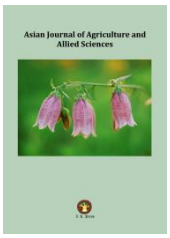

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Postharvest Treatments with Naphthalene Acetic Acid and Methyl Jasmonate to Maintain the Quality of 'Laetitia' Plums (*Prunus salicina* Lindl.) during Cold Storage

Paulo Sérgio Gularte [a*], Nair Cristiane dos Santos [b], Aquidauana Miqueloto Zanardi [b], Keli Cristina Fabiane [b], Willian Coser [a], Guilherme Stefanello [b] and Vanucci Marcos Santi [b]

[a] Agrovetergroveterinary Science Center, State University of Santa Catarina, Lages, Brazil.
[b] Federal Institute of Santa Catarina, São Miguel do Oeste, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work aimed to verify the efficiency of the phytoregulators naphthalene acetic acid (NAA) and methyl jasmonate (MeJa) in maintaining the postharvest quality of 'Laetitia' plum fruits, stored under refrigeration.
Study Design: The experimental design used was completely randomized with three treatments and five repetitions of 30 fruits/repetition.

**Corresponding author: E-mail: paulo.sgularte@gmail.com;*

Place and Duration of Study: The experiment was carried out with 'Laetitia' plums from a commercial orchard in the municipality of Catanduvas, SC (27° 4' 3'' S and 51° 39' 47'' W), during the 2017/18 harvest.
Methodology: The fruits were treated with distilled water (control), MeJa (10^{-4} M), and NAA (10 mg. L^{-1}); and stored in chambers with Biochemical Oxygen Demand (BOD), maintained at a temperature of 4±1°C and relative humidity of 85±3%, for 35 days and another three days at ambient conditions (23±1°C). The main quality attributes were evaluated after storage.
Results: Weight loss, soluble solids content, and pulp firmness are not affected by the application of phytoregulators. The application of NAA 10 mg.L^{-1} accelerated fruit ripening and showed no positive effects. Treatment of 'Laetitia' plums with MeJa 10^{-4} M decreases ethylene production, respiration, and lipid peroxidation. This treatment also increased the concentration of antioxidants and total phenolic compounds. Fruit treatment with MeJa 10^{-4} M significantly reduced the incidence and severity of internal browning of the 'Laetitia' plum.
Conclusion: The treatment of 'Laetitia' plums with MeJa 10^{-4} M has positive effects on post-harvest storage, mainly reducing internal browning.

Keywords: Phytoregulators; shelf-life; storage; stone fruit.

1. INTRODUCTION

Among the cultivated stone fruits, plums (*Prunus domestica* L.) are highly accepted by consumers due to their organoleptic characteristics and the presence of functional/nutraceutical compounds, such as antioxidants and vitamins [1]. The 'Laetitia' plum is the most planted cultivar in the Serrana region, in the West and Far-West of Santa Catarina, and Rio Grande do Sul, due to its high productivity, fruit quality, and low susceptibility to diseases [2]. However, plums are climacteric fruits that present a short period of supply to the consumer, due to accelerated metabolism, high respiration, and ethylene production, culminating in rapid loss of quality after harvest [3,4].

To increase the time of commercialization, regulate the supply and maintain the quality of the fruits for a long period, refrigerated storage (RS) has been the most viable alternative for the farmers, since this condition of storage delays metabolic processes and the action of enzymes, reduces respiration, ethylene biosynthesis, and water loss [5].

However, the RS of stone fruits for long periods at low temperatures can cause a reduction in firmness and an increase in the incidence of physiological disorders such as internal browning [4], causing a reduction in the acceptability of the fruits by consumers [6]. The fruits most susceptible to this damage are peach, nectarines, and plums, which are usually stored at temperatures of 2ºC to 7ºC [7].

The exogenous application of growth regulators such as jasmonates can reduce the occurrence of physiological disorders and maintain the quality of plums. The use of methyl jasmonate (MeJa) in post-harvest has been studied to prolong the conservation of fruits and vegetables [8]. In addition to being involved in protection against biotic and abiotic stresses, it plays a central role in inducing plant defense [9]. A previous study has shown that JAs can induce the production of chemical compounds such as antioxidants and cell protection molecules [10]. This fact is of great importance in protecting plum fruit tissues, as antioxidants can dissipate Reactive Oxygen Species (ROS) [11], which cause the degradation of cell membranes.

