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Controlled atmosphere associated with nitric oxide: A new alternative for preserving 'Prata' bananas

Atmosfera controlada associada ao óxido nítrico: Uma nova alternativa para a conservação de bananas 'Prata'

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RESUMO

O objetivo do presente trabalho foi avaliar o efeito da aplicação de óxido nítrico (NO) na qualidade pós-colheita de bananas 'Prata' durante o armazenamento em atmosfera controlada (AC). Os frutos foram separados em pencas e mantidas, durante 25 dias, em microcâmaras de AC com pressões parciais de 2 kPa de O_2 + 4 kPa de CO_2, 13 ± 1 °C e 92% ± 2% de umidade relativa (UR). Os tratamentos avaliados foram 0 (controle), 20 µL L^{-1} de NO, no início do armazenamento, 0,5 e 1 µL L^{-1} de NO aplicado diariamente e 1 e 5 µL L^{-1} de NO aplicado a cada cinco dias. Após o armazenamento, os frutos foram avaliados na saída da câmara e após seis dias em condições ambiente (23 ± 3°C e UR de 65% ± 5%) quanto as variáveis de coloração da epiderme, taxas respiratória e de produção de etileno, sólidos solúveis, acidez titulável, firmeza de polpa e incidência de podridões. O delineamento experimental foi inteiramente casualizado. A aplicação de NO, nas doses de 20 µL L^{-1} no início do armazenamento, 1 µL L^{-1} diariamente ou a cada cinco dias, proporcionou frutos com melhores atributos de qualidade. Os resultados evidenciam que 1 µL L^{-1} de NO aplicado diariamente ou a cada 5 dias apresenta-se como mais viável na manutenção da qualidade de bananas 'Prata'.

PALAVRAS-CHAVE *Musa sapientum L*.; etileno; senescência.

ABSTRACT

The objective of the present work was to evaluate the effect of nitric oxide (NO) application on the postharvest quality of 'Prata' bananas during controlled atmosphere (CA) storage. The fruit were separated into bunches and kept in CA microchambers with 2 kPa of O_2 plus 4 kPa of CO_2, 13 ± 1 °C and 92% ± 2% and relative humidity (RH) for 25 days. The fruit were treated with 0 (control), 20 µL L^{-1} of NO at the start of storage, 0.5 and 1 µL L^{-1} of NO applied daily and 1 and 5 µL L^{-1} applied every five days. After storage, fruit were evaluated at the time of chamber opening and, after six days in room conditions (23 ± 3°C and RH of 65% ± 5%) for skin color, respiratory and ethylene production rates, soluble solids, titratable acidity, flesh firmness and rot incidence. The experimental design was completely randomized. Treatments with application of 20 µL L^{-1} of NO at the start of storage, 1 µL L^{-1} of NO applied daily and every five days provided fruits with better quality attributes. The results show that 1 µL L^{-1} of NO applied daily or every 5 days is more viable for maintaining fruit quality.

KEYWORDS: *Musa sapientum L;* ethylene; senescence.

INTRODUCTION

Bananas reign as Brazil's most popular fruit, with the country ranking as the world's fourth-largest producer (CNA 2021). While Brazil's banana production is primarily consumed domestically, exports to distant markets, such as Europe, have been increasing in recent years (COLTRO & KARASKI 2019). Prata bananas are highly appealing due to their vibrant yellow peel with small brown spots and their flesh's high sugar content, resulting in a sweet flavor and high consumer acceptance (BHUIYAN et al.). 2020). Bananas are climacteric fruits that ripen rapidly, leading to significant changes in quality attributes and reduced postharvest shelf life, which limits long-distance maritime transport (GULARTE et al. 2022).

Controlled atmosphere (CA) is a widely employed technique in Central America for banana shipments to North America and Europe. However, storage solely in controlled atmosphere conditions results in diminished fruit flavor and aroma, incomplete ripening, uneven coloration, and limited efficacy in controlling decay. Therefore, identifying novel technologies to complement the effects of controlled atmosphere in delaying ripening and maintaining banana quality for extended periods is of paramount importance.

