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Nitric oxide supply reduces ethylene production, softening and weight loss in papaya fruit

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ABSTRACT: Nitric oxide application has been seen as a promising technique to extend the postharvest life of various fresh fruits and vegetables. This is the first work involving the spray application of nitric oxide donor *S*-nitrosoglutathione (GSNO) on Golden papaya. Considering that results are very distinct depending on the type of the nitric oxide donor, the form of application, the concentration used and the species studied, the application must be adapted to each necessity. The aim of this study was to relate the application of GSNO, spray applied at 10, 100, 1,000 μM, with the physiological, physical-chemical, and biochemical changes of Golden papaya, in the first 72 h of ripening. Control fruit was sprayed with distilled water. GSNO application did not interfere on color and chlorophyll fluorescence of the peel, on soluble solids, titratable acidity, lipid peroxidation, and in the level of *S*-nitrosothiols. Control fruit and 10-μM GSNO sprayed showed lower respiration. After 72 h of ripening at 25°C, all fruits showed an increase in ethylene biosynthesis, except for those treated with 10 μM GSNO. Papaya sprayed with 10-μM GSNO showed the highest pulp firmness and 52% less weight loss when compared to control fruit. GSNO was also responsible for increasing the ascorbic acid in papayas, besides showing an increase in total antioxidant activity production. The results indicated that the application of 10 μM of GSNO by spray can potentially preserve the quality characteristics of Golden papaya, mainly due to the lower ethylene production, the delay in the firmness loss, and the less weight loss.

Key words: postharvest, ripening, *S*-nitrosothiols, ethylene.

INTRODUCTION

The constant search for new post-harvest techniques that contribute to the conservation of fruits can result in benefits for product commercialization. Among them, the use of plant regulators has been shown to be efficient for many tropical fruits. Nitric oxide (NO) interacts with different cellular compounds, including other radicals, being considered a signaling molecule with antioxidant potential, and it has an antagonistic effect to ethylene during fruit ripening and senescence (Palma et al. 2019). Several studies have already reported effects of applying NO on decreased respiration (Steelheart et al. 2019), on the ethylene production (Corpas and Palma 2018, Shi et al. 2019), firmness and weight loss (Steelheart et al. 2019), and lipid peroxidation (Zhang et al. 2019) during fruit ripening, that can contribute to extend postharvest life. The literature suggests that NO effects occur in the first hour after fruit exposure, especially with regard to respiration and ethylene production (Huang et al. 2019, Steelheart et al. 2019).

Several NO donors were used on postharvest, with differences in composition, half-life, NO release rate under light conditions, and presence of reducing agents (Silveira et al. 2019). *S*-nitrosoglutathione (GSNO) is an *S*-nitrosothiol that spontaneously releases NO without harmful effects to plants (Manjunatha et al. 2012). In addition, oxidized forms of NO can react with thiol groups of cysteine residues forming *S*-nitrosothiols, by a reaction known as *S*-nitrosation (Lindermayr et al. 2006). *S*-nitrosation can, in turn, change the activity, stability, and conformation of target proteins, in addition to interacting with other molecules, regulating a wide range of functions and signaling events (Sevilla et al. 2015). However, reports of this post-translational modification on postharvest are scarce.

Besides the variation of NO donors, studies also show different responses in relation to the doses applied (Shi et al. 2019). According to Gheysarbigi et al. (2020), nitric oxide can have a beneficial effect, as well as a harmful effect, on the fruits, depending on the concentration used. Responses may also vary according to the application methods such as immersion (Shi et al. 2019), spraying (Grozeff et al. 2017) and fumigation (Ma et al. 2019). Although NO has well-established characteristics and functions, there are still many questions about the different formulations of NO donors and efficient application methods, considering the particularities of each fruit.

Papaya (*C. papaya* L.) is a fruit of great economic importance for Brazilian fruit production. Limitations for expanding markets include the fragility and deterioration of the fruit due to its rapid ripening during storage. In this sense, the fruits undergo intense changes, that are irreversible. The main changes are related to the loss of firmness, weight loss, change in the skin color, including changes in chlorophyll fluorescence and biochemical changes, such as increased oxidative damage (Resende et al. 2012, Zhang et al. 2019). Therefore, the search for the effectiveness of the application of GSNO as a NO donor through spraying can be a trigger for new formulations on postharvest.

Thus, the present study hypothesized that spraying Golden papaya with GSNO, a NO donor, would attenuate the short-term physiological, physical-chemical, and biochemical changes caused by ripening, in a dose-dependent mode. To the best of our knowledge, this is the first report to investigate the effects of sprayed administration of GSNO in papaya.

MATERIAL AND METHODS

Plant material and treatments

Papaya fruits (*C. papaya* L.) cv. Golden were harvested from a commercial orchard at Linhares, Espírito Santo state, southeastern Brazil, at maturity stage 1 (yellow color covers less than 15% of the skin's surface), and were then transported in a refrigerated truck (10°C) to Agronomic Institute, in Campinas, São Paulo state, Brazil. Before treatment, characterization analysis was performed (respiration, ethylene production, weight loss, firmness, total soluble solids, titratable acidity, ascorbic acid content, malondialdehyde and *S*-nitrosothiols content).