The naphthalene acetic acid (NAA) has high physiological activity and plays an important role in the regulation of plant growth and development [12,13]. Furthermore, this hormone maintains fruit quality [12,14], induces xylem vessel differentiation [15], and can increase the number of antioxidants and phenolic compounds in fruits.

Thus, looking for new alternatives that aim to reduce the internal browning of the flesh, and maintain the post-harvest quality of plum fruits are essential. Therefore, the use of phytoregulators such as NAA and MeJa associated with the condition of cold storage can be an alternative to guarantee the post-harvest conservation of fruits. However, few studies have been carried out verifying the effect of these phytoregulators in the maintenance of postharvest quality and physiological disorders in plums. Thus, the objective of this work was to verify the efficiency of the phytoregulators naphthalene acetic acid (NAA) and methyl

jasmonate (MeJa) in maintaining the postharvest quality of 'Laetitia' plum fruits, stored under refrigeration.

2. MATERIALS AND METHODS

The experiment was carried out with 'Laetitia' plums from a commercial orchard in the municipality of Catanduvas, SC (27° 4' 3" S and 51° 39' 47" W), during the 2017/18 harvest. The fruits were harvested and selected, discarding those that had injuries or morphological abnormalities. After harvesting and selection, the fruits were randomly separated into treatments and replications.

The treatments evaluated were control (distilled water), MeJa (10^{-4} M), and NAA (10 mg. L^{-1}). The application was carried out with the fruits arranged on a marble bench and the treatments distributed in the form of sprinkling, using a manual sprayer. After application, the fruits were kept under the bench until the surface had completely dried and then placed in vegetable nets. The plums were stored in chambers with Biochemical Oxygen Demand (BOD), maintained at a temperature of 4±1 °C and relative humidity of 85±3%, for 35 days and three more days at ambient conditions (23±1°C). The temperature and relative humidity were monitored using mercury thermometers introduced into the fruit flesh and a thermohygrometer (INCOTERM®, Porto Alegre RS, Brazil) installed inside the BOD.

Immediately after harvest, the fruits were evaluated for the initial weight of repetitions, total soluble solids (TSS), titratable acidity (TA), skin color, and flesh firmness, to verify the physicochemical characteristics before the storage period. At the time of harvesting the fruits from the RA, the following attributes were evaluated: weight loss, skin color, respiratory rate, and ethylene production. After 35 days of refrigerated storage and another three days at ambient conditions, the same attributes as at the time of harvest were evaluated, in addition to flesh browning (incidence and severity), total antioxidant activity (TAA), total phenolic compounds (TPC) and lipid peroxidation.

The weight loss (%) was measured with the aid of a digital scale (accuracy of 0.001g) at the beginning of storage, at the time of removal of the fruits from the RA, and after three days under ambient conditions.

The respiratory rates (nmol of CO_2 kg^{-1} s^{-1}) and ethylene production (nmol of C_2H_4 kg^{-1} s^{-1}) were determined according to Nunes et al. [16], using 15 fruits/repetition, closed in hermetic flasks, and obtained by the differences in the concentrations of CO_2 and ethylene inside the container, immediately after closing and after 90 minutes.

The TSS content (°Brix) was quantified with a precision digital refractometer (Atago®, Japan) and automatic temperature compensation to 20°C [17].

The TA (g^{-1} citric acid/100 g^{-1}) was quantified using 10 mL of juice prepared from fruit slices, diluted in 90 mL of distilled water, and titrated with 0.1 N NaOH, up to pH 8.1, using a digital burette (Boeco®, Germany) [17].

The flesh firmness (N) was determined with a penetrometer (GÜSS Manufacturing Ltd, South Africa) equipped with an 8 mm probe, after removing a small portion of the epidermis [17].

The incidence of flesh browning (FB) was assessed by counting the number of fruits that showed any browning [18,19].