Nitric oxide (NO) is a reactive nitrogen species naturally produced in living cells that can reduce ethylene production and delay fruit ripening, senescence and loss of quality when applied exogenously at low concentrations (PALMA et al. 2019). This gaseous compound has demonstrated senescence-inhibiting properties, extending the post-harvest shelf life of fruits. Furthermore, NO can also induce the activity of antioxidant enzymes, controlling oxidative stress and reducing the manifestation of physiological disorders and the incidence of post-harvest rot (SUN et al. 2021). The application of gas in CA storage is advantageous due to the low oxygen concentration, which helps maintain stable nitric oxide molecules for longer periods. This is crucial because nitric oxide is reactive to oxygen and can oxidize to nitrogen dioxide (NO_2) upon contact, thereby losing its functionality (SNYDER 1992).

Positive results from the application of NO have already been observed in 'Cavendish' bananas under similar conditions, reducing oxidative stress in the fruits and delaying their ripening (GULARTE et al. 2022). Furthermore, other climacteric fruits, such as 'Laetitia' plums treated with the gas during storage in CA, demonstrated lower respiratory rate and ethylene production, slower evolution of epidermal color and, at low doses, reduced internal browning (STEFFENS et al. 2022). Spray applications of NO donors (such as sodium nitroprusside and s-nitrosoglutathione) in papaya provided a reduction in ethylene production and less loss of pulp mass and firmness (MACHADO et al. 2022). Similarly, fumigation of peaches with NO gas prior to storage in a refrigerated atmosphere delayed fruit ripening, alleviated the occurrence of chilling injuries, and prevented the loss of volatile organic compounds (CAI et al. 2020). Satisfactory results were also achieved in 'Cripps Pink' apples using pre-storage NO application, delaying yellowing and reducing flesh browning (STEFFENS et al. 2021).

This study aimed to investigate the impact of NO application, considering both dosage and frequency, on the ripening process of 'Prata' bananas stored under controlled atmosphere conditions.

MATERIAL AND METHODS

The study utilized Prata cultivar bananas harvested from a commercial plantation in Luiz Alves, Santa Catarina, during the 2020/2021 growing season. Following harvest, banana hands were placed in 80 L experimental microchambers under controlled atmosphere conditions: 2 kPa O_2, 4 kPa CO_2, 13 ± 1°C, and 92% ± 2% relative humidity (RH). The partial pressure of gases within the experimental chambers was monitored and adjusted daily using an electronic gas analyzer (Schelle, Germany), with atmospheric corrections made as needed.

The treatments evaluated were control (0), 20 µL L^{-1} NO applied at the beginning of storage, 0.5 and 1 µL L^{-1} NO applied daily, and 1 and 5 µL L^{-1} NO applied every five days during storage. NO was sourced from a high-pressure gas cylinder (1000 µL L^{-1} NO balanced with N_2). The fruits were stored for 25 days under CA conditions, simulating the export transportation period, followed by an additional six days at room temperature (23 ± 3 °C and 65% ± 5% RH) to mimic retail conditions.

Before the storage period (immediately after harvest), the fruits presented the following physicochemical attributes: epidermal color (h°) of 120.2, respiratory rate of 50.2 nmol of CO_2 kg^{-1} s^{-1} and ethylene production of 0.28 nmol C_2H_4 kg^{-1} s^{-1} , soluble solids content of 3.9 °Brix, titratable acidity of 0.16% malic acid and pulp firmness of 54.3 N.

Following storage, upon opening the chamber, respiratory rate, ethylene production, skin color, and

incidence of decay were evaluated. After an additional six days of shelf life, the same evaluations performed at chamber opening were conducted (except for rot incidence), along with assessments of soluble solids content, titratable acidity, and pulp firmness.