Five fruits were used for characterization, and 54 fruits were analyzed per treatment. The fruits were sprayed with water or with GSNO solution at 10, 100, or 1,000 μM. These concentrations were based on a preliminary test (data not). The GSNOs were freshly prepared and sprayed with a semi-professional hand sprayer for 12 seconds for each fruit, up to the drainage point. Each fruit received about 6 mL of product. After the treatments, fruits were placed into hermetic and dark chambers (186 L), with air circulation, for 12 h, to decrease the volatility. After that, the fruits were removed from the chambers, and each treatment was kept, separately, at 25±2°C and 80-90% relative humidity (RH) for 72 h of storage. Except for chlorophyll fluorescence, with analysis performed at 48 h, all other analyses were performed at times 0 (characterization), 24, 48 and 72 h, after storage.

Synthesis of *S*-nitrosoglutathione

GSNO was synthesized and characterized as previously described by Silveira et al. (2016). Reduced glutathione (GSH) was reacted with equimolar amount of sodium nitrite in acidified aqueous solution, in an ice bath for 40 min, under magnetic stirring. The obtained GSNO was precipitated by the addition of acetone, filtrated, and washed with cold water. The obtained solid was freeze-dried for 24 h.

Ethylene production and respiration

To evaluate ethylene and CO_2 production, fruits with known weights were placed individually into 1,700 mL hermetic flasks and kept closed for 1 h. For ethylene evaluation, gas samples (1 mL) were collected through a silicone septum with a gastight syringe. The gas samples were analyzed with a gas chromatograph (Shimadzu GC-2010 Plus AF) fitted with a flame ionization detector (FID) and a Porapak N 80/100 mesh (2 m × 2 mm) column set at 100°C. Helium was used as the carrier gas at a flow rate of 40 $mL \cdot min^{-1}$. CO_2 measure was analyzed with PBI Dansensor® CheckMate gas analyzer (Dansensor, Ringsted, Denmark). After measurements, the flasks were opened, and the fruits removed. Respiration and ethylene production, expressed as $mL\ CO_2 \cdot kg^{-1} \cdot h^{-1}$ and $\mu L\ C_2H_4 \cdot kg^{-1} \cdot h^{-1}$, respectively, were determined by the difference between the initial and final gas concentrations (immediately after flasks were closed and after 1 h, respectively). The same fruits were used during the entire experimental period.

Firmness and weight loss

Fruit firmness was measured using a digital penetrometer (53200, Tr Turoni, Italy) fitted with an 8-mm diameter probe tip after the skin removal. Measurements were taken at two equatorial opposite sides of the fruit. Data were recorded in Newtons (N). Fruit weight loss was evaluated by weighing the same fruit daily on a semi-analytical balance, and the difference was calculated between the final and initial weights, and the results expressed as percentage (% w /w).

Skin color, soluble solids, titratable acidity, and ascorbic acid

Skin color was objectively measured with a colorimeter (Minolta CR-300, Osaka, Japan) by carrying out two readings per fruit on opposite sides at equatorial region, and results were expressed in hue angle (H°, 90° represents a totally yellow skin and 180° a totally green skin). Total soluble solids were determined by using a digital refractometer (Atago PR-101, Atago, Japan), with results expressed in °Brix. The ascorbic acid content and titratable acidity were determined according to Instituto Adolfo Lutz (2008), and results were expressed as $mg \cdot 100g^{-1}$ and $g \cdot 100g^{-1}$ FW, respectively.

Malondialdehyde and *S*-nitrosothiol contents

The malondialdehyde (MDA) was measured and used as an index of lipid peroxidation. Papaya pulps and skins (0.3 g) were macerated in 1.3 mL of 0.1% trichloroacetic acid (TCA) (w/v) and centrifuged at 10,000 g for 10 min. The supernatant was added to 1.5 mL of 0.5% thiobarbituric acid (w/v) in 20% TCA (w/v), and the mixture was incubated at 90°C. After that, the reaction was stopped in an ice bath. Then, a new centrifugation was performed at 10,000 g for 10 min, and after 30 min at room temperature the absorbance was measured at 532 and 600 nm. The absorbance at 600 nm was subtracted from the absorbance at 532 nm, and the MDA concentration was calculated using an extinction coefficient of $155 \cdot mM^{-1} \cdot cm^{-1}$ (Heath and Packer 1968).

For *S*-nitrosothiol (RSNO) content, the total proteins of the skin and pulp were homogenized, and an amperometric nitric oxide analyzer was used, as described by Santos et al. (2016). The measurements were performed with the WPI TBR 4100/1025 free radical analyzer (World Precision Instruments Inc., Sarasota, FL, United States of America) and a specific nitric oxide sensor, ISO-NOP (2 mm). Aliquots of 0.2 mL of aqueous suspension were added to the sample compartment with 10 mL of aqueous copper chloride solution ($0.1\ mol \cdot L^{-1}$). This condition allowed the detection of free NO released from the *S*-nitrosothiol present in the protein homogenate of both the skin and pulp tissue. The analyses were performed in triplicate, and the calibration curve was obtained with freshly prepared GSNO solutions. The data were compared with the standard curve obtained and normalized against fresh weight.

