90	18.46	19.94	25.28	13.99
VC_2 (%)	17.29	6.68	22.80	26.20	23.27	14.88

VC1: Residual variation coefficient associated with the effects of GA_3 doses. VC2: Residual variation coefficient associated with the effects of the evaluation days.

Table 3. Quality of model fit by Akaike's information criterion for 13 multiple regression models for characteristics evaluated on banana 'Nanicão' as a function of GA_3 doses and evaluation days.

Models	Fresh weight loss	Hue Angle	Chlorophyll content	Pulp firmness	Soluble solids	Titratable acidity
1	251.59[1/]	405.53	129.06	396.93	326.32	68.47
2	116.34	353.05	89.30	333.81	260.53	35.39
3	117.76	353.73	90.54	335.78	262.37	37.24
4	116.50	355.25	91.71	336.08	262.48	39.07
5	11.23	**313.63**	**89.03**	318.14	244.13	**28.29**
6	109.34	314.51	90.14	**317.60**	**243.27**	30.09
7	119.76	355.44	92.43	337.27	264.36	39.07
8	118.50	356.95	93.59	337.55	264.47	40.91
9	113.23	314.95	90.91	319.38	246.12	30.10
10	111.34	315.81	92.01	318.79	245.25	31.89
11	**106.37**	317.60	94.01	320.45	244.22	33.58
12	113.25	317.73	90.98	320.67	247.14	33.89
13	108.27	319.52	92.98	322.33	246.1	35.58

[1/] The lower the AIC (Akaike information criterion) estimates, the better the model fit

The loss of fresh matter increased as a function of the evaluation days (as the fruits matured), in the five doses of GA_3 (Figure 1). However, the loss of fresh matter was less in the fruits treated with 200 mg L^{-1}, and more significant in the fruits treated with the dose of 100 mg L^{-1}, which lost 24% more fresh weight, concerning the other doses. At the end of 31 days of storage, it appears that the loss of fresh mass was similar for all treatments, with an average loss of 9.78%, is greater than that reported by Aquino et al. (2016a) for 'Apple' banana, after 19 days of storage. Similarly, Al-Qurashi & Awad (2019) reported that the loss of fresh mass increased during eight days of storage of the 'Grand Naine' banana, reaching 9.9%, unaffected by the treatments, immersion of GA_3 (150 and 300 mg L^{-1}), 6-benzyl amino purine (BAP, 50 and 100 mg L^{-1}) or $CaCl_2$ (2 and 4%).

$z = 6.99*10^{-1} - 8.56*10^{-3}x + 3.76*10^{-5}x^2 + 1.46*10^{-1}y + 3.33*10^{-3}y^2 + 1.11*10^{-3}xy - 5.60*10^{-6}x^2y$ $R^2=95.52$

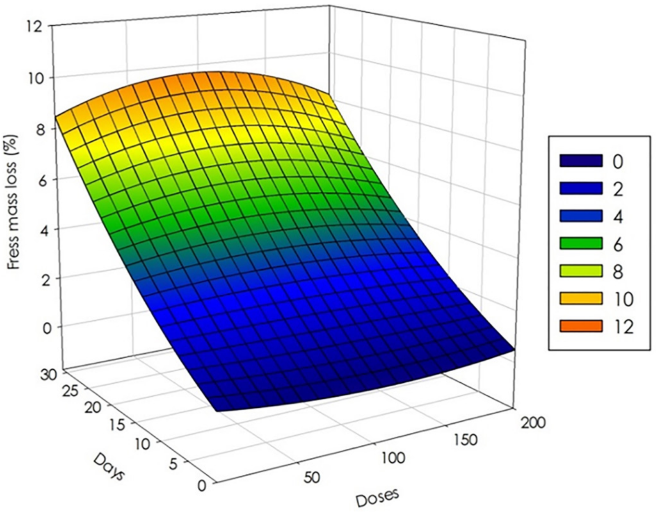

Figure 1. Response surface of fresh weight loss (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

The data pointed out that those treatments displaying a more significant loss of fresh mass were also those displaying a lower Hue angle, indicating a small effect in anticipating the ripening of the fruits, corroborating Finger et al. (1995). These authors verified that loss of fresh matter affects the pre-climacteric phase of 'Robusta' banana (*Musa acuminata* Colla), with the loss of 5% of fresh matter decreasing in seven days, and the loss of 10% decreasing in ten days the pre-climacteric period.

