Ethylene and changes during ripening in ‘Horvin’ plum (Prunus salicina Lindl.) fruits

El etileno y los cambios durante la maduración en frutos de ciruela (Prunus salicina Lindl.) ‘Horvin’

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ABSTRACT

The plum is a fruit prized for its nutraceutical properties because of its high content of fiber and sorbitol, which aid in digestion; furthermore, it is characterized as containing antioxidant pigments and an antiseptic action with anthocyanins. These fruits are classified as climacteric and continue the process of respiration and ripening after harvesting; because of this, it is necessary to harvest early so they can withstand transport, which often causes the fruits to not reach the consumption maturity required by consumers. The effect of ethylene on the ripening of plum fruits was evaluated, for which a completely randomized design with 10 treatments was used, which resulted from the combination of two factors plus a control without applications. The first factor was the ethylene dose (100; 1,000 and 2,000 mg L⁻¹) and the second factor was the length of exposure to the ethylene (5, 10 and 15 minutes). The fruits were stored at room temperature for 9 days. In the fruits treated with ethylene, a significant increase in the respiration index and total soluble solids was observed. Additionally, greater total titratable acids and firmness values were obtained with the control treatment than with the ethylene; similarly, the ethylene application increased the fresh mass loss in the plum fruits. Overall, differences between the fruits treated with ethylene and the control were observed, but not between the doses, indicating that the lowest ethylene dose (100 mg L⁻¹) can obtain the desired uniformity in plum fruits.

Key words: postharvest, storage, peel color, fruits.

RESUMEN

La ciruela es un fruto muy apreciado por sus propiedades nutraceuticas, ya que por su alto contenido de fibra y sorbitol ayuda a la digestión, además se caracteriza por contener pigmentos de acción antioxidante y antiséptica como las antocianinas. Estos frutos se clasifican como climatéricos, y continúan su proceso de respiración y maduración después de ser cosechados, debido a esto es necesario cosecharlos en etapas tempranas, para que puedan soportar el transporte, lo cual ocasiona que muchas veces los frutos no alcancen la madurez de consumo requerida por el consumidor. Se evaluó el efecto del etileno sobre la maduración de frutos de ciruela, para lo cual se utilizó un diseño completamente aleatorizado con 10 tratamientos, los cuales resultaron de la combinación de dos factores más un testigo (sin aplicación). El primer factor fue la dosis de etileno (100, 1,000 y 2,000 mg L⁻¹) y el segundo factor el tiempo de exposición al etileno (5, 10 y 15 minutos). Los frutos fueron almacenados a temperatura ambiente durante 9 días. En los frutos tratados con etileno se observó un aumento significativo en el índice de respiración y los sólidos solubles totales. Adicionalmente, en las variables de acidez total titulable y firmeza se obtuvieron valores mayores en el tratamiento testigo que en los tratamientos con etileno, del mismo modo la aplicación de etileno aumentó la pérdida de masa fresca en los frutos de ciruela. En general se observaron diferencias entre los frutos tratados con etileno y el testigo, pero no entre las dosis, lo que indica que con la menor dosis de etileno (100 mg L⁻¹) se puede obtener la uniformidad deseada en los frutos de etileno.

Palabras clave: poscosecha, almacenamiento, color de la epidermis, frutas.

Introduction

The Horvin plum fruit variety comes from a deciduous fruit tree widely cultivated in Colombia (Campos, 2013; Álvarez-Herrera et al., 2015); the fruit is a drupe that usually presents wax on the epidermis (Baugher, 2003). Plum fruits can measure between 2 and 8 cm and the shape can be round, oblong or elliptical (Baugher, 2003). Their principal component is water and they are enriched with phosphorus, calcium and potassium (Calvo-Villegas, 2009). They also contain compounds that are of nutritional interest, including sorbitol, which promotes a laxative characteristic and a high fiber content (Calvo-Villegas, 2009). The same author also mentioned that they have antiseptic, antioxidant...
pigments, such as anthocyanins. However, their consumption is low, which is attributed to under ripe when marketed (Crisosto et al., 2004) because consumers value the fruits of plum in color, flavor and aroma (Louw and Theron, 2012) and even though they are many nutritional benefits they provide plums, postharvest handling is not appropriate because the fruits are often harvested early in order to facilitate transport and distribution (Abdi et al., 2002).