Antioxidant activity

The antioxidant activity (AA) was measured using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical photometric assay (Mishra et al. 2012, Pelegrino et al. 2020). A volume of 300 μL of DPPH ethanolic solution (0.06 $mmol{\cdot}L^{-1}$) was added to 300 μL of tissue sample homogenized and 0.5 mL of phosphate buffered saline (PBS). The control group was prepared with DPPH and PBS. The final mixtures were kept at room temperature, protected from light for 30 min, and placed into quartz cuvettes. The absorbance intensities at 517 nm were recorded for all samples by using a ultraviolet (UV)-vis spectrophotometer (Thermo Fisher Scientific, Genesys 10S, Waltham, MA, United States of America). The percentage of AA was calculated according to Eq. 1.

$$\%AA = 100 - \frac{(\text{Abs sample}) \text{ x } 100}{\text{Abs control}} \tag{1}$$

In which: Abs sample = the absorbance of the sample in ethanolic DPPH; Abs control = the absorbance of ethanolic DPPH and PBS, without sample.

The assay was normalized by skin or pulp weight.

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed using a modulated fluorometer (6400-40 LCF, Li-Cor, Lincoln, NE, United States of America), as carried out previously by Bron et al. (2004), with modifications. The minimal and maximal chlorophyll fluorescence (Fo and Fm, respectively), variable chlorophyll fluorescence (Fv = Fm - Fo), and potential quantum yield of photosystem II (Fv/Fm) were obtained. Before chlorophyll fluorescence measurements, fruits were dark-adapted during 30 min in a darkroom. The distance between the fiber optic terminus and the fruit exocarp was the same for all treatments. Measurements were taken in two opposite positions of each fruit at the same location in the fruit surface and then averaged.

Data analysis

The experimental design was conducted in a factorial design (doses × time of storage), and data were subjected to the analysis of variance (ANOVA). The Scott-Knott test was used to compare treatments when significance was found ($p<0.05$). The results presented are the mean, and the number of replicates is stated in each figure caption.

RESULTS

Control fruits and those treated with GSNO at 100 and 1,000 μM showed increasing ethylene production during storage. After 72 h at 25°C, these fruits produced approximately three times more ethylene when compared to the initial values, reaching the average of 3.3 μL $C_2H_4{\cdot}kg^{-1}{\cdot}h^{-1}$ ($p<0.05$) (Fig. 1a). This increase in ethylene production after 72 h was not observed in fruits treated with GSNO at 10 μM. In this case, papayas maintained a constant ethylene production after 48 h ($p\geq0.05$), reaching 1.3 μL $C_2H_4{\cdot}kg^{-1}{\cdot}h^{-1}$ at 72 h.

There was reduction in the respiration of all fruits when considering the first 48 h of storage at 25°C. After this period, an increase in respiration was observed, and after 72 h it was found that fruits treated with water and with GSNO at 10 μM showed lower respiration rate, average of 27.1 mL $CO_2{\cdot}kg^{-1}{\cdot}h^{-1}$, while fruits treated with GSNO at 100 and 1,000 μM reached higher values, with average of 33 mL $CO_2{\cdot}kg^{-1}{\cdot}h^{-1}$ ($p<0.05$) (Fig. 1b).

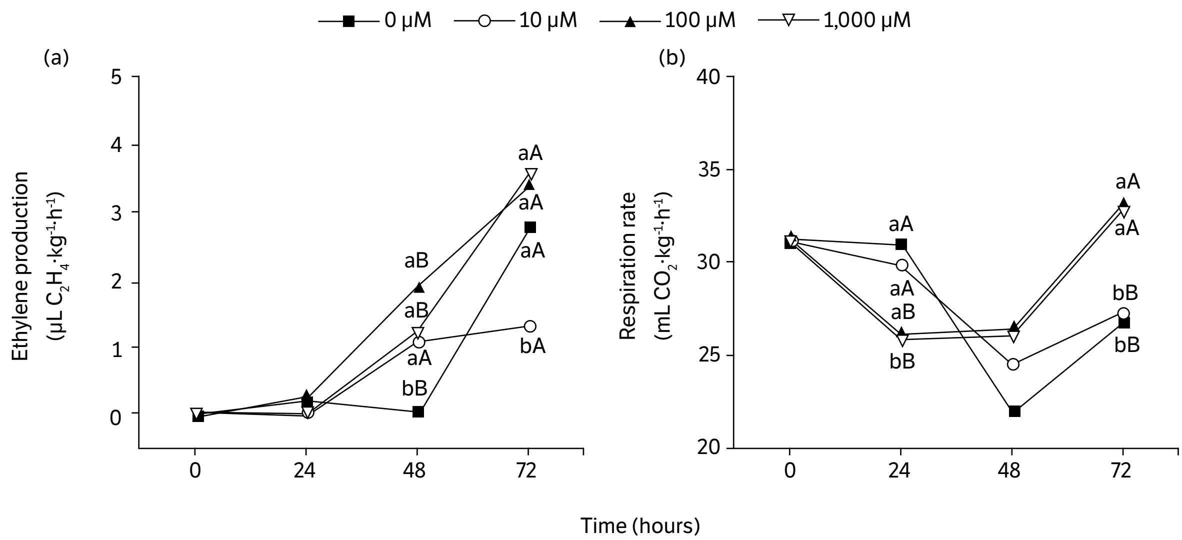

Figure 1. (a) Ethylene production and (b) respiration rate of Golden papaya sprayed with water (0 μM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, and 1,000 μM and stored at 25±2°C and 80-90% relative humidity for 72 h. Data represent the mean value of six replications. Different lowercase letters indicate significant differences among doses, and different uppercase letters indicate significant differences among the evaluation times (Scott-Knott test, $p<0.05$).