It appears that as increasing the doses of GA3, there was an increase in the Hue angle; therefore, the fruits remained green colored (Figure 2). At the end of the storage period, the fruits treated with 200 mg L^{-1} showed the highest Hue angle value (89.52), compared to the other doses that presented an average of 80.17, indicating the yellow color of the peel. Aquino et al. (2016a), found in banana fruits 'Apple,' treated with 100 mg L^{-1} of GA_3, at 16 days of storage, Hue angle of 92.82, while in the control fruits, the angle was 87.39, the which is indicative of a possible action of GA_3 to delay the degradation of chlorophyll, since the gibberellins have the function of delaying the ripening of the fruits, which mainly affects the color changes of the peel (Yang et al., 2009; Taiz et al., 2017).

$z = 111.65 + 1.78*10^{-2}x + 0.99y - 6.7*10^{-2}y^2$ $R^2=85.44$

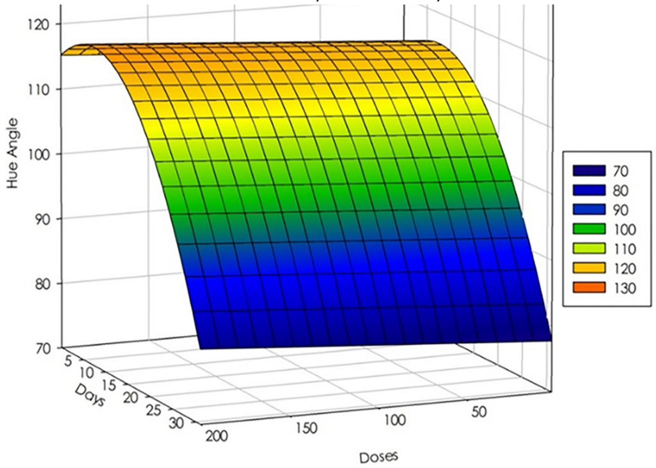

Figure 2. Response surface of the Hue angle (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

Huang et al. (2014) observed that bananas treated with 50 mg L^{-1} of GA_3, combined with 10 mg L^{-1} of phenylurea, delayed the change in the color of the skin to the control, probably due to the action of gibberellins in delaying the degradation of chlorophyll, which mainly affects peel color changes (Taiz et al., 2017). This is associated with phenylurea, as a competitive factor and irreversible inhibitor of cytokinin oxidase and dehydrogenase (Kopecny et al., 2010), which could block the activity of these target enzymes, acting as an inhibitor to maintain the endogenous level of cytokinin (Huang et al., 2014). Additionally, the level of cytokinin affects chloroplast activity, and regulates leaf senescence (Riefler et al., 2006). Furthermore, Al-Qurashi & Awad (2019) found that 150 mg L^{-1} of GA_3 was more effective in reducing peel browning and retaining green color than in other treatments, after eight days postharvest. Rossetto et al. (2004) found that slices of 'Nanicão' banana treated with GA_3 showed a delay in ripening, observed by the absence of aroma of ripe banana and by the color of the peel that remained green for a longer time. The color of the banana peel generally is directly correlated with the ripening of the fruits, which is an essential parameter for the shelf life and the consumers' purchase decision (Matsuura et al., 2004).

The chlorophyll content decreased over the storage period, with a slope on the surface for the highest doses of GA_3, namely, the highest doses favorably influenced the maintenance of the green color of the shell, delaying the degradation of chlorophyll in the peel over the entire fruit storage period (Figure 3), corroborating Aquino et al. (2016a) for 'Apple' banana and Al-Qurashi & Awad (2019) for 'Grand naine' banana. A decrease in chlorophyll contents characterizes the color change of the peel from color stage 1 (totally green rind) to 6 (totally yellow rind), evidencing the appearance of yellow coloration, as a consequence of the enzymatic action in the chlorophyll structure, allowing the disclosure of carotenoids (Newilah et al., 2009).

