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Treatment with nitric oxide in controlled atmosphere storage to preserve the quality of 'Laetitia' plums

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ABSTRACT

This study aims to evaluate the effect of nitric oxide (NO) treatment under controlled atmosphere (CA) on quality of 'Laetitia' plums, mainly on internal browning. The experiment followed a completely randomized design, with five repetitions and a 25-fruit experimental unit. The evaluated treatments were: control (no NO); NO at 2 μL L^{-1} (low dose) applied every 5 days of storage; NO at 5 μL L^{-1} (medium dose) applied every 5 days of CA storage; and NO at 10 μL L^{-1} (high dose) applied only at the beginning of CA storage. The treatments with NO in CA storage did not delay the loss of flesh firmness and compression force of the fruit. Treatments with NO reduced ethylene production and respiratory rates and delayed the skin color evolution, especially when applied at medium dose every 5 days of CA storage and high dose at the beginning of CA storage. The treatment with low dose of NO every 5 days of CA storage was the most effective to reduce the internal browning of 'Laetitia' plums.

1. Introduction

The 'Laetitia' plum is a very attractive fruit, medium to large in size and oval shaped, with purple-red epidermis coloration and yellow colored flesh (Fioravanço, Nachtigall & Andolfato, 2015). The fruit exhibits a climacteric pattern of respiration and ethylene production and rapid color and texture change after harvest (Argenta, Amarante, Shirayama, Scolaro, & Ayub, 2011; Steffens et al., 2017). The fruit matures quickly, which implies the supply of a large volume of fruit in a short period of time. To increase and regulate the supply period and decrease postharvest losses, it is necessary to store part of the production (Alves, Steffens, Amarante, Pavanello, & Brackmann, 2009).

Controlled atmosphere (CA) storage delays the ripening of 'Laetitia' plums stored for up to 60 days. CA storage delays the loss of flesh firmness and skin color evolution and decreases the severity of the internal browning (Steffens et al., 2017). However, after storage, there was a rapid skin color evolution and a high incidence of internal browning. Internal browning is considered a cold damage in stored plums (Singh & Singh, 2013b) and the main problem in the storage of 'Laetitia' plums. It arises from an oxidative process related to the production of reactive oxygen species (ROS), which cause lipid peroxidation, and a decrease in the efficiency of antioxidant systems, resulting in damage to cell membranes (Singh & Singh, 2013a; 2013b).

Since internal browning results from oxidative stress induced by low temperature storage and is aggravated by ethylene (Steffens et al., 2017), postharvest management strategies that decrease the ethylene synthesis and/or action and that increase the fruit's antioxidant activity may become promising alternatives to improve the quality maintenance of stored plums (Martin et al., 2018; Nunes et al., 2019).

Nitric oxide (NO) is a reactive nitrogen species naturally produced in living cells and involved in the regulation of several processes during plant development (Manjunatha, Lokesh & Neelwame, 2010; Buet et al., 2021). Its action on ripening and senescence can be attributed, at least in part, to the decrease in ethylene synthesis through inhibition of the enzymes 1-aminocyclopropane 1-carboxylic acid synthase (ACS) and 1-aminocyclopropane 1-carboxylic acid oxidase (ACO), and ethylene sensitivity (Manjunatha, Gupta, Lokesh, Mur, & Neelwame, 2012; Zuccarelli et al., 2021). A possible mechanism of action of NO on the ripening was purpose by Buet et al. (2021). According to authors, NO blocks ethylene increase mediated by the inhibition of ACO activity, probably through metal-nitrosylation. Another proposed mechanism is the inhibition of the activity of ACS and S-adenosylmethionine

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synthetase (SAMS), through nitrosation. NO is also capable of impact on redox balance, influencing ROS in fruit ripening. Through these actions, NO contributes to the modulation of fruit ripening during postharvest storage.