Pulp firmness was reduced after 72 h regardless of treatments ($p<0.05$), and control fruit, with an initial firmness of 75.4 N, reached 22 N after 72 h of storage at 25°C. Fruits treated with GSNO at 100 and 1,000 μM also showed reduced firmness during storage, reaching 25.8 N after 72 h. Papaya treated with 10-μM GSNO showed higher pulp firmness in all evaluations ($p<0.05$), reaching 46.5 N after 72 h (Fig. 2a), a firmness two times greater when compared to the average of the other treatments.

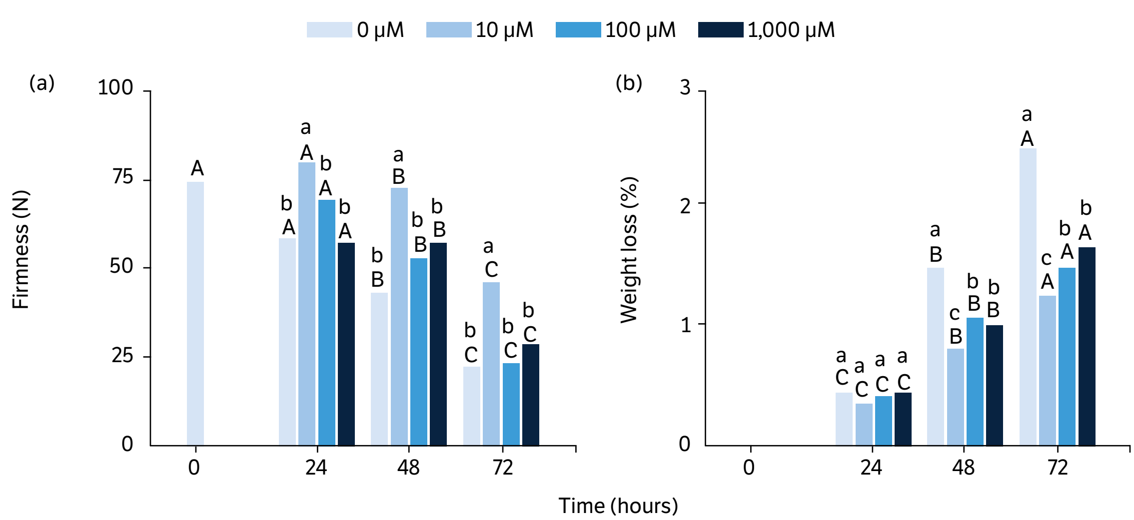

Figure 2. (a) Firmness and (b) weight loss of Golden papaya sprayed with water (0 μM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, and 1,000 μM and stored at 25±2°C and 80-90% relative humidity for 72 h. Data represent the mean value of five (firmness) and ten (weight loss) replications. Different lowercase letters indicate significant differences among doses, and different uppercase letters indicate significant differences among the evaluation times (Scott-Knott test, $p < 0.05$).

Regardless of the concentration, the application of GSNO reduced the fruit weight loss after 48 h of storage at 25°C. Control fruits and treated with 10-μM GSNO showed, respectively, the highest and the lowest weight loss in all evaluations. After 72 h, untreated fruits had lost 2.5% of weight, and those treated with GSNO at 100 and

1,000 µM lost average of 1.6%. Fruits treated with 10 µM GSNO lost 1.2% of weight, 48% less than then control fruits weight loss ($p<0.05$) (Fig. 2b).

The GSNO did not interfere on skin color of papaya. Regardless of the treatment, the fruits lost a little green color, reaching 92.1 °Hue after 72 h at 25°C (Figs. 3a and S1). Chlorophyll fluorescence, after 48 h of storage, did not change, regardless the treatment ($p\geq0.05$) (Table S1).

There was no variation of soluble solids (SS) along the 72-h storage at 23ºC. Comparing GSNO doses, there were no significant differences among the treatments. Non-treated fruits had the lowest soluble solids content from the 24th h up to the end of storage ($p\geq0.05$) (Fig. 3b). From the 48-h storage, there was reduction of titratable acidity in all fruits, regardless the applied treatment. There were no significant differences among the treatments ($p\geq0.05$) (Fig. 3c). GSNO was responsible for increasing the concentration of ascorbic acid in papayas. Considering the entire period analyzed, control fruits had average of 47.4 mg·100g^{-1}, while the GSNO treated fruits had average of 62.4 mg·100g^{-1} (Fig. 3d).