$z = 2.23+9.67*10^{-4}x-1.11*10^{-2}y^{-1}.67*10^{-3}y^2$ R^2=58.83

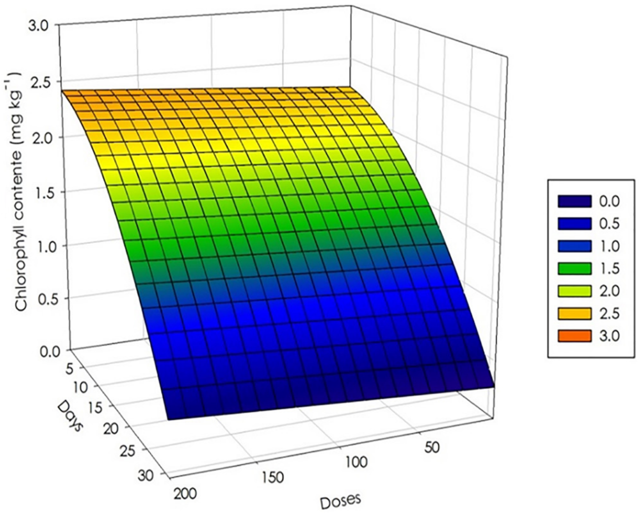

Figure 3. Response surface of chlorophyll content (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

The pulp remained firm until around the 19th day of storage in all treatments. After that, there was a decrease in the firmness of the pulp (Figure 4). From the 19th day on, there was a decrease in the firmness of the pulp. At the end of storage, fruits treated with 200 mg L^{-1} of GA_3 were firmer, with an average of 8.18N (Figure 4).

Huang et al. (2014) assessed the effect of GA_3 in postharvest, in maintaining quality and extending the shelf-life of bananas (AAA) during storage, found that fruits treated with 50 mg L^{-1} of GA_3, combined with 10 mg L^{-1} of phenylurea, remained firm after 16 days of storage at 23 °C and 75-90% relative humidity. These data corroborated with Al-Qurashi & Awad (2019) when they observed that the firmness of the pulp decreased during storage, being higher in the treated fruits than in control.

The loss of pulp firmness is associated with enzymatic activities related to the degradation of pectic components of the cell wall and middle lamella and with the conversion of starch into sugars during ripening (Ruiz et al., 2016). According to Rossetto et al. (2004), gibberellic acid affects enzymes that degrade starch, especially amylases, and as starch has a structural function in the banana pulp, there was a delay in the loss of firmness in the pulp of fruits treated with GA_3.

$z = 37.38+5.4*10^{-2}x-2.80*10^{-4}x^2+0.27y – 4.24*10^{-2}y^2$ R^2=81.86

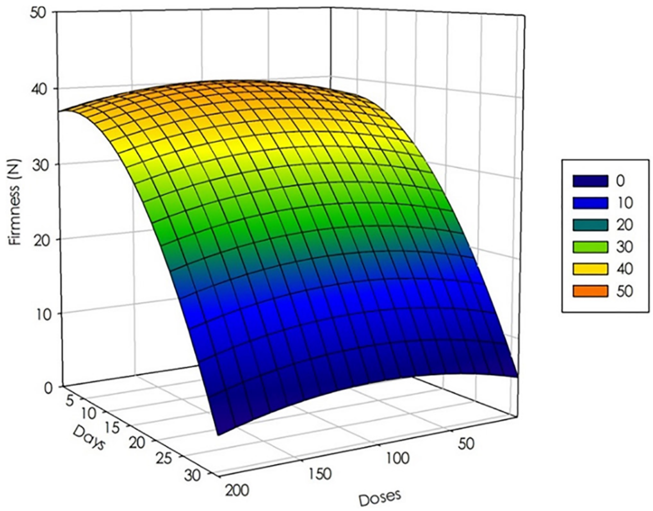

Figure 4. Pulp firmness response surface (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