In peaches, NO gas decreased ethylene production, delayed loss of flesh firmness, and maintained overall fruit quality (Flores et al., 2008; Han et al., 2018; Huang, Ullah, Zhou, Yi, & Zhao, 2019; Cai, Han, Yu, Ma, & Yu, 2020). In 'Amber Jewel' plums, NO applied early in cold storage delayed ripening and reduced internal browning (Singh, Singh, & Swinny, 2009). However, NO in an O_2 environment is unstable and turns into NO_2 (Liu, 2016). Under CA storage conditions, due to the low partial pressure of O_2 (1 kPa), the treatment with NO might guarantee its persistence and effectiveness throughout storage. In addition, we propose the hypothesis that the fruit's exposure to lower concentrations of NO (1–5 μL L^{-1}) for a longer period of time has a greater effect on delaying ripening and decreasing physiological disorders than a higher dose (10 μL L^{-1}) applied at the beginning of CA storage. The CA conditions for 'Laetitia' plum storage, with 1 kPa of O_2, provide a suitable environment for the NO application and reapplication at lower doses. The NO's effect depends on its concentration, the fruit species (Manjunatha, Lokesh, & Neelwarne, 2010), and possibly the time of exposure to the gas (Buet et al., 2021). There are no studies evaluating the effect of NO on the quality maintenance of 'Laetitia' plums, and as an alternative to enhance the beneficial effects of CA storage.

This study aims to evaluate the effect of NO applied at different doses and storage times in CA on the quality maintenance of 'Laetitia' plums, mainly on the internal browning.

2. Materials and method

2.1. Plant material, experimental design and treatments

'Laetitia' plums (*Prunus salicina*) were harvested (2019/2020 crop) at the ripening stage when 40–50% of the epidermis surface had a red coloration, in a commercial orchard located in Urubici (28°00′54″ S; 49°35′30″ W), State of Santa Catarina, Southern Brazil. Soon after harvest, the fruit were transported to the laboratory. Fruits with mechanical damage or damage caused by insects and diseases were discarded. The experiment followed a completely randomized design, with five repetitions and a 25-fruit experimental unit. The fruit showed the following ripening attributes at harvest: skin color ($h°$) on more intense and less intense red regions of 65.3 and 82.4, respectively, flesh firmness of 77.1 N, soluble solids content of 10.8° Brix, and titratable acidity of 1.62% malic acid.

The following treatments were evaluated: control (0 μL L^{-1} of NO); 2 μL L^{-1} of NO applied every 5 days of CA storage; 5 μL L^{-1} of NO applied every 5 days of CA storage; and 10 μL L^{-1} of NO applied only at the beginning of CA storage. All fruit were stored in 60 L capacity experimental micro chambers placed inside cold storage for 55 days. CA condition in all treatments was 1 kPa of O_2 + < 0.5 kPa of CO_2, 1.5 ± 0.2 °C, and RH of 92 ± 2%.

CA condition was stablished by injecting gaseous nitrogen from high-pressure cylinders to reduce O_2 down to 5 kPa, and over the next 3 days of storage the O_2 gradually decreased to 1 kPa through O_2 consumption by fruit respiration.

NO was applied with a standard gas mixture (1000 μL L^{-1} of NO + balance N_2), stored in a high-pressure cylinder. NO was added inside the CA micro chambers in an amount necessary to reach the pre-established NO concentrations (2, 5, and 10 μL L^{-1}). We performed the first application on the third day of storage when the O_2 partial pressure reached 1 kPa. NO was reapplied every 5 days, from the first application, in the treatments with NO concentrations of 2 and 5 μL L^{-1}.

2.2. Measurements

Fruits were evaluated for respiratory and ethylene production rates and peel color at the chamber opening. After plus 3 days of shelf life, fruit were evaluated for respiratory and ethylene production rates, flesh firmness, fruit compression force, soluble solids content (SSC), titratable acidity (TA), skin color, and incidence and severity of internal browning (browning index and flesh color attributes).