At present, deciduous fruit crops present several challenges, some related to the handling of fruit in the pre- and postharvest periods, due to the high demand for quality in specialized markets (Fachinello et al., 2011) that obliges producers and marketers to implement appropriate technologies that help conserve the physical and chemical characteristics of fresh produce for a longer period of time. Among the alternatives for fruit storage, the use of 1-MCP has presented major effects in terms of the preservation of the postharvest shelf-life of fruits. Velardo et al. (2012) reported that 1-MCP treatments did not affect weight loss, but retained flesh firmness; 1-MCP helped to maintain the characteristic fruit color regardless of the temperature for more than 30 d of shelf-life during storage. Dong et al. (2001) reported in fruits of ‘Red Rosa’ plum was treated 1-MCP low ethylene production and softened more slowly than untreated fruits. They also showed lower exopolypgalacturonase (exo-PG) and endo-glucanase (EGase) activities and the respiration rates, pectin esterase (PE) and endo-PG were similar in 1-MCP-treated fruits and control. Ozturk (2015) reported the effects of pre-harvest methyl jasmonate (MJ) treatments on the ethylene production, respiration rate, bioactive compounds and physico-chemical parameters of plum fruits (Prunus salicina Lindl. cv. Fortune and Friar); in both plum cultivars, 1,120 mg L⁻¹ MJ increased the hue angle of the fruits. The fruit mass and geometric mean diameter were lower in the MJ treatments, while the flesh firmness was higher, except on the initial harvest date. The soluble solids concentration increased and the titratable acidity decreased with the MJ treatments. The MJ-treated fruits exhibited higher levels of ethylene production and respiration rates. MJ was more effective in increasing the water-soluble antioxidant activity, water-soluble phenolics and individual phenolics. The chlorogenic acid, caffeic acid, rutin, ferulic acid, naringenin and kaempferol contents increased significantly with 2,240 mg L⁻¹ MJ.

In plum crops, fruit harvesting is done manually based on the fruit coloration, according to the proportion of color in the epidermis (green-red), which generates different organoleptic characteristics when marketing and harvest maturity plays a very important role in determining the quality and shelf-life of the product and consumer acceptance (Singh and Khan, 2010).

In climacteric fruits such as the plum, ethylene is one of the promoters and coordinates the principal changes during the growth period; this action promotes fruit softening and accelerates the metabolic processes (Palou et al., 2003). The action of this hormone is produced by the binding of the molecule and the receptors located in the endoplasmic reticulum membrane (Serek et al., 2006). This process activates a receptor that sends transduction signals that generate a physiological and generic response (Pereira et al., 2008). The ethylene presence before harvest improves the stimulation of maturation, offering consumers better tasting plums that are fully ripened (Kader, 2008), because the marketing of plum fruits are valued for their color, flavor and aroma (Louw and Theron, 2012).

The side effects of ethylene doses on plums is unknown; as a perishable fruit, it can diminish its shelf-life, but, if the treatment is done after a prolonged refrigeration period or with crops recently harvested at different maturity levels, they are not ready to be commercialized. It could be a management option with greater opportunity for distributing products in the international market.

Moreover, this study aimed to assess the effect of ethylene on harvested Horvin variety plums and to establish the acceleration of fruit ripening through ethylene doses, as well as maintain the physical and organoleptic characteristics of plum fruits.

Materials and methods

This study was performed at the Vegetal Physiology Laboratory of the Universidad Pedagogica y Tecnologica de Colombia, in Tunja (Colombia). The plum fruit samples were taken from a commercial crop located at 2,600 m a.s.l., 05° 41’ 36”N and 73° 14’ 51”W in the Tuta municipality of the department of Boyaca. Furthermore, this location has an average precipitation of 935 mm and temperature between 12 and 14°C.