The soluble solids content increased from 5.5 ° Brix to 9.1 °Brix, from day zero until the 15th day of storage for all treatments. After 15 days, the levels began to rise sharply until the end of storage, always with the highest levels observed in fruits not treated with GA_3, with an average of 22.6 °Brix versus 21 °Brix for the others doses (Figure 5), which was also observed by Al-Qurashi & Awad (2019). The sharp increase observed confirms the positive effect of GA_3 in delaying the conversion of starch to soluble sugars. Green fruits have a high percentage of starch, which is hydrolyzed with ripening, resulting in the accumulation of sugars, the soluble solids content being indicative of the sugar content in the fruit (Aquino et al. 2016b).

Treatment with 0.1 mM gibberellic acid in 6 mm thick slices of green banana pulp delayed the rate of starch degradation and the accumulation of soluble sugars by at least two days compared to untreated slices (Rossetto et al., 2004).

There was an increase in the titratable acidity content with advancing ripening, with fruits with a zero dose and fruits treated with 100 mg L^{-1} of GA3, with the highest levels observed at the end of storage, with an average of 0.35 g of acid (100 g^{-1}) of pulp. In the fruits treated with 150 and 200 mg L^{-1} of GA3, the average was

0.24 g of malic acid (100 g)$^{-1}$ of pulp (Figure 6).

The increase of the titratable acidity demonstrates the positive effect of GA_3 in delaying fruit ripening. Furthermore, Al-Qurashi & Awad (2019) report higher levels of control fruits. Aquino et al. (2017) observed in the mature pulp of 'Nanicão,' average titratable acidity content 0.37 g of malic acid (100 g^{-1}) of pulp. According to Bleinroth (1995), the banana in the green stage has low acidity, which increases with the ripening process until reaching a maximum, when the peel is entirely yellow, to later decrease, indicating the beginning of senescence. This phenomenon occurs due to the solubilization of pectic substances because of enzymatic activity, with the ripening of fruits (Prill et al., 2012).

The fruits of all treatments presented a typical breathing pattern, regardless of the dose of GA_3 used (Figure 7). There were no changes in respiratory rate up to 15 days of storage for any dose of GA_3. The doses of GA_3 were efficient in delaying the cause of the respiratory ascension, without affecting the magnitude of this process, unlike what was observed by Rossetto et al. (2004), who did not find differences in the respiratory profile of banana slices 'Nanicão' treated with GA_3 and those of the control, during ripening. Fruits treated with zero doses were the first to start respiratory ascension, after 15 days of storage. In the other doses, respiratory ascension was observed after 19 days of storage. Huang et al. (2014) found that treatment with 50 mg L^{-1} of GA_3, combined with 10 mg L^{-1} of phenylurea, delayed the onset of the respiratory peak, unlike the control fruits, which exhibited significantly higher peaks. In the same vein, Archana & Sivachandiran, (2015) found that fruits of the cultivar Kathali, treated with GA_3, resulted in a delay in ethylene production (7th day), while the control fruits showed earlier production of ethylene (2nd day).

$z = 6.84 -3.0*10^{-3}x-1.4*10^{-4}x^2 -0.12^2y +2.0*10-2\ y^2 \quad R^2= 83.17$

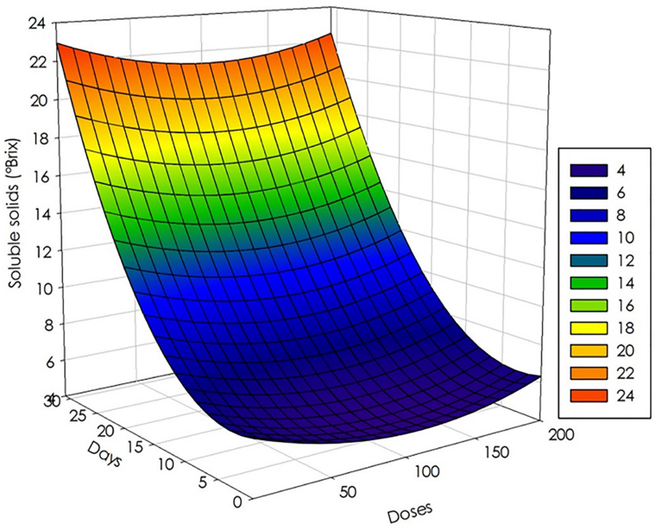

Figure 5. Response surface of soluble solids content (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