Respiratory and ethylene production rates were evaluated by analyzing CO_2 and C_2H_4 concentrations accumulated inside containers (4.1 L volume with approximately 1000 g of fruit) closed for 30 min. CO_2 reading was performed in an electronic gas analyzer (Schelle®, Germany). C_2H_4 was analyzed with a gas chromatograph (PerkinElmer, Clarus® 580 model, USA), equipped with a 3 m long Porapak N® column (80–100 mesh) and flame ionization detector. The column, detector, and injector temperatures were 90, 240, and 120 °C, respectively. The nitrogen, hydrogen, and synthetic air flows were 70, 45, and 450 mL min^{-1}, respectively. Respiratory and ethylene production rates values were expressed as ηmol CO_2 kg^{-1} s^{-1} and ρmol C_2H_4 kg^{-1} s^{-1}, respectively.

Skin and flesh color (attributes L, C, and $h°$, corresponding to lightness, chroma and hue angle, respectively), SSC (%), TA (%), red color index (RCI; 1–4), flesh firmness (N), fruit compression force (N), and internal browning incidence (%) and severity (1–3) were evaluated according to the methodology described in Stanger, Steffens, Amarante, Brackmann, and Anese (2017).

2.3. Statistical analysis

Data were subjected to analysis of variance (ANOVA) and means of treatments compare by LSD test ($p < 0.05$). Percentage data were transformed through the arcsine formula ($[(x+0.5)/100]^{1/2}$) before ANOVA.

3. Results and discussion

The ethylene production rate at the chamber opening was lower in plums treated with 2 μL L^{-1} of NO every 5 days of CA storage than the other treatments. However, after 3 days of shelf life, all treatments with NO showed a lower ethylene production rate than the control (Table 1). NO effects on ethylene biosynthesis is due to the stoichiometric decrease of ACC to 1-(malonylamino) cyclopropane-1-carboxylic acid (MACC) and to the formation of a stable ternary complex "ACC - ACC oxidase - NO", which hinders the oxidation of ACC to ethylene (Manjunatha et al., 2012). NO pre-treatment to storage also reduced ethylene production in 'Cripps Pink' apples (Steffens, Miqueloto, et al., 2021) and tomato

Table 1
Ethylene production and respiratory rates of 'Laetitia' plums stored in controlled atmosphere (1.0 kPa O_2 + <0.5 kPa CO_2; 1.5 ± 0.2 °C and RH 92 ± 2%) and treated with nitric oxide (NO), after 55 days of storage, followed by 3 days of shelf life (23 ± 3 °C and RH 60 ± 5%).

Treatment	Ethylene production rate (ρmol C_2H_4 kg^{-1} s^{-1})		Respiratory rate (ηmol CO_2 kg^{-1} s^{-1})	
	Removal from CA storage	3 days of shelf life	Removal from CA storage	3 days of shelf life
NO 0 μL L^{-1}	0.51a[c]	1.30a	269.4 a	65.8 a
NO 2 μL L^{-1}[a]	0.41 b	0.78b	253.2 a	50.2 b
NO 5 μL L^{-1}[a]	0.54 a	0.33c	187.7 b	47.0 b
NO 10 μL L^{-1}[b]	0.54 a	0.30c	213.5 b	47.1 b
CV (%)	13.8	43.8	11.1	10.1

[c] Means followed by the same letters are not different by the LSD test ($p < 0.05$).
[a] Nitric oxide applied at the establishment of the CA storage and every 5 days until the end of CA storage.
[b] Nitric oxide applied at the beginning of CA storage (when the storage atmosphere was established).

(Zuccareli et al., 2021).

The respiratory rate at the chamber opening was lower in plums treated with 5 μL L^{-1} of NO every 5 days and with 10 μL L^{-1} of NO at the beginning of CA storage. However, after 3 days of shelf life, all fruits treated with NO showed a lower respiratory rate than the control (Table 1). Similar results have been reported in mango, kiwi, papaya, peach, apples and plum, where NO-treated fruit had reduced ethylene production and respiratory rates during storage (Guo et al., 2013; Huang et al., 2019; Singh et al., 2009; Zaharah and Singh, 2011; Zhu, Zhou, & Zhu, 2010; Steffens et al., 2021a, 2021b). Other studies also reported that treatment with NO decreases the respiratory rate of 'Galaxy' apples (Brackmann et al., 2017) and 'Amber Jewel' plums (Singh et al., 2009). NO decreases cellular respiration through the reversible inhibition of cytochrome oxidase (Pandey et al., 2019).

