

Quality maintenance of 'Laetitia' plum by application of 1- methylcyclopropene, ethanol vapor and heat treatment

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Abstract

The aim of this study was to evaluate the effect of 1-methylcyclopropene (1-MCP), ethanol and heat treatment on ripening behavior and internal browning occurrence of 'Laetitia' plum stored under regular air. The treatments were: control, 1-MCP (1,0 $\mu\text{L L}^{-1}$), ethanol (500 and 1500 $\mu\text{L L}^{-1}$) and heat treatment (37 and 40 °C) for 24 h. Fruit were stored under regular air for 25 or 35 d at $0.5\pm 0,1$ °C and $96\pm 1\%$ relative humidity (RH), and assessed for respiration rate, ethylene production, skin color, flesh firmness, titratable acidity, soluble solids content, incidence of decay and incidence and severity of internal flesh browning. In both storage periods, immediately at removal from cold room, fruit from all the treatments showed lower respiration rate and ethylene production than control fruit. Ethanol vapor at 1500 $\mu\text{L L}^{-1}$, 1-MCP at 1,0 $\mu\text{L L}^{-1}$ and heat treatment at 40 °C allowed a less reddish skin after 35 d of storage, as well as higher flesh firmness and lower intensity of internal flesh browning than control fruit, in both evaluated storage periods. In either 25 or 35 d of storage period, heat treatment at 40 °C increased the occurrence of decay. All the treatments provided lower incidence of internal flesh browning than control fruit in both evaluations. The postharvest application of ethanol at 1500 $\mu\text{L L}^{-1}$, 1-MCP at 1,0 $\mu\text{L L}^{-1}$ and heat treatment at 40 °C for 24 h delay the ripening process and reduce the incidence of internal flesh browning of 'Laetitia' plum.

Keywords: Ethylene, fruit ripening, internal browning, postharvest quality, *Prunus salicina*

Introduction

'Laetitia' plum is a cultivar of late fruit maturity and a large production of high quality fruit (Stanger et al., 2014). For this cultivar, the period of commercialization of fruit is short due to the fast ripening process. Thus, in order to regulate the supply and price, storage at low temperature is a viable alternative. However, the regular air storage of plums for prolonged periods of time results in fruit with low flesh firmness and higher incidence of internal flesh browning, which reduces the acceptability by the consumers (Manganaris et al., 2008; Alves et al., 2009).

During the regular air storage, the low temperature reduces the activity of many

enzymes associated with the membranes, causing changes in the permeability and inducing an accumulation of toxic intermediates, such as reactive oxygen species (Jiang et al., 2014). These changes, in turn, affect negatively the fruit tissues and induce the occurrence of damage caused by low temperature conditions, which manifest as internal flesh browning (Argenta et al., 2011; Hendges et al., 2013). Although flesh browning is a disorder caused by low temperature (Singh et al., 2009; Singh & Singh, 2013a), its manifestation may increase with ethylene (Corrêa et al. 2011). The high ethylene production damages the membranes, inducing senescence in plum and consequently internal browning (Larrigaudière et

Received: 27 April 2017
Accepted: 19 December 2017

al., 2009). It has been verified that the presence of ethylene in 'Laetitia' plum alters the quality of fruit, causing a rapid softening, a reduction in acidity and an increase in internal flesh browning (Alves et al., 2009). Therefore, the reduction of ethylene synthesis or action is essential to maintain fruit quality.

Treatment with 1-methylcyclopropene (1-MCP), which inhibits the action of ethylene, is an excellent alternative to enhance the potential of plum storage (Steffens et al., 2013). Many authors have verified that 1-MCP delays the ripening and reduces the incidence of internal flesh browning in 'Laetitia' plum (Alves et al., 2010; Argenta et al., 2011; Hendges et al., 2013).

Studies have also demonstrated that the exogenous application of ethanol vapor has an inhibitory effect on ethylene production and can be applied in order to extend the storage period and preserve the quality of horticultural products (Asoda et al., 2009; Liu et al., 2012). However, the efficiency of ethanol vapor application is directly associated with the specie, cultivar and even the concentration used (Jin et al., 2013).

Heat treatment can also contribute in reducing postharvest losses and extending the storage period, since it can inhibit ripening, induce resistance to damage caused by low temperature conditions and contribute to the maintenance of the external appearance of fruits during storage (Aghdam et al., 2013; Wu et al., 2015). The effects of heat treatment on 'Laetitia' plums, regarding the quality and the occurrence of internal flesh browning, are currently unknown.