$z = 2.15*10^{-1}-3.55*10^{-5}x+1.12*10^{-2}y-2.25*10^{-4}y^2 \quad R^2=58.74$

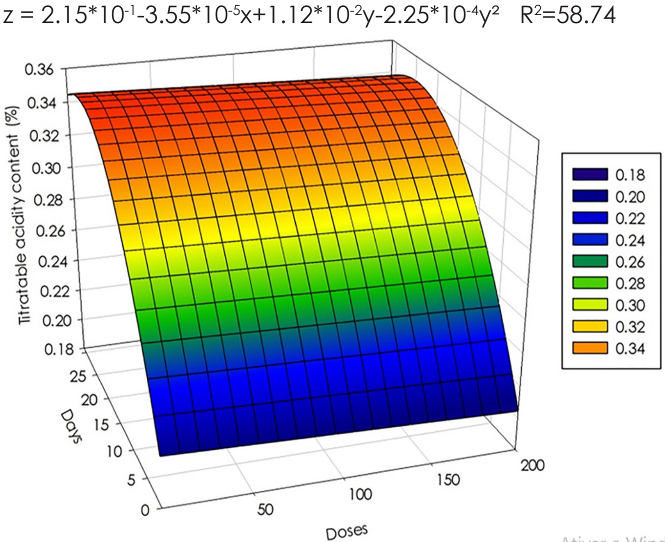

Figure 6. Response surface of the titratable acidity content (z) in 'Nanicão' banana fruits as a function of storage days (y) and doses of GA_3 (x).

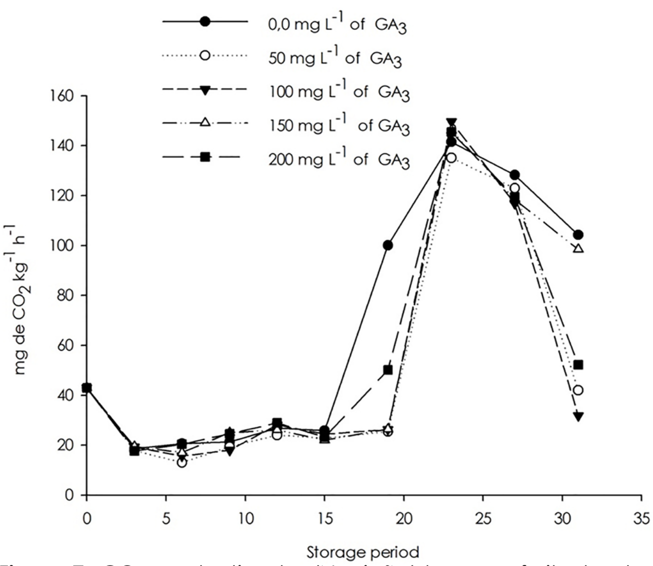

Figure 7. CO_2 production by 'Nanicão' banana fruits, treated with different doses of gibberellic acid (GA_3), depending on the storage period.

The use of GA3 to extend the shelf life of 'Nanicão' bananas can be promising, as it prolongs the shelf life of the fruits. Future researches, with application in pre-harvest or longer times of fruit immersion in GA_3 associated with higher doses of this plant regulator, must be carried out to control fruit ripening.

Conclusions

The GA3 untreated fruits started ripening after 15 days of storage, while in the other treatments, ripening started from the 19th day of storage.

The doses of 150 and 200 mg L^{-1} of GA_3 positively influence the postharvest conservation of 'Nanicão' bananas, increasing the shelf life.

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