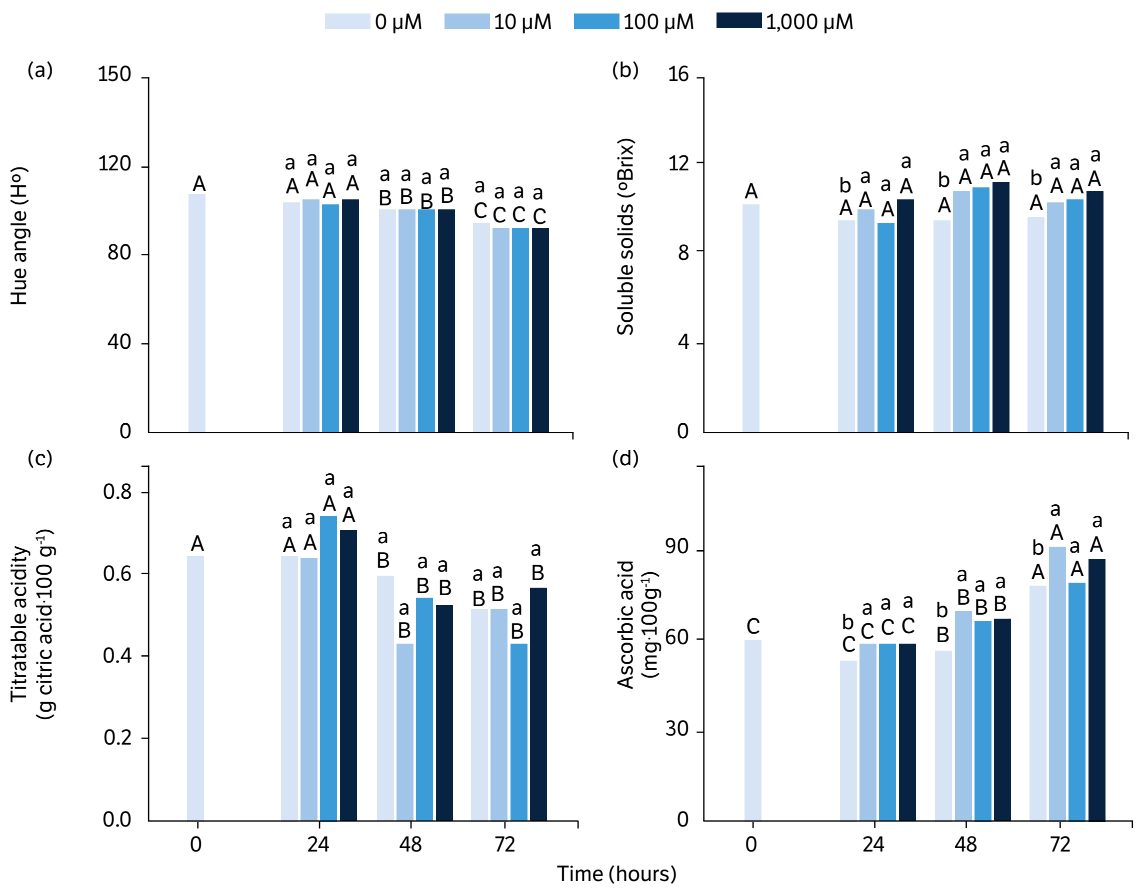

Figure 3. (a) Skin color, (b) soluble solids, (c) titratable acidity and (d) ascorbic acid of Golden papaya sprayed with water (0 µM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, and 1,000 µM and stored at 25±2 °C and 80-90% relative humidity for 72 h. Data represent the mean value of eight (skin color) and five (soluble solids, titratable acidity, and ascorbic acid) replications. Different lowercase letters indicate significant differences among doses, and different uppercase letters indicate significant differences among the evaluation times (Scott-Knott test, $p<0.05$).

The application of GSNO did not interfere in the content of MDA, in the skin and pulp of papayas. There was decrease in lipid peroxidation in the pulp during the 72 h of evaluation, regardless the applied GSNO dose (Fig. 4).

It was observed that in GSNO treated fruits the content of RSNO in the skin was lower when compared to the control fruits. After 72 h, the control fruits showed 0.69 µmol·mg^{-1}, while GSNO treated fruits showed average of 0.37 µmol·mg^{-1}, with decrease in relation to the initial values (1.22 µmol·mg^{-1}). The application of GSNO did not affect the RSNO content of the papaya pulp. Regardless the dose, there was reduction in the amount of RSNO assessed during the analysis period (Fig. 4).