O delineamento experimental foi inteiramente casualizado. Each treatment consisted of six replicates, with an experimental unit comprising a cluster of 12 fruits. The data were initially subjected to Bartlett's and Shapiro-Wilk tests. When data met the assumptions of normality, analysis of variance (ANOVA) was performed, and significant variables ($p<0.05$) were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Upon chamber opening and after six additional days at ambient conditions, treatments with 20 µL L^{-1} NO applied at storage initiation and 1 µL L^{-1} NO applied daily or every five days resulted in lower respiratory rates and ethylene production compared to the control and other treatments (Table 1). When applied to climacteric fruits, NO can inhibit ACC oxidase enzyme activity and downregulate ACC oxidase gene expression. Furthermore, a stable ternary complex, “ACC-ACC oxidase-NO”, is formed, which prevents the oxidation of ACC to ethylene (MANJUNATHA et al. 2012). The conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylate (ACC), and consequently ethylene production, can be regulated at a potential point, which is affected by nitric oxide's (NO) ability to differentially modulate enzymes involved in SAM synthesis and utilization as precursors, as observed in a study by BUET et al. 2021

Table 1. Respiration and ethylene production rates of 'Prata' bananas stored in a controlled atmosphere (2 kPa O_2 + 4 kPa CO_2 ; 13±1 °C; RH of 92±2%) and treated with nitric oxide (NO), after 25 days of storage and an additional six days of shelf life (23±3 °C and RH of 65±5%).

	Ethylene production (nmol C_2H_4 kg^{-1} s^{-1})		Respiratory rate (nmol CO_2 kg^{-1} s^{-1})	
Treatment	Chamber exit	After 6 days on the shelf	Chamber exit	After 6 days on the shelf
0 µL L^{-1}	73.6 a	50.6 a	526.7 a	651.7 a
20 µL L^{-1} [a]	0.8c	12.8 b	65.3 b	90.3 b
0.5 µL L^{-1} [b]	65.5 ab	65.5 to	568.7 a	643.7 a
1 µL L^{-1} [b]	0.6 c	13.5 b	71.7 b	79.2 b
1 µL L^{-1} [c]	8.5c	9.3 b	78.9 b	95.8 b
5 µL L^{-1} [c]	43.1 b	71.6 a	492.8 a	692.8 a
CV (%)	14.8	15.9	16.1	17.8

Means followed by different letters in the columns differ from each other by the Tukey test ($p<0.05$). CV = coefficient of variation. [a] NO applied at the onset of CA storage (when storage atmosphere was established). [b] NO applied at CA storage establishment and daily until the end of CA storage. [c] NO applied at CA storage establishment and every 5 days until the end of CA storage.

The reduction in ethylene production is also attributed to NO's interaction with other plant signaling molecules and phytoregulators, such as salicylic acid, which modulates and inhibits the expression of genes involved in ethylene biosynthesis (PALMA et al. 2019). Furthermore, NO inhibits mitochondrial respiration through reversible binding to cytochrome c oxidase (PANDEY et al. 2019). The lower respiratory activity of fruits treated with NO may also be associated with lower ethylene production, since the presence of this phytohormone increases respiration during the ripening of climacteric fruits (PALMA et al. 2019).

Applications of 20 µL L^{-1} of NO at the beginning of storage and 1 µL L^{-1} of NO daily or every five days of storage provided a reduction in SS and AT contents, as well as greater pulp firmness (Table 2). Unlike other climacteric fruits, bananas exhibit an increase in soluble solids and titratable acidity during ripening due to the conversion of starch into reducing sugars and organic acids (DEDO ADI et al. 2019). Therefore, the lower values of SC and TA in NO-treated fruits indicate delayed ripening due to reduced starch degradation and lower levels of soluble sugars and organic acids in the fruit.

Table 2. Soluble solids content (SC), titratable acidity (TA), pulp firmness (PF), skin color and incidence of rot in 'Prata' bananas stored in a controlled atmosphere (2 kPa O_2 + 4 kPa CO_2 ; 13±1°C; RH of 92±2%) and treated with nitric oxide (NO), after 25 days of storage.

	SC	AT	PF	Epidermal coloration (h°)		Incidence of rot
Treatment	(°Brix)	(%)	(N)	Chamber exit	After 6 days on the shelf	(%)
0 µL L^{-1}	19.9 a	0.77 to	8.8 b	101.4 b	89.0 b	63 to
20 µL L^{-1} [a]	5.6c	0.32 b	48.9 a	116.1 a	111.9 a	66 a
0.5 µL L^{-1} [b]	18.8 ab	0.67 to	11.8 b	103.0 b	85.4 b	69 to
1 µL L^{-1} [b]	7.5c	0.43 b	39.5 to	115.8 a	108.7 a	35 b
1 µL L^{-1} [c]	5.2c	0.33 b	43.3 a	114.9 a	110.0 a	33 b
5 µL L^{-1} [c]	15.2 b	0.63 to	15.8 b	97.3 b	85.7 b	63 to
CV (%)	4.5	1.3	4.9	5.2	6.6	26.5

Means followed by different letters in the columns differ from each other by the Tukey test ($p<0.05$). CV = coefficient of variation. [a] NO applied at the onset of CA storage (when storage atmosphere was established). [b] NO applied at CA storage establishment and daily until the end of CA storage. [c] NO applied at CA storage establishment and every 5 days until the end of CA storage.