The objective of this study was to evaluate the effect of 1-MCP, ethanol and heat treatment on ripening and internal browning of 'Laetitia' plums under cold storage.

Materials and Methods

'Laetitia' plums were harvested from a commercial orchard located in Vacaria, Rio Grande do Sul State, southern Brazil, during the 2013/2014 season. In order to obtain the experimental units, the fruit homogenization was carried out after removing damaged and defective fruit. The fruit were harvested at the commercial maturity, when around 50% of the fruit surface displayed a red color.

Before the treatments were applied, three samples of 10 fruit each were evaluated for the determination of the initial quality of the plums, which presented average flesh firmness of 35 N, soluble solids content (SSC) of 11.2 °Brix, titratable acidity (TA) of 0.26%, and h° values of 33.98 and 83.3 for the most and least red skin surfaces of the fruit, respectively.

The treatments evaluated were: control, 1-MCP at 1.0 $\mu\text{L L}^{-1}$, ethanol vapor (500 $\mu\text{L L}^{-1}$ and 1500 $\mu\text{L L}^{-1}$; v/v) and heat treatment at 37 and 40 °C.

The commercial product SmartFresh®, in the form of powder, was used for the application of 1-MCP. After the weighing, the product was diluted in water and then placed in a hermetically sealed mini-chamber (180 L) with the fruit. The treatment was applied to the fruit for 24 h under ambient conditions of 20±5 °C and 63±2% relative humidity (RH). The concentration of 1-MCP used was 1.0 $\mu\text{L L}^{-1}$.

For the ethanol vapor application, the fruit of each sample were weighed and stored in hermetically sealed pots of 4100 mL volume. The volume of liquid ethanol needed to reach the established concentration for each treatment was added in 250 mL Gerbox plastic boxes (11.0 x 11.0 x 3.5 cm), which were placed inside the pots before being sealed. The average of ethanol volume kg^{-1} of fruit ratios were 1.2 and 3.5 mL kg^{-1} for the treatments with 500 $\mu\text{L L}^{-1}$ and 1500 $\mu\text{L L}^{-1}$ ethanol, respectively. The applications were performed under ambient conditions (20±5 °C and 63±2% RH for 24 h).

The heat treatments were applied to the plum in biochemical oxygen demand (BOD) incubation chambers, at temperatures set according to the treatment (37 and 40 °C) and 85 -90% RH. The fruit were kept under BOD chambers for 24 h.

After the application of the treatments, the fruit were immediately stored at 0.5±0.1 °C and 96±1% RH for 25 or 35 d. The respiration rate and ethylene production were determined after fruit removal from the chamber and after four days of shelf life at 20±5 °C and 63±2% RH. The determination of skin color (red color index – RCI; h°), flesh firmness, TA, SSC, decay incidence and the intensity and incidence of internal browning

(*L* value for the pulp) was carried out only after four days of shelf life.

Respiratory rate (nmol of CO₂ kg⁻¹ s⁻¹) and ethylene production (pmol C₂H₄ kg⁻¹ s⁻¹) were quantified by gas chromatography. Ten fruits of each replicate were packed in 4.1 L containers, hermetically sealed. Respiratory rate and ethylene production were obtained by the difference in the CO₂ and C₂H₄ content, respectively, inside the container, immediately after its closure and after half an hour. After this period, using a 1.0 mL plastic syringe, two samples were collected from the free space of these containers, which were injected into a Varian® gas chromatograph, model CP-3800 (Palo Alto, USA), equipped with a 3 m long Porapak N® column (80-100 mesh), methanator and flame ionization detector. The column, detector, methanator and injector temperatures were 70, 250, 380 and 130°C, respectively. Nitrogen, hydrogen and synthetic air fluxes used were 70; 30 and 300 mL min⁻¹, respectively.

The red color index (RCI) was evaluated in terms of amount of the fruit skin which displays a red color. Grades of 1, 2, 3 and 4 were attributed to fruit with 0-25%, 26-50%, 51-75% and 76-100% of red pigmented on the skin, respectively.

The skin color was evaluated in terms of the hue angle (*h*°) values obtained in the equatorial region and the most and least red surfaces of the fruit, using a colorimeter (model CR-400), manufactured by Minolta® (Konica Minolta, Tokyo, Japan). The *h*° values corresponding to the color of the surface tissue were as follows: 0°/red, 90°/yellow, 180°/green and 270°/blue.