The plum (Prunus salicina Lindl.) variety Horvin was used, which was collected in four different maturity stages (2, 3, 4 and 5) according to the classification reported by Sánchez and Saavedra (2013). The fruits were selected with homogenous sizes, a lack of damage and clean conditions. A commercial ethylene was used, known as Ethrel®. The fruits were disinfected with a 1% sodium hypochlorite solution, leaving them to air dry for 30 min, followed by
immersion in an ethylene solution, depending on the dose and time of the treatment. The storage of the plum fruits was done at room temperature (18°C).

A completely random design was used, in which three different ethylene doses were used (100; 1,000 and 2,000 mg L⁻¹), according to the doses used by Arévalo-Galarza et al. (2007) and Balaguera-López et al. (2013), along with a control without any applications, for three different exposure times (5, 10 and 15 min), for a total of 10 treatments with four replications, corresponding to 40 experimental units (EU). Each EU was composed of approximately 400 g of fruit.

The measurements were taken every 3 days after the harvest (dah) using the methodology described by Deaquiz et al. (2014); the color was evaluated with a Minolta CR 300 digital colorimeter (Minolta, Osaka, Japan). The CIELAB “L”, “a” and “b” system parameters were determined with three readings for each fruit in the equatorial diameter. L indicates the brightness, where 0 is black and 100 white; values of “a” <0 indicate a tendency towards green and >0 towards red; “b” has the same range, but values <0 indicate a tendency towards blue and >0 towards yellow. For this variable, two fruits were labeled per experimental unit, with which the measurements were taken over time, always with the same fruits and the same area. The mass loss was measured with an Acculab VIC 612 electronic scale with 0.01 g precision (Sartorius Group, Goettingen, Germany), the respiratory intensity (RI) was measured with a labquest 2 (Vernier Software & Technology, Beaverton, OR), the firmness was measured in the equatorial part of the fruit with a PCE-PTR200 digital penetrometer digital (PCE-Iberica, Tobarra, Spain), and the total titratable acidity (TTA) was determined using the formula: % acidity = (A * B * C) / 100/D, where: A = volume of spent NaOH; B = normality of NaOH (0.097); C = equivalent weight in g of the fruit’s predominant acid (0.067 g meq citric malic acid); and D = weight in grams of the sample (5 g) and total soluble solids (TSS) using a refractometer Hanna HI 96803 (Hanna Instruments, Eibar, Spain). This phase process lasted 9 d, in which the plum fruits lost their organoleptic quality.

With the obtained data, a normality test and a homogeneity of variance test (Bartlett) were carried out (Shapiro-Wilk). An analysis of variance (Anova) was done to determine the statistical differences and establish better treatments through the comparisons of Duncan (P≤0.05). This analysis and the statistics were done with the statistic program SAS v. 9.2e (SAS Institute Inc., Cary, NC).

Results and discussion

Respiratory intensity (RI)

The variance analysis showed significance differences between the control and the ethylene treatments (Fig. 1). Furthermore, at the beginning of the experiment, there were no significant differences due to the fact that all of the fruits were at the same ripening stage (stage 3: 50% V-50% R) with a starting RI average of 21.41 mg CO₂ kg⁻¹ h⁻¹. After the third day, there was an RI increase that continued on day 6 and day 9 of storage although some of the treatments had a decrease in their RI average.

On the other hand, the control treatment showed an RI increase of 19.10 mg CO₂ kg⁻¹ h⁻¹ after the third day of its storage, in contrast to the other treatments that showed a considerable increase in their respiratory rate, obtaining an average of 35.9 mg CO₂ kg⁻¹ h⁻¹. It was also observed that the same tendency occurred after the sixth day of storage of the control treatment, in which the RI was lower (29.08 mg CO₂ kg⁻¹ h⁻¹) in comparison with the other treatments (39.18 mg CO₂ kg⁻¹ h⁻¹). After the ninth day of storage, both the control treatment and the 100 mg L⁻¹ treatment had an RI that decreased with 5 and 10 min of immersion due the natural behavior of the climacteric fruits. Once fruits reach the highest climacteric, when the highest respiratory rate also occurs, the aging process and death of fruits starts and the RI decreases (Rodriguez and Restrepo, 2011).
Significant differences between the ethylene doses were observed on the third day of the fruit storage experiment, but not so much at the beginning nor on the sixth day or ninth day of the fruit storage. Moreover, the Anova showed that there was not a variant between the immersion time in which similar values were obtained, contrary to the control test that presented variants (Fig. 2).