The preservation of pulp firmness can be attributed to reduced ethylene production in fruits treated with 20 µL L^{-1} applied at the onset of storage, daily applications of 1 µL L^{-1} NO, and 1 µL L^{-1} NO applied every five days during controlled atmosphere storage. During climacteric fruit ripening, ethylene activates cell wall enzymes responsiblethrough the solubilization of pectic substances and the conversion of insoluble pectin to soluble pectin, ultimately resulting in loss of pulp firmness and fruit softening (PALMA et al. 2019).

Upon removal from the chamber and after six additional days under ambient conditions, fruits treated with 20 µL L^{-1} NO at the start of storage and 1 µL L^{-1} NO applied daily or every five days during storage exhibited higher h° values, indicating a greener coloration (Table 2). These findings demonstrate that the treatments effectively delayed the progression of banana peel color changes. The observed outcomes are linked to decreased ethylene production resulting from these NO application conditions, as ethylene promotes chlorophyll degradation and fruit yellowing (PALMA et al. 2019).

Daily or every five days application of 1 µL L^{-1} NO reduced fruit decay incidence after storage (Table 2). Nitric oxide has emerged as a promising method for inducing resistance against pathogenic fungi and extending the post-harvest shelf life of fruits. The antifungal activity of NO is linked to its ability to regulate hydrogen peroxide levels, stimulate phenolic compound synthesis, and induce activities of phenylalanine ammonia-lyase, peroxidase, polyphenol oxidase, catalase, and the ascorbate-glutathione cycle (GULARTE et al. 2023). Along with salicylic acid (SA), NO plays a crucial role in plant immune responses, collaborating with reactive oxygen species to trigger hypersensitive responses and cellular death during plant-pathogen interactions (SCHELER et al. 2013).

Studies indicate that NO is incorporated into this immune network, coordinating with several classical pathways, such as signaling events related to SA or jasmonic acid/ethylene (LEÓN et al. 2013). NO may serve as a key signal in establishing systemic acquired resistance, functioning as a secondary messenger in various plant hormone signaling pathways and conferring disease resistance benefits, as demonstrated across multiple species (PALMA et al. 2019). There was a reduction in the severity of *Penicillium expansum* (blue mold) in 'Cripps Pink' apples (GULARTE et al. 2023), and significant inhibition of *Monilinia fructicola*, in peaches (LI et al. 2016) with application of NO, and increased defense responses and inhibition of anthracnose in pitaya by NO (HU et al. 2019).

However, NO has a short half-life, around 2 hours, degrading rapidly in AC chambers (BUET et al. 2021). The 20 µL/L treatment likely exacerbated oxidative stress, as NO is a reactive oxygen species that can damage cell membranes at high concentrations. The proliferation of decay in this treatment can be attributed to multiple factors. For instance, elevated NO concentrations may exacerbate oxidative stress in fruit cells, compromising cellular structures and weakening natural pathogen defenses, thereby increasing susceptibility to decay.

Furthermore, nitric oxide exhibits hormetic effects, with low concentrations promoting beneficial adaptive responses in plants, while higher levels may prove detrimental. Consequently, excessive NO concentrations may surpass beneficial thresholds, leading to adverse effects such as increased decay. Furthermore, elevated

NO concentrations may compromise banana cell integrity, increasing susceptibility to pathogenic infection and decay (BUET et al.). 2021).

CONCLUSION

Daily or every five days applications of 1 µL L^{-1} NO in CA delay ripening and reduce decay incidence in 'Prata' bananas after 25 days in CA plus 6 days at ambient conditions, presenting a viable alternative for storage or during export.

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