The skin color (h°) and the severity of FB determined using a Delta Vista 450 G colorimeter (Delta Color Indústria e Comércio de Equipamentos Eletrônicos Ltda., Brazil). For the staining of the epidermis, readings were taken in the equatorial region, on the less and more red sides. The h˚ defines the basic coloring, where 0˚ = red, 90˚ = yellow, and 180˚ = green. The severity of FB was evaluated using parameters L and C [18,19].

Lipid peroxidation (nmol malonaldehyde g^{-1}/FW) was determined following the procedure described by HEATH & PACKER [20]. TPC (mg EAG/100 g^{-1}) and TAA (µg of Trolox equivalent. g^{-1}/FW) were evaluated using extracts obtained with 5 g^{-1} of crushed flesh, homogenized with 10 mL of acidified methanol (0.01% HCL), followed by centrifugation at 10.000 RPM at 4°C for 10 minutes [18]. After filtering, the supernatant was read. The determination of TPC was performed using the Folin-Ciocalteau reagent [21], whereas the TAA was based on the capture of Free Radical DPPH as described by Rufino et al. [22]. In both analyses, the reading of the samples was performed in a K37-UV/VIS spectrophotometer (KASVI® Products and Equipment for Laboratories, Brazil).

The experimental design used was completely randomized with three treatments, and five repetitions of 30 fruits/repetition. The data

obtained in the different variables were initially submitted to Bartlett [23] and Shapiro-Wilk [24] analysis to verify the homogeneity of variances and normality of the residuals, respectively. The means were compared using the Tukey test ($p \leq 0.05$). All analyzes were performed using R statistical software, version 3.6.1 [25].

3. RESULTS AND DISCUSSION

At harvest, the plums presented the following physicochemical characteristics: flesh firmness of 39.40 N, TA of 18 g^{-1} citric acid/100g^{-1} of flesh, SS of 10.1 °Brix, skin color (h^o) on the red plus and minus side of 26.4 and 75.0, respectively.

Weight loss was not influenced by the application of phytoregulators (Fig. 1). Currently, there are no studies that prove the effectiveness of auxins in preventing the loss of fruit mass, however, in jabuticabas, MeJa was efficient in reducing the loss of fresh mass [8]. The loss of fresh mass occurs due to metabolic reactions such as transpiration, and directly affects the quality of the fruit, since wilting compromises the appearance and quality of the fruit [19].

The application of phytoregulators influenced the respiratory rate and ethylene production of plums. At the time of removal of the fruits from the refrigerated atmosphere, the plums that received the application of MeJa (10^{-4} M) presented lower respiratory rate and lower ethylene production, when compared to the control and the treatment with NAA 10 mg.L^{-1} (Table 1). After three days in ambient conditions, the fruits treated with MeJa also presented lower respiration, when compared to the control and the treatment with NAA (10 mg. L^{-1}), with no significant difference in ethylene production (Table 1).

The results were found to corroborate the effects reported by other authors who applied MeJa to climacteric fruits. 'Golden' papayas treated with MeJa also showed lower respiration throughout storage and delayed climacteric peaks [26]. In apple, the treatment with MeJa reduced the production of ethylene in the post-climacteric phase [27]. In 'Nanguo' pears, the application of MeJa suppressed the respiratory rate of the fruits [28].

The effects of exogenous application of MeJa on ethylene production and respiration are not yet sufficiently clear and seem to be dependent on the species, cultivar, and physiological state of the fruits at the time of application of the regulator [29,30,31].

The epidermis color did not differ between treatments at the exit of the chamber (Table 2). However, after 3 days in the environment, the fruits that received NAA application showed lower h^o values on the more and less red sides (Table 2). On the other hand, the fruits that received the application of NAA 10 mg.L^{-1} obtained lower values of $h°$ in the skin color, demonstrating more advanced maturation than those treated with MeJa and the control. Similar results were found by Ramos et al. [11] in which NAA-treated 'galaxy' apple fruits obtained lower $h°$ values.