The flesh firmness (N) was quantified in the equatorial region of the fruit, at two opposite sides, after the removal of a small portion of the skin. An electronic penetrometer (GÜSS Manufacturing Ltd., Cape Town, South Africa), equipped with a 7.9 mm diameter tip was used for this task.

The TA (% of citric acid) was quantified using 10 mL of juice extracted using a centrifuge. The juice was diluted in 90 mL of distilled water and the titration was carried out with a solution of 0.1 N NaOH up to pH 8.1, using an automatic titrator (TitroLine® easy, Schott Instruments, Mainz, Germany). The SSC (°Brix) was determined with

a digital refractometer (PE201a, Atago®, Tokyo, Japan), using an aliquot of the juice obtained for the TA quantification.

The decay incidence (%) was evaluated through counting the fruit with external lesions greater than 5 mm of diameter, showing the characteristics of infection by pathogens.

The internal flesh browning incidence (%) was evaluated by counting the fruit which showed this physiological disorder, which is characterized by a difference in the color of the flesh, visualized in the region of the equatorial cut of the fruit. The severity of the internal browning was evaluated in terms of lightness (*L*) values, quantified with a colorimeter model CR 400 (Konica Minolta®, Tokyo, Japan). Readings were carried out on the left and right sides of the flesh, after the transversal cut coincident with the groove characteristic of the anatomy of 'Laetitia' plums. The *L* value expresses the luminosity on a scale from 0, which corresponds to black, to 100, which corresponds to white. Thus, the lower the *L* value the more darkened the flesh will be.

The experimental design used was completely randomized, with four replicates, and the experimental units composed by 15 fruit each. The data were submitted to analysis of variance (ANOVA) and the averages were compared applying the Tukey test (*p*<0.05) using SAS statistical program (SAS Institute, Cary, NC, USA). Data obtained in percentages were transformed using the arcsine formula [(*x*+1)/100]^{1/2} before they were submitted to ANOVA.

Results and Discussion

After 25 d of storage, after their removal from the chamber, the fruit treated with 1-MCP (1.0 µL L⁻¹), ethanol vapor (500 and 1500 µL L⁻¹) and heat treatment (37 and 40 °C) showed a lower production of ethylene than the control fruit (Table 1). The same data indicated that the fruit submitted to the higher concentration of ethanol vapor (1500 µL L⁻¹) or to heat treatment at the higher temperature (40 °C), had a lower production of ethylene than 1-MCP treated fruit or those subjected to heat treatment at 37 °C. After four days of shelf life, only fruit treated with 1-MCP, ethanol vapor (1500 µL L⁻¹) and heat treatment (40 °C) showed lower ethylene

production than the non-treated fruit.

After 35 d of storage, the control fruit showed a higher ethylene production than all other treatments, on removing the fruit from the chamber (Table 1). The application of 1-MCP and the heat treatment at 40 °C were more efficient at reducing the ethylene production rate when compared to the other treatments. The effect of 1-MCP on the reduction of ethylene production

in 'Laetitia' plum has also been reported by other authors (Alves et al., 2010; Argenta et al., 2011). This result is possibly associated with the inhibitory effect of 1-MCP on ethylene action, as well as the reduction that it causes on the activity of the enzymes ACC-synthase and ACC-oxidase, reducing the autocatalytic ethylene production in fruit (Villalobos-Acuña et al., 2011).

Table 1. Ethylene production and respiration rate in 'Laetitia' plum after 25 and 35 d of regular air storage (0.5±0.1 °C and 96±1% RH) followed by four days of shelf life at 20±5 °C and 63±2% RH.