NO, regardless of the dose and time of application, decreased fruits' RCI at the chamber opening in comparison to the control. The lower RCI in the fruits treated with NO indicate delayed development of the red color on the fruit surface. However, after 3 days of shelf life, RCI was not different between treatments (Table 2). The color attributes *L*, *C*, and *h*° show less intensity of the red color in the skin of the fruits treated with NO, especially in the treatments with 10 μL L^{-1} of NO applied at the beginning of CA storage and 5 μL L^{-1} of NO applied every 5 days of CA storage (Table 2). The change in the skin color of the plums involves the degradation of chlorophylls and the synthesis of carotenoid pigments, with the transformation of chloroplasts into chromoplasts. In broccoli, NO delayed the loss of chlorophyll *a* (Eum, Kim, Choi, & Lee, 2009). Therefore, the less intense red color of the skin in NO-treated fruit might reflect a higher chlorophyll content. NO could be a protective molecule, preserving the chloroplast membrane against the toxicity of reactive oxygen species (Lazxalt, Beligni & Lamattina, 1997). Singh et al. (2009) also observed that NO applied at the beginning of cold storage delayed skin color changes in 'Amber Jewel' plums.

Table 2
Red color index (RCI) and skin color attributes of 'Laetitia' plums stored in controlled atmosphere (1.0 kPa O_2 + <0.5 kPa CO_2, 1.5 ± 0.2 °C, and RH 92 ± 2%) and treated with nitric oxide (NO), after 55 days of storage, followed by 3 days of shelf life (23 ± 3 °C and RH 60 ± 5%).

Treatment	RCI (1–4)	Skin color attributes					
		On more intense red region			On less intense red region		
		L	*C*	*h*°	*L*	*C*	*h*°
Removal from CA storage							
NO 0 μL L^{-1}	3.9 a[c]	35.9 b	35.7 a	22.0 a	50.8 c	34.8 a	51.1 c
NO 2 μL L^{-1}[a]	3.7 b	36.4 b	36.8 a	22.4 a	52.0 bc	34.3 ab	49.7 c
NO 5 μL L^{-1}[a]	3.7 b	36.6 ab	35.9 a	23.5 a	53.7 ab	32.3 c	57.8 b
NO 10 μL L^{-1}[b]	3.7 b	38.4 a	35.7 a	23.8 a	56.1 a	33.3 bc	65.0 a
CV (%)	4.1	3.9	3.4	8.7	3.9	2.4	5.2
3 days of shelf life							
NO 0 μL L^{-1}	3.9 a	33.2 b	33.5 a	21.5 a	46.2 b	33.8 a	47.3 b
NO 2 μL L^{-1}[a]	3.8 a	33.7 ab	34.2 a	22.0 a	46.4 ab	33.2 ab	48.2 b
NO 5 μL L^{-1}[a]	3.8 a	34.1 ab	33.9 a	21.7 a	46.6 ab	32.1 b	49.8 ab
NO 10 μL L^{-1}[b]	3.8 a	35.4 a	34.9 a	23.0 a	49.7 a	32.4 ab	55.5 a
CV (%)	2.2	3.8	4.1	5.5	5.3	3.3	5.6

[c] Means followed by the same letters are not different by the LSD test ($p < 0.05$).

[a] Nitric oxide applied at the establishment of the CA storage and every 5 days until the end of CA storage.

[b] Nitric oxide applied at the beginning of CA storage (when the storage atmosphere was established).