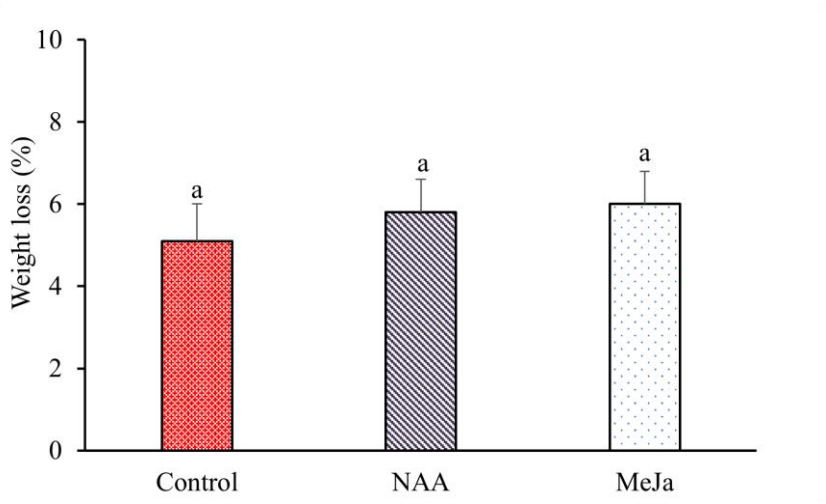

Fig. 1. Fresh weight loss percentage of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C and relative humidity of 85±3% for 35 days

Table 1. Respiratory rate and ethylene production of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C for 35 days, followed by another three days in ambient conditions (23±1 °C)

	Respiration (ηmol of CO_2 kg^{-1} s^{-1})		Ethylene (ρmol of C_2H_4 $kg^{-1}h^{-1}$)	
	RA removal	**+ 3 ambient days**	**RA removal**	**+ 3 ambient days**
Control	150.9 a	170.3 a	5.7 a	5.5 a
NAA 10 $mg.L^{-1}$	190.3 a	170.9 a	5.4 a	5.1 a
MeJa 10^{-4} M	140.1 b	150.8 b	3.5 b	5.2 a
CV (%)	9.2	8.7	5.7	9.7

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation*

Table 2. Coloring of the epidermis (hue angle) of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C for 35 days, followed by another three days in ambient conditions (23±1°C)

	***h*° redder side**		***h*° less red side**	
	RA removal	**+ 3 ambient days**	**RA removal**	**+ 3 ambient days**
Control	26.1 a	24.2 a	54.1 a	48.7 a
NAA 10 $mg.L^{-1}$	23.1 a	22.3 b	53.1 a	45.3 b
MeJa 10^{-4} M	26.1 a	24.1 a	54.4 a	49.2 a
CV (%)	8.9	3.9	5.3	2.4

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation*

The fruits during prolonged storage and/or with the application of phytoregulators may change the color of the epidermis, mainly caused by the degradation of chlorophyll and the synthesis of pigments, such as anthocyanins and carotenoids [19]. The accumulation of anthocyanins in the fruit epidermis causes a reduction in *h*° values and reflects the change from green to red color [32]. Thus, it can be inferred that the application of NAA stimulated the degradation of chlorophylls and the synthesis of anthocyanins in plums, accelerating the fruit ripening process.

There was no significant difference in the soluble solid's contents (Fig. 2). However, control fruits have lower TA values after storage (Fig. 2). The phytoregulators, depending on the concentration and period of application, can alter the respiratory metabolism of fruits. The application of phytoregulators altered the soluble solids content and the titratable acidity of apples [11]. Thus, the higher TA values presented by those treated with phytoregulators are explained by the ability of jasmonates and auxins to reduce respiration and consumption of organic acids in respiratory and metabolic processes for cell maintenance [33,34,19].

The application of phytoregulators did not change the flesh firmness of 'Laetitia' plums (Fig. 3). Fruit firmness is one of the aspects most valued by consumers during fruit acquisition, and the reduction in its values during storage occurs mainly due to the activity of cell wall enzymes, responsible for softening [19,35]. In 'Packham's Triumph' pears, the application of phytoregulators antagonistic to ethylene also did not change the flesh firmness of the fruits [36].

The application of MeJa provided higher antioxidant activity and lower lipid peroxidation in 'Laetitia' plums when compared to control fruits and NAA treatment (Table 3). Likewise, the application of MeJa provided a higher content of phenolic compounds when compared to the control (Table 3).