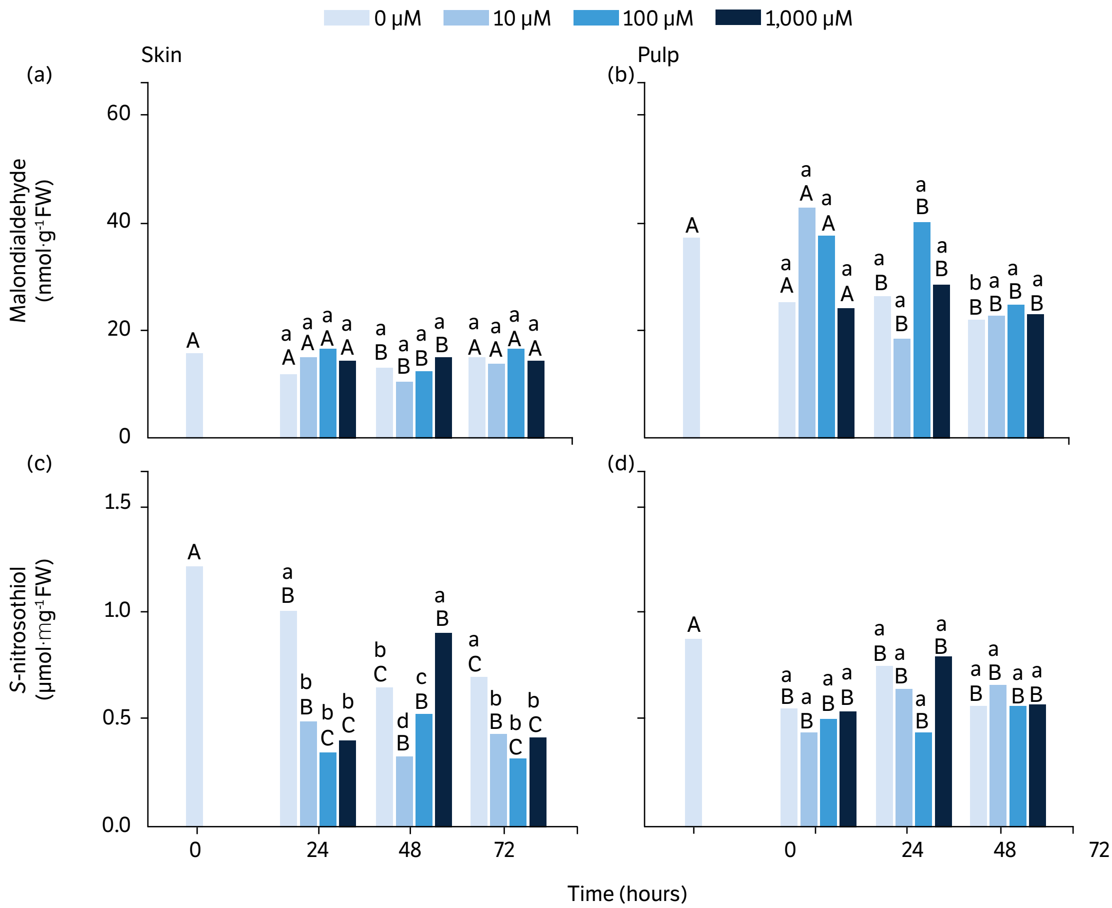

Figure 4. (a and b) Malondialdehyde (MDA) and (c and d) *S*-nitrosothiols (RSNO) in (a and c) skin and (b and d) pulp of Golden papaya sprayed with water (0 µM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, and 1,000 µM and stored at 25±2°C and 80-90% relative humidity for 72 h. Data represent the mean value of four replications. Different lowercase letters indicate significant differences among doses, and different uppercase letters indicate significant differences among the evaluation times (Scott-Knott test, $p<0.05$).

The antioxidant activity of the skin of GSNO-treated fruit was higher when compared to control fruit after 24 h, no matter the concentration. In addition, there was increase AA in the pulp of the fruits treated with 10- and 100-µM GSNO, while AA of the 1,000-µM GSNO treated fruits showed decline (Fig. 5).

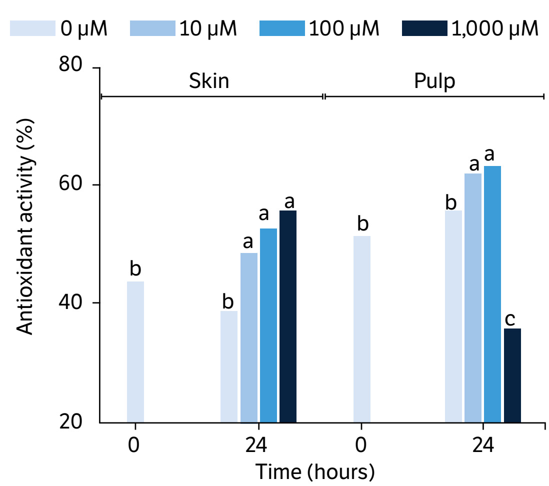

Figure 5. Total antioxidant activity (AA) in skin and pulp of Golden papaya sprayed with water (0 µM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, and 1,000 µM and stored at 25±2°C and 80-90% relative humidity for 24 h. Data represent the mean value of four replications. Different lowercase letters indicate significant differences among doses, including the characterization (0 h) (Scott-Knott test, $p<0.05$).

DISCUSSION

The results obtained in this study showed that papayas sprayed with 10-μM GSNO produced almost three times less ethylene when compared to the fruits of the other treatments (Fig. 1a). Generally, when applied for a short period and at low concentrations, NO delays fruits ripening (Corpas and Palma 2018). NO application activates the antioxidant system (Rodríguez-Ruiz et al. 2017, Shi et al. 2019, Zhao et al. 2020), induces the defense system against pathogens (Stangarlin et al. 2011), and regulates sugar and energy metabolism (Manjunatha et al. 2012, Shi et al. 2019). Countless studies have concluded that NO operates as an antagonistic molecule to ethylene, reducing its production and, therefore, delaying ripening (Shi et al. 2019, Steelheart et al. 2019). NO can decrease ethylene production by direct and indirect routes. The direct pathways can be:

- Through the transcriptional regulation of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase enzyme genes (Nakatsuka et al. 1998);
- Through *S*-nitrosation causing post-translational modification of the enzyme methionine adenosyltransferase (MAT) (Zhu and Zhou 2007);
- By inhibiting hydrogenation of C_2H_6 (Manjunatha et al. 2012);
- Stoichiometric reduction of ACC in 1-malonylaminocyclopropane-1-carboxylic acid (MACC) (Lindermayr et al. 2006);

NO and 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO) form a binary complex (ACCO-NO) that is chelated by ACC to produce a stable ternary ACC-ACCO-NO complex, preventing the formation of ethylene (Manjunatha et al. 2012, Shi et al. 2019, Zhao et al. 2020).

The indirect pathways can regulate ethylene through the coordination of other signal molecules such as salicylic acid (Zottini et al. 2007), polyamines (Rümer et al. 2009), and cytokinins (Manjunatha et al. 2012).

Papaya that was sprayed with higher doses of GSNO (100 and 1,000 μM) had no reduction in ethylene production and even higher respiration (Fig. 1b). These doses did not promote reduction in firmness loss either (Fig. 2a). According to some studies (Steelheart et al. 2019, Zhao et al. 2020), considering the physical and physiological characteristics of the fruits, lower doses tend to be more effective in controlling ripening. Zhu and Zhou (2007), studying the ripening of peaches NO fumigated at 5 and 10 $\mu L \cdot L^{-1}$ and stored at 25°C, observed reduction in ACC and ethylene production. In higher doses (15 $\mu L \cdot L^{-1}$), fruit toxicity was found when the authors analyzed the interaction of reactive oxygen species with nitric oxide.