Treatments	Ethylene production (pmol C ₂ H ₄ kg ⁻¹ s ⁻¹)	Respiration rate (nmol CO ₂ kg ⁻¹ s ⁻¹)	Ethylene production (pmol C ₂ H ₄ kg ⁻¹ s ⁻¹)	Respiration rate (nmol CO ₂ kg ⁻¹ s ⁻¹)
	25 days at 0.5 °C		35 days at 0.5 °C	
	25 days at 0.5 °C + 4 days at 20 °C		35 days at 0.5 °C + 4 days at 20 °C	
Control	4.27 a	118.40 a	7.17 a	132.52 a
1-MCP (1 µL L ⁻¹)	2.30 b	59.21 c	0.04 c	97.75 b
Ethanol (500 µL L ⁻¹)	1.12 cb	84.13 b	3.02 b	85.99 c
Ethanol (1500 µL L ⁻¹)	0.29 c	68.77 cb	1.51 b	107.65 b
Heat treatment (37 °C)	2.11 b	74.14 cb	2.07 b	81.93 c
Heat treatment (40 °C)	0.31 c	67.35 cb	0.81 c	99.74 b
CV (%)	37.21	11.11	26.15	8.25
Control	20.35 a	63.84 a	28.66 a	162.94 a
1-MCP (1 µL L ⁻¹)	0.78 b	65.88 a	12.38 b	116.04 b
Ethanol (500 µL L ⁻¹)	17.75 a	54.99 ab	32.04 a	61.19 c
Ethanol (1500 µL L ⁻¹)	1.10 b	40.50 c	8.92 cb	56.32 c
Heat treatment (37 °C)	17.01 a	50.14 bc	14.16 b	56.18 c
Heat treatment (40 °C)	3.98 b	44.49 bc	4.58 c	33.69 c
CV (%)	31.11	10.63	18.04	25.33

*Averages followed by the same letter in the columns were not different from one another according to the Tukey test (p<0.05)

A reduction in ethylene production following heat treatment has been verified in peach (Budde et al., 2006; Vitti et al., 2007) and *Myrica rubra* (Luo et al., 2009), and is related to the exposure of the fruit to temperatures higher than 35 °C (Budde et al., 2006). The heat treatment response on ethylene production is usually related to the inhibitory effect of the heat treatment on the enzyme ACC-oxidase, reducing the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) into ethylene (Antunes & Sfakiotakis, 2002; Budde et al., 2006).

After 35 d of storage, followed by four days of shelf life, the heat treatment at 40 °C provided a lower ethylene production in relation to the other treatments, with exception of the application of ethanol vapor at 1500 µL L⁻¹, followed by 1-MCP (1,0 µL L⁻¹) and the heat treatment at 37 °C. The ethanol vapor seems to be associated with the reduction of ethylene

synthesis. In melon 'Yumeiren', the ethanol vapor (0.5 mL kg⁻¹ and 3 mL kg⁻¹) decreased the activity of the enzymes ACC-synthase and ACC-oxidase, reducing the ethylene concentration inside the fruit during storage (Jin et al., 2013). According to the same authors, both ethanol vapor treatments suppresses the gene expression associated with the synthesis of these enzymes, such as the *CM-ACO1*, *CM-ACO2*, *CM-ACO3* and *CM-ACS1*, *CM-ACS2*, *CM-ACS3*. Asoda et al. (2009), in a study on broccoli, also observed that the regulation mechanism of ethylene production associated with the application of ethanol occurs at molecular level, through suppressing the expression of the genes *BO-ACO1*, *BO-ACO2* and *BO-ACS1*.

After 25 d of storage, the respiration rate of the fruit measured immediately after removal fruit from the chamber was lower in samples treated with 1-MCP when compared to the

application of ethanol vapor at 500 $\mu\text{L L}^{-1}$ and the control fruit, and did not differ from the other treatments (Table 1). However, after four days of shelf life, the fruit submitted to the application of 1-MCP showed a respiration rate similar to the control, with values higher than those of fruit treated with ethanol vapor at 1500 $\mu\text{L L}^{-1}$ and the heat treatment at both temperatures. After 35 d of storage, all the treatments provided a reduction in the respiration rate when compared to the control, on the removal of the fruit from the chamber as well as after four days of shelf life. After removing the samples from the chamber, the ethanol vapor at a concentration of 500 $\mu\text{L L}^{-1}$ and the heat treatment at 37 °C showed lower values for the respiration rate than other treatments. After four days of shelf life, the heat treatment (at both temperatures) and the application of the ethanol vapor (at both doses evaluated) provided the lowest respiration rates.

A lower respiration rate was also observed by Alves et al. (2009; 2010) in 'Laetitia' plum treated with 1-MCP (1,0 $\mu\text{L L}^{-1}$), associated with the action of the product on the reduction of

ethylene production. The reductions in respiration rate and the ethylene production could be related, since the increase in the respiration rate is a secondary event, which is stimulated by a higher ethylene production rate during the fruit ripening (De Martin et al., 2015). In peach, heat treatment applied by immersion in water at 48 °C for 10 min, reduced the respiration metabolism through the inhibition of the glycolysis and of the tricarboxylic acid cycle (Jiang et al., 2014).