The MeJa can increase the antioxidant capacity of the fruit, causing the resistance to oxidative stress to also increase [37]. Other studies also showed an increase in the content of bioactive compounds in fruits treated with MeJa [38,33]. The antioxidants are substances that can contribute to the dissipation of reactive oxygen species and reduce tissue browning and explains why fruits that received methyl jasmonate had less flesh browning [39].

The lipid peroxidation is an indicator of cell membrane damage, mainly caused by the accumulation of reactive oxygen species (ROS), which culminates in oxidative stress [19,40].

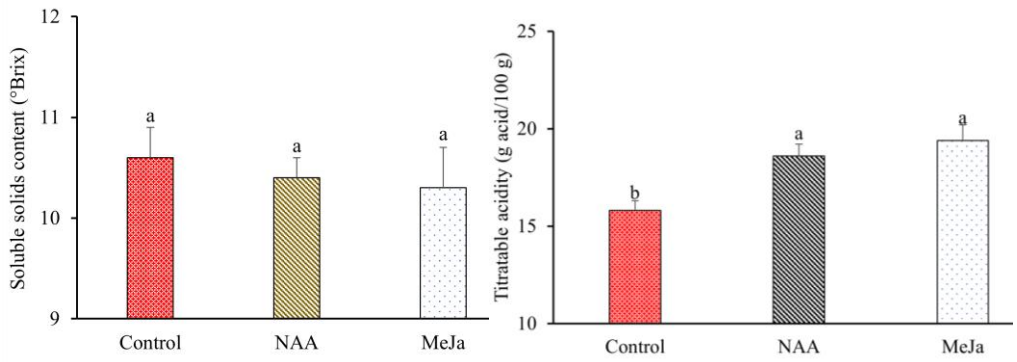

Fig. 2. Content of soluble solids and titratable acidity of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C and relative humidity of 85±3% for 35 days, followed by another three days in ambient conditions (23±1 °C)

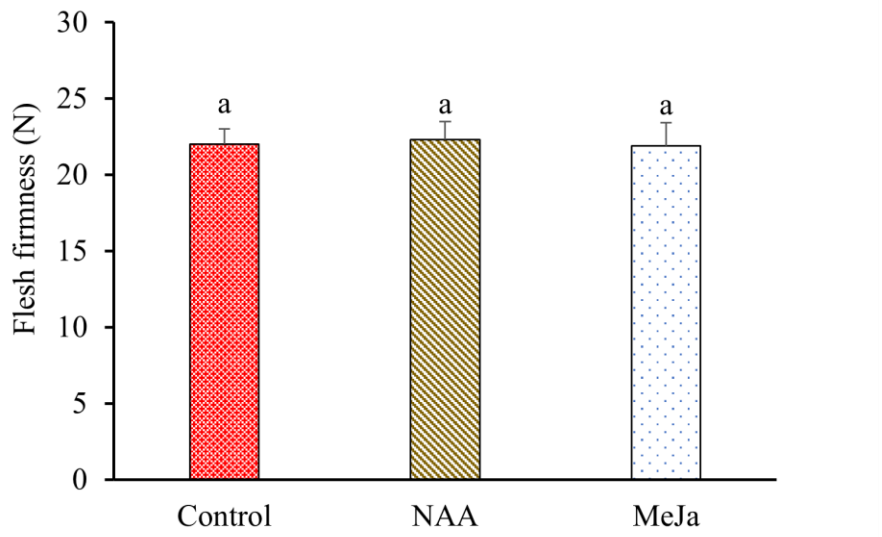

Fig. 3. Flesh firmness of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C and relative humidity of 85±3% for 35 days, followed by another three days in ambient conditions (23±1°C)

During fruit ripening, several changes occur in membranes, such as decreased fluidity of the plasma membrane, loss of integrity, and accumulation of reactive species [41,42]. The peroxidation of membrane lipids compromises cell function and structure, causing ion extravasation and cell death [26,42]. In other climacteric fruits, the application of MeJa also reduced lipid peroxidation [26].

The MeJa application reduced the incidence and severity of flesh browning when compared to NAA treatment and the control (Fig. 4). On the other hand, the application of NAA was not effective in reducing internal browning, probably due to the non-reduction of oxidative stress (Fig. 4).