It is also interesting to notice that papayas sprayed with 10-μM GSNO, in addition to producing less ethylene (Fig. 1a), maintained the firmness during all evaluations, when compared to the other treatments (Fig. 2a). In the softening process of papaya, several hydrolytic enzymes are responsible for this solubilization, mainly polygalacturonase, β-galactosidase and pectinamethylesterase (PME) (Lazan et al. 1995). The loss of firmness is closely related to the activity of these pectinolytic enzymes, whose activity is related to ethylene with different degrees of dependence (Jeong et al. 2002). In addition to the integrity of the cell wall, the fruit firmness can also be determined by cell turgor (Luza et al. 1992).

All GSNO sprayed fruits showed less weight loss (Fig. 2b), but only fruits that received GSNO at 10 μM maintained its firmness (Fig. 2a). Therefore, we can indicate that the firmness maintenance probably occurred due to the reduced ethylene production, and not due to the reduced fruit transpiration. In any case, the lower weight loss observed in all treatments can be a benefit when considering postharvest management, since the loss of fresh weight is an important factor in determining fruit quality. The loss of 7% of the initial weight is sufficient for losses in the brightness and appearance of papayas skin (Paull and Chen 1989). As the velocity of weight loss in untreated papayas was much higher than other treatments in the first 72 h, this benefit should be considered. Ku et al. (2000) studied the weight loss in various NO treated vegetables and attested to a reduction of 14 to 26%. The authors concluded that the exogenous NO application could then stimulate vegetables to act in a way to reduce transpiration. For Deng et al. (2013), NO treatment in apples significantly preserved the membrane integrity and maintained cell compartmentalization, also reducing the water and weight loss.

Even with a decrease in ethylene production and a delay in firmness loss, fruits treated with 10-μM GSNO did not have any change in the skin color (Fig. 3a). Some studies have observed that ethylene does not always directly influence fruit color changes. According to Flores et al. (2001), while the loss of green color in melon is totally dependent on ethylene, the synthesis of yellow pigments is not, happening even without the presence of the hormone. Bron and Jacomino (2006) suggest that pulp softening in Golden papaya is more dependent on ethylene than the development of the skin color.

The chlorophyll fluorescence technique is a complementary and non-destructive evaluation, which can be combined and associated with other fruit quality evaluations (Bron et al. 2004). Bron et al. (2004), evaluating stages of ripening of papayas, observed that the chlorophyll fluorescence parameters decreased as the °Hue decreased, and other authors also reported this correlation (DeEll et al. 1996). In this study, there were no changes neither in the fluorescence parameters of chlorophyll (Table S1) nor in the skin color (Fig. 4a).

Unlike what was detected for color and titratable acidity, the amount of ascorbic acid and soluble solids was influenced by NO application (Figs. 3b and 3d). Despite the small difference, there was an increase in SS in NO sprayed fruits (Fig. 3b). Recent studies have shown that treatment with exogenous NO is related to changes in sugar metabolism due to increased activities of sucrose phosphate synthase (SPS), SS and neutral invertase (NI), decreased activity of acid invertase (AI), maintaining higher levels of glucose, fructose and sucrose (Ma et al. 2019, Shi et al. 2019, Zhao et al. 2020).

Considering the entire period analyzed, NO treated fruits showed little more than 20% ascorbic acid, when compared to control fruit (Fig. 3d). It has been shown that NO at 5 ppm causes an increase of about 40% in the ascorbate content in peppers (Rodríguez-Ruiz et al. 2017), thus increasing the nutritional fruit content. According to Rodríguez-Ruiz et al. (2017), NO has a strong influence on the last stage of ascorbate synthesis in mitochondria during fruit ripening as a consequence of a simultaneous increase in the activity of *L*-galactone-1, 4-lactone dehydrogenase (GalLDH), and gene expression.

Regarding biochemical changes, it is known that the ripening process itself induces an oxidative burst (Manjunatha et al. 2012, Ma et al. 2019, Zhang et al. 2019, Zhao et al. 2020), which can damage the cellular composition, such as proteins and lipids, leading to loss of membrane integrity and functionality. Thus, maintaining the balance of reactive oxygen species (ROS) production at the cellular level is important for the conservation of fruit quality. In this sense, there is evidence indicating the role of NO in suppressing these ROS (Zhang et al. 2019, Zhao et al. 2020), although their effects are paradoxical and seem to depend on concentration (Delledonne et al. 2001). In the present study, lipid peroxidation was not verified in the skin and pulp of papaya for 72 h, since the MDA levels were similar to those seen in the fruit characterization, regardless of GSNO application (Figs. 4a and 4b). However, the effects of oxidative stress on proteins and on the integrity and function of DNA cannot be excluded (Oliveira et al. 2010). In addition, it has been reported that, during the early stages of fruit ripening, the antioxidant system protects the fruits from the destructive effects of progressive oxidative stress.