For all of the evaluations carried out there were no differences between the treatments in relation to the RCI (data not shown). Also, the skin color (h°) did not differ between treatments in the evaluations carried out on fruit stored for 25 d (Table 2). However, after being stored for 35 d, the fruit treated with 1-MCP (1,0 $\mu\text{L L}^{-1}$), ethanol vapor (1500 $\mu\text{L L}^{-1}$) and heat treatment at 40 °C showed a less reddish skin color (lower h° value) when compared to the control and the fruit submitted to the application of ethanol vapor at 500 $\mu\text{L L}^{-1}$. Fruit subjected to heat treatment at 37 °C showed intermediate results.

Table 2. Skin color, flesh firmness, titratable acidity (TA), soluble solids content (SSC) and decay incidence in 'Laetitia' plums after 25 and 35 d of regular air storage (0.5±0.1 °C and 96±1% RH) followed by four days of shelf life (20±5 °C and 63±2% RH).

Treatments	Skin color (h°)	Flesh	TA (meq 100	SSC (°Brix)	Decay
		firmness (N)	mL ⁻¹)		
25 days at 0.5 °C + 4 days at 20 °C					
Control	21.29 ^{ns}	7.62 d	23.69 a	10.70 ^{ns}	0.0 b
1-MCP (1 $\mu\text{L L}^{-1}$)	24.11	13.18 a	22.98 ab	11.12	0.0 b
Ethanol (500 $\mu\text{L L}^{-1}$)	20.94	8.05 d	20.48 ab	11.00	0.0 b
Ethanol (1500 $\mu\text{L L}^{-1}$)	22.56	10.42 b	20.68 ab	11.00	0.0 b
Heat treatment (37 °C)	21.38	8.66 cd	19.22 b	10.85	0.0 b
Heat treatment (40 °C)	23.39	9.77 cb	19.23 b	10.77	6.67 a
CV (%)	8.06	7.67	8.81	3.07	52.59
35 days at 0.5 °C + 4 days at 20 °C					
Control	18.62 b	4.95 c	8.66 ab	10.52 ^{ns}	0.0 b
1-MCP (1 $\mu\text{L L}^{-1}$)	22.60 a	8.92 b	8.94 ab	10.95	0.0 b
Ethanol (500 $\mu\text{L L}^{-1}$)	18.47 b	6.13 c	6.50 b	10.70	0.0 b
Ethanol (1500 $\mu\text{L L}^{-1}$)	22.78 a	11.28 a	10.57 a	10.75	0.0 b
Heat treatment (37 °C)	21.14 ab	6.36 c	8.95 ab	10.40	0.0 b
Heat treatment (40 °C)	23.91 a	9.43 b	7.42 b	10.52	39.87 a
CV (%)	6.09	9.73	15.09	3.06	12.59

*Averages followed by the same letter in the columns were not different from one another according to the Tukey test ($p < 0.05$)

The color change process occurring during the ripening of plum is a dependent event on ethylene action (Alves et al., 2009). Thus, the lower h° values observed for the skin of fruit treated with 1-MCP, ethanol vapor (1500 $\mu\text{L L}^{-1}$) and heat treatment at 40 °C may be

associated with a lower ethylene production and action. In the case of broccoli, treatment with ethanol (500 $\mu\text{L L}^{-1}$) caused a decrease in chlorophylls degradation through the inhibition of chlorophyllase, Mg-dechelataase and peroxidase activity, leading to higher h° values in florets (Xu

et al., 2012).

The application of ethanol vapor at 500 $\mu\text{L L}^{-1}$ and heat treatment at 37 °C led to low flesh firmness with values similar to the control after 25 and 35 d of storage (Table 2). After 25 d of storage plus four days of shelf life, 1-MCP application provided the highest flesh firmness, followed by ethanol vapor (1500 $\mu\text{L L}^{-1}$), which did not differ only from the heat treatment at 40 °C. In the case of the fruit stored for 35 d plus four days of shelf life, the flesh firmness was higher in fruit treated with ethanol vapor at 1500 $\mu\text{L L}^{-1}$, followed by 1-MCP and heat treatment at 40 °C.