Based on the observations, it was clear that the physicochemical changes that took place during the ripening process of the plum fruits were initiated and coordinated by ethylene. In general, most of the fruits were very receptive to the endogenous ethylene and exogenous ethylene in which plum fruit was not different based on the results acquired in this study.

It is known that the plum fruit is a climacteric fruit that starts its ripening process with the autocatalysis of ethylene production and an increase in the respiratory rate (Park et al., 2006). In the beginning of the ripening phase, this translates into physical changes, such as a loss of firmness, loss of green color, decrease in acidity, development of a scent and irreversible aging (Palou et al., 2003). In addition, according to the above, Park et al. (2006) confirmed that the production of ethylene throughout the catalysis effect of the endogenous ethylene process is a well-known climacteric fruit trait found in kiwi and plum fruits. In this study, the respiratory behavior of the plum fruits treated with ethylene started with a prolonged increase during the first 6 d of storage, where it reached the climacteric peak; then, an RI decrease initiation was observed, which is consistent with the assertion of Fonseca et al. (2002) that the rate of respiration in climacteric fruits is high in the early stages of fruit development, reaching peaks, but decreases as the fruit ripens, approaching senescence. Larsen and Vangdal (2013) evaluated the RI and ethylene production in nine plum cultivars that showed relatively high rates of respiration and were strongly influenced, more so by the storage temperature than by the production of endogenous ethylene, confirming the assertion of Zuzunaga et al. (2001) that most plum cultivars exhibit typical climacteric changes of the RI; however, some cultivars show a different pattern called suppressed climacteric, which is not the case for the Horvin variety, which presents the typical climacteric peak of the species, rather prematurely due applications of exogenous ethylene.

**Fresh mass loss**

There were no significant differences between the treatments; however, a normal pattern occurred when the fruits were stored. As time passed, the percentage of loss increased 5.12% the first 3 d and 12.42% after 9 d of storage. On the other hand, in the plum fruits that were treated with 2,000 mg L⁻¹, the lost mass decreased 5.87% within the first 3 d and 14.34% after 9 d of storage (Fig. 3).

The increase in the mass loss of the fruits and vegetables during storage is a pattern that originates in the natural process of transpiration and respiration, in which water loss occurs (Znidarcic et al., 2010), during the postharvest period, more so in climacteric fruits such as plum fruits. In addition, the use of exogenous ethylene can reduce the quality of fruits (Wills and Warton, 2004) due to the fact that it causes degradation of the cellular walls, speeding up the transpiration and respiration processes and leading to greater water loss (Kader, 2002). This greatly affected
the quality of the plum fruits tested due to the use of the ethylene (2,000 mg L⁻¹), which caused greater mass loss over time.

**Firmness**

This variable showed differences between the control and the ethylene treatment due to the fact that the fruits treated with ethylene had greater firmness loss over time, with 87.2% at 9 d of storage for 2,000 mg L⁻¹, very similar to the data from the treatments with doses of 100 and 1,000 mg L⁻¹ (87.34 and 88.01%, respectively). For the control treatment, the loss of firmness was less dramatic, with a reduction in the firmness of 64.40% (Fig. 4). This behavior during the plum fruit ripening process has also been observed in several kinds of fruits and vegetables when treated with ethylene, its firmness was reduced this becoming in fruits acceptable to the market. However, when treated with ethylene for long periods of time, the ripening process could become more rapid and the aging could occur faster, leading to fruit pulp that is too soft and an unacceptable product for consumers. The degradation of the polymer carbohydrates, more precisely cellulose and pectin, weakened the cell walls and the cohesive forces that keep the walls together, producing firmness loss (Reid, 2002).

On this subject, Sánchez and Saavedra (2013) agreed that Horvin fruits lose firmness at every stage of their

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**FIGURE 3.** Mass loss of 'Horvin' plum fruits during the postharvest period with application ethylene. A, ethylene doses; B, immersion times. Means with different letters in treatment indicate significant differences according to the Duncan test ($ P \leq 0.05$) ($ n = 4$).