In this study, GSNO application increased the antioxidant activity of the fruit skin and pulp. NO treated fruits showed little more than 20% AA in skin, when compared to control fruit (Fig. 5). High contents of antioxidant activity in NO-treated peaches could also improve the fruit qualities (Saba and Moradi 2017). Fruits treated with 1,000 μM of GSNO, on the other hand, negatively affected AA, with reduction of 50% AA, when compared to other doses (Fig. 5). This result demonstrates that higher than optimal doses can have undesirable effects.

In addition, higher levels of MDA were found in the pulp compared to the fruit skin (Figs. 4a and 4b). In fact, this difference between tissues occurs. Resende et al. (2012) found a higher content of MDA in the papaya pulp than in the peel, due to the lower activity of antioxidant enzymes such as catalase (CAT) in the pulp. Also, in relation to short-term biochemical changes, there was reduction in the content of S-nitrosothiols (RSNO), in the pulp and in the skin, in all treatments compared to the characterization. This decrease was maintained throughout the experimental period, with no differences between treatments (Figs. 4c and 4d). S-nitrosation is an important post-translational modification that occurs due to the covalent addition of a portion of NO to a cysteine residue, affecting the activity and stability of the protein (Silveira et al. 2019). Studies have shown that the content of RSNO is involved in the regulation of the activity of S-nitrosoglutathione reductase (GSNOR), a NO-degrading enzyme, and therefore this enzyme could be inhibited via S-nitrosation, with a consequent increase in total RSNOs (Guerra et al. 2016).

CONCLUSION

The GSNO application at 10 μM by spray can potentially preserve the quality characteristics of Golden papaya, mainly due to the lower ethylene production, reduction in fruit softening and weight loss, besides showing an increase in total antioxidant activity production. In addition, the greatest short-term changes were observed for the physical-chemical and physiological variables in these experimental conditions. Thus, understanding the short-term physiological, physical-chemical, and biochemical regulations in the face of exogenous supplementation of NO donors, such as GSNO, is of great interest, since they may contribute to the development of efficient strategies, aiming to reduce the limitations of commercialization.

AUTHORS' CONTRIBUTION

Conceptualization: Bron, I. U.; Valentini, S. R. T.; Silveira, N. M.; Seabra, A.B.; Veiga, J. C.; Machado, M. R.; **Formal Analysis:** Machado, M. R.; Veiga, J. C.; Silveira, N. M.; Boza, Y. E. A. G.; Pelegrino, M. T.; **Supervision:** Bron, I. U.; Valentini, S. R. T.; **Resources:** Seabra, A. B.; Bron, I. U.; **Writing – Original Draft:** Bron, I. U.; Seabra, A. B.; Machado, M. R.; Veiga, J. C.; Silveira, N. M.; Cia P.; **Writing – Review & Editing:** Cia, P.; Bron, I. U.; Valentini, S. R. T.; Silveira, N. M.; Veiga, J. C.; Machado, M. R.; Boza, Y. E. A. G.; Pelegrino, M. T.; Seabra, A. B.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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SUPPLEMENTARY MATERIAL

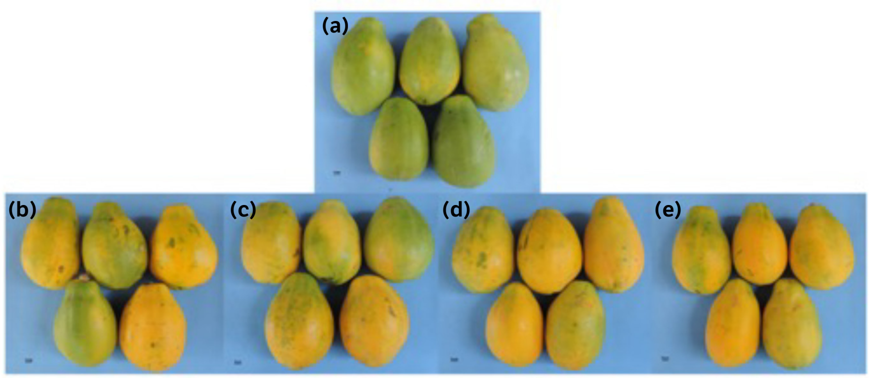

Figure S1. (a) Visual aspect of Golden papaya before treatment with nitric oxide donor *S*-nitrosoglutathione (GSNO) (characterization), and 72 h after GSNO treatment at (b) 0, (c) 10, (d) 100, and (e) 1,000 μM at 25±2 °C and 80-90% relative humidity.

Table S1. Hue angle (H°) and minimal (Fo), maximal (Fm), variable (Fv) and variable maximal (Fv/Fm) fluorescence yield in papaya fruits sprayed with water (0 μM) or nitric oxide donor *S*-nitrosoglutathione (GSNO) at 10, 100, or 1,000 μM and stored at 25±2ºC and 80-90% relative humidity for 48 h*.

Variables	0 μM	10 μM	100 μM	1,000 μM
Hue angle (H°)	100.88±2.66a	100.48±3.33a	99.95±2.80a	100.59±3.43a
Fo	179.43±29.17a	188.74±22.67a	180.51±26.33a	157.79±19.20a
Fm	891.49±112.25a	946.33±84.09a	891.97±66.75a	1039.00±185.75a
Fv	712.06±86.52a	757.59±74.25a	711.46±43.68a	881.22±170.62a
Fv/Fm	0.79±0.016a	0.78±0.027a	0.79±0.017a	0.83±0.013a

*The data represent the mean value of ten replications ± standard deviation. Different lowercase letters indicate statistical difference among treatments (Scott-Knott test, p <0.05).