Fruit treated with 5 μL L^{-1} of NO every 5 days of CA storage had the lowest flesh firmness and fruit compression force values, evaluated after CA storage, followed by 3 days of shelf life (Table 3). This might be due to an overdose of NO, with 10 applications of the 5 μL L^{-1} of NO during the whole CA storage period. Also, high concentration of NO applied at the beginning of cold storage caused a decrease in flesh firmness in peaches (15 μL L^{-1}), possibly by the formation of reactive nitrogen species with superoxide ions (Zhu, Liu, & Zhou, 2006). However, in 'Amber Jewel' plums, Singh et al. (2009) obtained higher flesh firmness in fruit treated with NO at the beginning of cold storage. The authors attributed this effect to lower cell wall enzyme activity resulting from reduced ethylene biosynthesis. Positive results of NO on the maintenance of flesh firmness were also observed in peaches (Flores et al., 2008) and kiwi (Zhu, Sun, Liu, & Zhou, 2008). In our study, the absence of the NO's positive effect on the flesh firmness and fruit compression force may be related to the fact that fruit were stored in a CA storage system that has been shown effective to maintain the flesh firmness of 'Laetitia' plums (Steffens et al., 2017). Therefore, dose response to NO should be evaluated for each crop and cultivar, as well as storage condition.

TA evaluated after CA storage, followed by 3 days of shelf life, was not affected by treatments, while SS was highest in the control (Table 3). Fruit treated with 10 μL L^{-1} of NO at the beginning of CA storage had the lowest SS value, which did not differ from the treatment with 5 μL L^{-1} of NO applied every 5 days of CA storage. Treatment with 2 μL L^{-1} of NO every 5 days of CA storage showed intermediate results (Table 3). Different results were reported by Singh et al. (2009), applying NO only at the beginning of cold storage and keeping the fruit in air. The authors observed that NO application did not influence SS content but did maintain higher TA. According to Singh et al. (2009), the ripening of plums involves a slight increase in SS levels, so the lower SS values in 'Laetitia' plums treated with NO may be due to the action of the gas in delaying fruit ripening.

The treatment with 2 μL L^{-1} of NO every 5 days of CA storage had the lowest incidence and severity (lower flesh browning index, lower *h*° value, and higher flesh *L* value) of internal browning, assessed after storage, followed by 3 days of shelf life (Table 3). The treatments with 10 and 20 μL L^{-1} of NO at the beginning of cold storage reduced the severity of internal browning in 'Amber Jewel' plums (Singh et al., 2009). Internal browning is due to oxidative stress that causes damage to cellular membranes and subsequent loss of cellular compartmentalization. The internal browning may be related to the disintegration of tissue membranes resulting in enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to *o*-quinones, which are brown-colored polymers (Singh et al., 2009). Studies have shown that NO can reduce the oxidative stress by inducing enzymes such as SOD, POD and CAT, and suppressing LOX (Manjunatha et al., 2010, 2012; Buet et al., 2021; Jiménez-Muñoz et al., 2021). Pristijono, Wills, and Golding (2006) reported a decrease of flesh browning in minimally processed 'Granny Smith', 'Royal Gala', 'Golden Delicious', 'Sundowner', 'Fuji', and 'Red Delicious' apples treated with NO. The authors suggested that NO modulates the oxidative activity of PPO and other oxidative enzymes, minimizing the browning reactions. Pre-storage sodium nitroprusside (a NO-donor) treatment with 25 μM reduced chilling injury of zucchini fruit by S-nytrosilation of proteins and modulation of the antioxidant response (Jiménez-Muñoz et al., 2021).