Other authors have reported the effect of 1-MCP 0,5 $\mu\text{L L}^{-1}$ (Argenta et al., 2011) or 1,0 $\mu\text{L L}^{-1}$ (Alves et al., 2010) in delaying the loss of flesh firmness in 'Laetitia' plum during cold storage, similarly to the results of this study. The endogenous ethylene produced by plums regulates the enzyme activity associated with the degradation of cellular wall components and the loss of flesh firmness (Dong et al., 2001). Thus, the lower production of ethylene resulting from the treatment with 1-MCP reduces the loss of flesh firmness in plum (Argenta et al., 2011). The effect of ethanol vapor, reducing the loss of flesh firmness, also seems to be associated with the inhibition of ethylene synthesis (Jin et al., 2013). Delayed loss in flesh firmness and in the ripening with heat treatment have been previously reported for peach (Budde et al., 2006; Jiang et al., 2014). Heat treatment delays the loss of flesh firmness and changes in the texture of fruit by the inhibition of the activity of the enzymes pectin methylesterase and polygalacturonase (Luo et al., 2009). However, it is possible that the effect of the heat treatment in maintaining the flesh firmness is also related to the reduction in the biosynthesis of ethylene.

After 25 d of regular air storage followed by four days of shelf life, the fruit submitted to heat treatment (37 and 40 °C) showed lower TA than the control fruit (Table 2). After 35 d of regular air storage followed by four days of shelf life, the fruit treated with ethanol vapor at 1500 $\mu\text{L L}^{-1}$ showed higher TA, compared to those treated with 500 $\mu\text{L L}^{-1}$ of ethanol, or to heat treatment at 40 °C, but did not differ from other treatments. These results differ from those reported by Alves et al. (2010) for

'Laetitia' plum, in which the application of 1-MCP (1,0 $\mu\text{L L}^{-1}$) kept higher TA than the control fruit stored under regular air for 60 d, followed by four days of shelf life. The effect of ethanol vapor at 1500 $\mu\text{L L}^{-1}$ on the maintenance of the TA may be associated with a reduction in the consumption of acids in the tricarboxylic acid cycle (Alves et al., 2010) since the respiration rate was lower in fruit treated with ethanol vapor (1500 $\mu\text{L L}^{-1}$) for most evaluations carried out (Table 1).

Differences between the treatments were not verified after 25 and 35 d of storage in relation to the SSC (Table 2). Other authors have reported that 1-MCP does not influence the SSC of 'Laetitia' plum (Argenta et al., 2011; Corrêa et al., 2011). The SSC in melon was also found to be unaffected by the application of ethanol vapor (Jin et al., 2013). In addition, Budde et al. (2006) verified that heat treatment for 24 or 36 h at 39 °C had no effect on the SSC of 'Dixieland' peach.

For both storage periods evaluated, heat treatment at 40 °C increased the occurrence of decay (Table 2). Inadequate heat treatment can cause undesired effects in fruits and vegetables, which can include the darkening or yellowing of the skin, internal browning, impairment of ripening and an increase in the decay incidence (Aghdam et al., 2014). According to the same authors, the fruit tolerance to heat treatment depends on the species, cultivar, picking date of the fruit and preharvest factors. A precise determination of the most appropriate combination of time and temperature to be applied in the process is therefore needed to avoid damage to the fruits. Thus, in this investigation, the temperature of 40 °C was excessively high, causing damage to the skin tissue, which contributes to increasing the decay incidence.

After 25 d of storage followed by four days of shelf life, the control fruit showed greater incidence of internal flesh browning compared to the other treatments, except for the heat treatment at 37 °C (Figure 1). The fruit submitted to the heat treatment at 40 °C, 1-MCP or ethanol vapor (1500 $\mu\text{L L}^{-1}$) had a lower incidence of internal flesh browning, which did not differ, however, from the result for the application of ethanol vapor (500 $\mu\text{L L}^{-1}$). After 35 d of regular air storage followed by four days of shelf life all

of the treatments provided low incidence of internal flesh browning than the control.

The occurrence of internal browning in stone fruits can result from the loss of selective permeability of membranes, leading to interaction between phenols and phenol oxidases (which, in general, are separated, being present in different

cellular compartments), this being associated with the senescence of the tissues (Lurie & Crisosto, 2005; Argenta et al., 2011), inadequate controlled and modified atmospheres conditions (Singh & Singh, 2013b) and cold storage-induced oxidative stress (Singh & Singh, 2013a).