The vulnerability of the 'Laetitia' plum to internal browning is the main problem in its storage [2]. 'Laetitia' plums developed more severe browning of flesh tissues near the pit [43]. The Internal occurs mainly due to oxidative stress that causes damage to cell membranes and subsequent loss of cell compartmentation [4]. Furthermore, the internal browning may be related to the enzymatic oxidation of phenolic compounds by the enzyme polyphenol oxidase (PPO) [4].

The MeJA has a potential antioxidant effect, contributing to the preservation of phenolic compounds and inducing the production of defense compounds against oxidative stress [9].

Table 3. Total antioxidant activity, total phenolic compounds, and lipid peroxidation (malondialdehyde content) of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C for 35 days, followed by another three days in ambient conditions (23±1 °C)

	TAA (µg Trolox.g^{-1}/FW)	TPC (mg EAG.100 g^{-1})	MDA (nmol g^{-1}/FW)
Control	6085.8 b*	180.0 b	12.4 a
NAA 10 mg.L^{-1}	6012.5 b	183.0 ab	15.2 a
MeJa 10^{-4} M	6930.8 a	204.0 a	6.8 b
CV (%)	8.2	9.5	10.8

**Means followed by the same letter, in the columns, do not differ statistically by the Tukey test or t (5% probability). CV (%): Coefficient of variation*

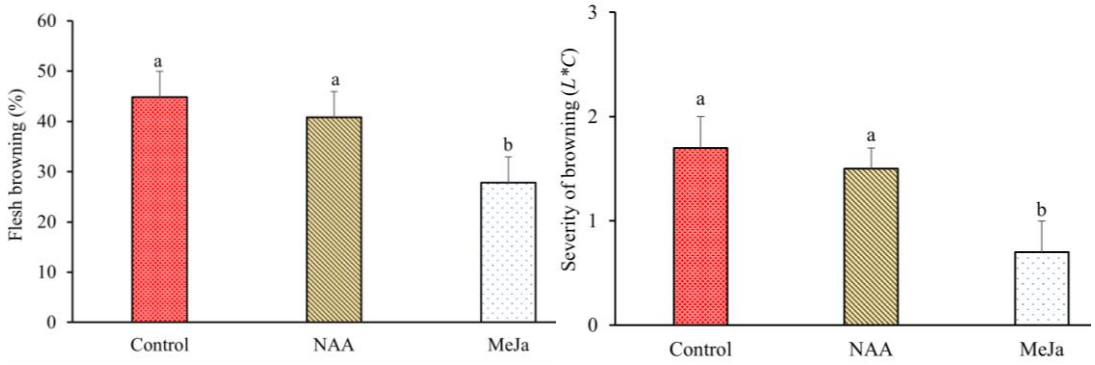

Fig. 4. Incidence and severity of internal browning of 'Laetitia' plums treated with different growth regulators and stored at 4±1 °C and relative humidity of 85±3% for 35 days, followed by another three days in ambient conditions (23±1°C)

The results obtained in the present study corroborate the data found by other authors, who also showed reductions in the internal browning of the flesh with the application of MeJa [44]. In 'Queen' pineapple, MeJA application alleviated the symptoms of internal browning [38]. Likewise, the application of MeJa also increased the cold storage potential of pomegranate fruits, reducing the symptoms of chilling injury, maintaining plasma membrane stability, and increasing the content of bioactive compounds with antioxidant activity [45]. In blueberry, the application of MeJA also maintained the sensory and nutritional qualities and extended the shelf life of the fruit [33].

With the results obtained, it is possible to infer that the plums treated with MeJa 10^{-4} M exhibited less flesh browning, due to the greater number of antioxidants and less oxidative stress (Table 3 and Fig. 4).

4. CONCLUSION

Weight loss, soluble solids content, and flesh firmness are not affected by the application of phytoregulators;

The application of NAA 10 mg.L^{-1} is not recommended in the post-harvest of 'Laetitia' plums, as it accelerates the ripening of the fruits and has no positive effects;

The treatment of 'Laetitia' plums with MeJa 10^{-4} M provides better conservation and postharvest quality, decreasing ethylene production, respiration and lipid peroxidation, and increasing the concentration of antioxidants and total phenolic compounds;

Fruit treatment with MeJa 10^{-4} M greatly reduced the incidence and severity of internal browning of 'Laetitia' plum fruit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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