**FIGURE 4.** Firmness of 'Horvin' plum fruits during the postharvest period with application ethylene. A, ethylene doses; B, immersion times. Means with different letters in treatment indicate significant differences according to the Duncan test ($ P \leq 0.05$) ($ n = 4$).
ripening process when the storage time is increased. The following values were taken for 100% red plum fruits: 27.21 N at the beginning of harvest and 3.61 N 15 d after they were harvested. In contrast, the 100% green plum fruits showed greater firmness, with a beginning value of 50 and a finishing value of 19 N at day 29 after the harvest. When comparing the above mentioned values with the obtained values in this study; it was perceived that, since the plum fruits were treated with ethylene, all of the values were lower in all of the treatments, except in the control treatment. In addition, Park et al. (2006) observed that kiwi fruits treated with ethylene lost their firmness quickly during their ripening process. These values correlated with the results obtained by Palou et al. (2003) in peach fruits treated with ethylene doses of 1, 10 and 100 µL L⁻¹, which showed a softening in their texture in contrast to the fruits that were not treated. The occurrence of these changes is important because firmness is a key parameter in fruit quality that is directly related to maturity and is often a good indicator of the potential shelf-life in fruits such as plums (Parra-Coronado et al., 2007; Valero et al., 2007). In the specific case of plums, there have been no studies on ethylene applications to promote maturation, but with respect to the firmness, Usenik et al. (2014) stated that, for good consumer acceptance, the firmness of the plum fruit must be close to 15 N, but also that these values depend not only on the cultivar but also to the environmental conditions because fruits harvested at periods of high rainfall showed values inferior to those obtained at periods of low rainfall.

Color
When comparing the averages obtained in the different treatments, it was observed that there was only differences between the control treatment (without ethylene applications) and the treatments with ethylene applications, regardless of the dose used; therefore, it was inferred that either the lowest dose (100 mg L⁻¹) or the highest dose (2000 mg L⁻¹) can obtain the same results for the change in peel color (Tab. 1).

The data obtained for the color index (CI = 1000 x a/L x b) indicated that the application of ethylene accelerated the color change in the plum fruits, comparing the average of the control (33.79 and 69.42) at 3 and 6 d, respectively, with the averages obtained in the treatment with an ethylene dose of 100 mg L⁻¹ on the same measurement day (84.03 and 111.81). In the plum fruits, the anthocyanin content increased during development and maturation, reaching a maximum at full maturity (Hagen et al., 2006). In this period, synthesis is stimulated by light through two mechanisms: one due to an increase in the photosynthetic activity and the second by the activation of phytochrome, which is an intermediate in various processes (Bastías and Corelli-Grappadelli, 2012).

In the last measurement, no difference was observed between the treatments; therefore, it was inferred that, regardless of the application of ethylene, the color index will be the same as that obtained in the fruits without

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Means with different letters in each row indicate significant differences according to the Duncan test (P ≤0.05) (n=6).

Rozo-Romero, Álvarez-Herrera, and Balaguera-López: Ethylene and changes during ripening in ‘Horvin’ plum (Prunus salicina Lindl.) fruits
applications. Therefore, ethylene applications induce a color change more rapidly, coinciding with Deaquiz et al. (2014) who studied pitahaya fruits where the application of ethylene accelerated the color change.

**Total soluble solids (TSS)**

The Anova test only showed significant differences between the treatments with the 2,000 mg L\(^{-1}\) ethylene application and a 10 min immersion, which had a difference in the measurements taken at 6 d; in which, it showed a lower average than the other treatments. On the third day of the storage, a difference was observed between the control treatment with a value of 15.37 °Brix and the 2,000 mg L\(^{-1}\) treatment with a value of 14.3; the other two treatments (100 and 1,000 mg L\(^{-1}\)) do not show differences between them or with the other ones. In this same tendency, it was observed that the measurements taken on day 6 of storage presented a clear difference between the control treatment and the 2,000 mg L\(^{-1}\) treatment; however, the 100 mg L\(^{-1}\) treatment did not have any differences with the control. On day 9 of storage, there was not any difference between the average values of each treatment (Fig. 5). A similar behavior was observed in degreening citrus fruits by being exposed to ethylene where it does not affect the quality parameters, when it's compared against the de degreening citrus fruits without ethylene (Sdiri et al., 2012). Other studies agree that fruits treated with ethylene present similar biochemical and physical changes as non-treated fruits (Plaza et al., 2004; Martínez-Jávega et al., 2008; Tietel et al., 2010).