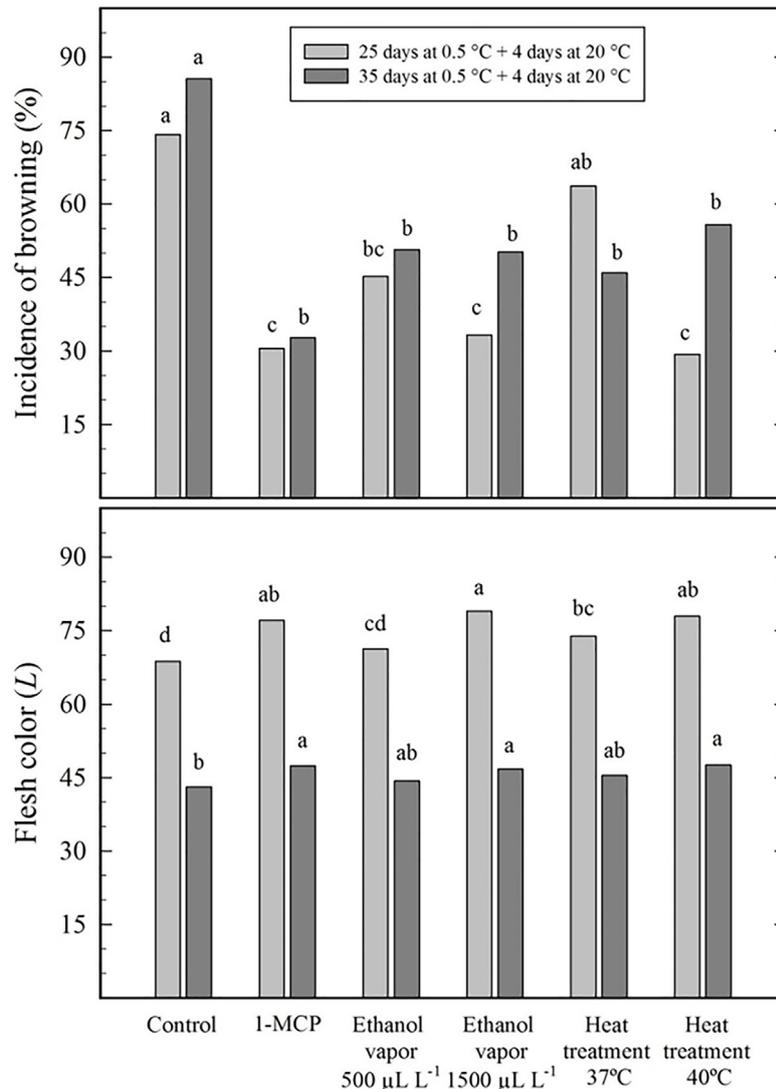


Figure 1. Incidence (%) and severity (L attribute of the flesh color) of internal flesh browning in 'Laetitia' plum after 25 and 35 d of regular air storage (0.5±0.1 °C and 96±1% RH) followed by four days of shelf life (20±5 °C and 63±2% RH). Bars followed by the same letter are not different from one another according to the Tukey test (p<0.05).

A reduction in the incidence of internal flesh browning in 'Laetitia' plum with the use of 1-MCP has been reported by other authors (Alves et al., 2010; Corrêa et al., 2011). This result may be associated with the inhibitory effect of this compound on the action of ethylene, delaying the ripening and senescence of the fruit and, therefore, reducing the development of internal browning (Argenta et al., 2011).

The effect of ethanol in reducing the damage caused by cold conditions has been reported for other kinds of fruit and can be associated with a changes in membrane properties and function (Pesis, 2005). Working with broccoli, Han et al. (2006) verified that the activity of enzymes peroxidase, superoxide dismutase and catalase was higher in florets treated with ethanol vapor, increasing antioxidant activity

and, consequently, improving the preservation of membranes integrity in cells. Furthermore, the positive effect of ethanol vapor on the incidence of internal flesh browning may occur indirectly through the inhibition of ethylene synthesis, since this plant hormone is associated with the occurrence of internal browning in plum (Alves et al., 2009).