The treatment with 10 μL L^{-1} of NO at the beginning of CA storage provided a higher incidence of internal browning and lower values for the flesh color attributes *L* and h^o, which did not differ from the treatment with 5 μL L^{-1} of NO applied every 5 days of CA storage. The treatment with 10 μL L^{-1} of NO at the beginning of CA storage also had a higher internal browning index (Table 3). These results demonstrate a possible toxic effect of higher NO concentrations on 'Laetitia' plums. These results contradict those reported by Singh et al. (2009), showing a positive effect of NO at concentrations of 10 and 20 μL L^{-1}. However, these authors applied NO for 2 h, as opposed to our study, with NO

Table 3
Flesh firmness, fruit compression force, soluble solids (SS), titratable acidity (TA), and incidence and severity (internal browning index and flesh color attributes) of internal browning in 'Laetitia' plums stored in controlled atmosphere (1.0 kPa O_2 + <0.5 kPa CO_2, 1.5 ± 0.2 °C, and RH 92 ± 2%) and treated with nitric oxide (NO), after 55 days of storage, followed by 3 days of shelf life (23 ± 3 °C and RH 60 ± 5%).

Treatment	Flesh firmness (N)	Fruit compression force (N)	SS (%)	TA (% malic acid)	SSC/TA ratio	Internal browning (%)	Internal browning severity			
							Internal browning index (1–3)	Flesh color attributes		
								L	*C*	*h*°
NO 0 μL L^{-1}	15.1 a[c]	41.2 a	11.7 a	0.88 a	13.5 a	40.1 b[c]	1.52 b	49.9 b	21.9 a	84.6 b
NO 2 μL L^{-1}[a]	16.0 a	42.6 a	11.0 b	0.88 a	12.6 a	4.9 c	1.06 c	54.4 a	23.0 a	87.1 a
NO 5 μL L^{-1}[a]	12.4 b	33.2 b	10.9 bc	0.81 a	14.0 a	51.1 ab	1.73 b	45.1 c	20.3 b	83.7 bc
NO 10 μL L^{-1}[b]	16.1 a	43.2 a	10.4 c	0.79 a	12.8 a	64.1 a	2.09 a	42.4 c	19.1 b	82.6 c
CV (%)	8.0	6.7	3.7	12.2	12.4	29.8	12.1	4.4	5.2	1.2

[c] Means followed by the same letters are not different by the LSD test ($p < 0.05$).
[a] Nitric oxide applied at the establishment of the CA storage and at every 5 days until the end of CA storage.
[b] Nitric oxide applied at the beginning of CA storage (when the storage atmosphere was established).

applied in the CA storage environment that possibly has a long lasting effect of NO in the fruit. Thus, the NO's toxic effects might depend on dose, exposure time and the differential sensitivity among cultivars.

The treatment with 5 μL L^{-1} of NO, applied every 5 days of CA storage, showed positive results on the quality maintenance of the 'Laetitia' plum. However, control of NO concentrations throughout CA storage for each treatment was not performed. Future work should be conducted to verify the effect of fixed and controlled concentrations of NO throughout the storage period in the CA, by monitoring (and correcting if required) in the atmosphere O_2, CO_2 and NO. In this regard, a CA storage system (low O_2 and/or high CO_2 partial pressures) can be proposed to achieve the best benefits of NO.

4. Conclusions

NO had no effect on flesh firmness, but reduced ethylene production and respiratory rates, delayed changes of skin color, especially by treating with medium dose (5 μL L^{-1}) every 5 days of CA storage, and high dose (10 μL L^{-1}) at the beginning of CA storage. Low dose (2 μL L^{-1}) of NO, every 5 days of CA storage, was the most effective in decreasing the internal browning of 'Laetitia' plums.

Credit author statement

Cristiano André Steffens: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition **Grez Roberta Oliveira Santana:** Methodology, Formal analysis, Investigation, Writing – original draft **Cassandro Vidal Talamini do Amarante:** Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition **Josias Lenon Antonovviski:** Methodology, Formal analysis, Investigation, Writing – original draft **Tiago Miqueloto:** Methodology, Formal analysis, Investigation, Writing – original draft **Jéssica Mayumi Anami:** Methodology, Formal analysis, Investigation, Writing – original draft **Cristhian Leonardo Fenili:** Methodology, Formal analysis, Investigation, Writing – original draft.

Declaration of competing interest

The authors have declared no conflicts of interest for this article.

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