Exposure to high nonlethal temperatures induces in plants the synthesis of a characteristic group of proteins which are responsive to stress, known as the heat shock proteins (HSPs) (Lara et al., 2009; Aghdam et al., 2013). Thus, the tolerance of horticulture products to the damage caused by low temperature during regular air storage has been attributed to the production and accumulation of HSPs in fruits submitted to heat treatment, suggesting an important role of these proteins in increase the fruit tolerance to stress during the regular air storage. Low quantities of HSPs can act as membrane stabilizing agents and can also remove reactive oxygen species or act synergistically with the antioxidant system (Aghdam et al., 2013). In this regard, HSPs play an important role in relation to the fluidity and the selective permeability of the membranes under stress conditions caused by cold storage (Aghdam et al., 2013). Lara et al. (2009) reported that three days of exposure to 39 °C induced the synthesis of HSP in peach, as well as inhibiting the enzymatic activity of polyphenol oxidase. The combination of the heat treatment of fruit at 48 °C for ten minutes with the application of 10 $\mu\text{L L}^{-1}$ of 1-MCP increased the levels of glutathione peroxidases and thioredoxin peroxidases in peach, enhancing the antioxidant capacity and contributing to the elimination of free radicals (Jiang et al., 2014).

The severity of internal flesh browning (L attribute of the flesh color) of fruit stored for 25 d followed by four days of shelf life was greater (darker flesh) in control fruit when compared to those submitted to the other treatments, not differing only from the application of ethanol vapor (500 $\mu\text{L L}^{-1}$) (Figure 1). The fruit treated with ethanol vapor at 1500 $\mu\text{L L}^{-1}$ showed lighter flesh color than control, ethanol vapor at 500 $\mu\text{L L}^{-1}$ and heat treatment at 37 °C, but did not differ from other treatments. After 35 d of regular air

storage plus four days of shelf life, the fruit treated with 1-MCP, ethanol vapor (1500 $\mu\text{L L}^{-1}$) and heat treatment at 40 °C obtained higher L values (lighter flesh color) than the control fruit.

A reduction in the intensity of internal flesh browning with the use of 1-MCP (1.0 $\mu\text{L L}^{-1}$) was observed by Steffens et al. (2013) in 'Laetitia' plum. According to these authors, storage under controlled atmosphere at 1.0 kPa O_2 + 1.0 kPa CO_2 , combined with the use of 1-MCP (1.0 $\mu\text{L L}^{-1}$) reduced the severity of internal flesh browning, avoiding a reduction in the sensorial quality caused when this disorder is severe. According to Corrêa et al. (2011), 'Laetitia' plum with low severity of internal flesh browning present higher acceptability regarding the flesh color and fruit flavor in sensory analysis than fruit with high severity.

This study demonstrates that the application of ethanol vapor and heat treatment show potential for use as alternatives to 1-MCP, with regard to delaying the ripening and reducing the internal browning of plum. Among the advantages of these treatments are food safe and the fact that there is no period of residual effectiveness of a toxic compound, which make these treatments suitable for use in postharvest. Ethanol is a natural component which is classified as a safe substance, and it also has the advantage that its application is of low cost (Pesis, 2005; Xu et al., 2012) compared to other approaches, such as the use of 1-MCP or heat treatment. However, it is important to highlight that the concentration used in ethanol treatment, as well as the temperature and exposure time used in heat treatment influence the efficiency of the treatments (Jin et al., 2013; Aghdam et al., 2013).

In the present study, an ethanol concentration of 1500 $\mu\text{L L}^{-1}$ showed better efficiency in terms of the maintenance of the postharvest quality of 'Laetitia' plum when compared to a concentration of 500 $\mu\text{L L}^{-1}$. Regarding to the heat treatment, new studies aiming to evaluate temperatures between 37 and 40 °C as well as different periods of exposure to high temperature need to be carried out, with the purpose of preserving the fruit quality without increasing the incidence of decay.

Conclusions

1.The postharvest application of ethanol vapor at a concentration of 1500 $\mu\text{L L}^{-1}$ (v/v), 1-MCP at 1.0 $\mu\text{L L}^{-1}$ and the heat treatment at 40 °C for 24 h delay the ripening and reduce the occurrence of internal flesh browning in 'Laetitia' plum.

2.The heat treatment at 37 °C and the application of ethanol at 500 $\mu\text{L L}^{-1}$ (v/v) for 24 h reduce the occurrence of internal flesh browning in 'Laetitia' plum, however, these treatments are not efficient in delaying the ripening process.

3.The heat treatment at 40 °C for 24 h increases the incidence of decay in 'Laetitia' plum after 35 d of regular air storage plus four days of shelf life conditions.

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