A considerable increase was noticed in the quantity of TSS obtained on day 3 of storage in all of the treatments. On day 6, there was a small decrease in this value and at the end of the experiment, all of the treatment values had a similar tendency. This increase was attributed to starch hydrolysis and other carbohydrates during the produce ripening stage (Goñi et al., 1997). The increase in this value during the ripening stage coincided with the results obtained by Park et al. (2006), who found that the TSS was significantly higher in kiwi treated with ethylene, while the TSS gradually decreased independent of the applied treatments.

This behavior is important considering that the calculation of the TSS content is a key variable for fruit quality (Di Miceli et al., 2010; Infante et al., 2011), which, together with the acidity and the maturity index (TSS/TTA), has been established as one the most determining maturity indices for plums (Prasanna et al., 2007; Casquero and Guerra, 2009). It was also observed that, the in the 2,000 mg L\(^{-1}\) ethylene treatment, the TSS levels were lower than in the other treatments, which was attributable to an excess application; as stated by Atta-Aly et al. (2000), a large amount of exogenous ethylene may inhibit a proper maturation system.

In general, excluding the data obtained in the treatment with the highest ethylene dose (2,000 mg L\(^{-1}\)), there was no significant difference between the treatments with ethylene and the control, which coincided with the results of Palou et al. (2003), where the internal quality of fruits exposed to ethylene as climacteric (grapes) and non-climacteric (stone fruits) were unaffected by treatment with exogenous ethylene. Furthermore, Singh and Singh (2012) observed that there were no differences in the TSS of Japanese plums treated with 1-MCP in cool and controlled atmospheres,

**FIGURE 5.** Total soluble solids (TSS) of ‘Horvin’ plum fruits during the postharvest period with application ethylene. A, ethylene doses; B, immersion times. Means with different letters in treatment indicate significant differences according to the Duncan test (\(P \leq 0.05\)) (\(n=4\)).
as compared to the control. This contrasts with the report of Park et al. (2006) who showed that the contents of fructose, glucose and sucrose in ethylene-treated kiwi fruits increased, as compared to the untreated samples, deducing that the ethylene treatment improved the free sugar by increasing the metabolism of the kiwis.

Total titratable acidity (TTA)

This variable showed the same behavior as the respiration, where, at the beginning of the experiment, there was no significant change between the treatments; however, from day 3 of storage, the fruit response was different according to the applied treatment. The comparison between the results showed that, at day 3, there were significant differences in the control treatment, with a TTA of 1.18 %, while the 2,000 mg L\(^{-1}\) ethylene treatment obtained 0.85%. In the other two treatments (100 mg L\(^{-1}\) and 1,000 mg L\(^{-1}\)), there was no difference between them or with the other two treatments (Fig. 6).

Therefore, it was demonstrated that the highest ethylene dose helped to greatly reduce the TTA, a factor that is very important because plums contain high levels of acids, mainly malic acid, which decrease during ripening as they are used as respiratory substrates, and with increasing IR by ethylene action, be increase spend of acid that are used in the respiration process (Rodríguez et al., 2005), which benefits the fruits due to the fact that consumers prefer fruits with a great appearance and low levels of acids.

These results agree Sdiri et al. (2012) who evaluated the result of combined of degreening treatments with ethylene for the quality of ‘clementines’ tangerines and ‘Navelina’ oranges, both of which showed an increase in the TSS and a reduction in the TTA during the entire shelf-life, in comparison to the initial harvest results. With respect to the immersion time factor, there was no statistical difference for the TTA.

Conclusions

The application of ethylene accelerated the plum fruit ripening, especially during the first days of storage (day 3 to 6). Moreover, the application of ethylene increased the amount of TSS, improved fruit color, and decreased the TTA and firmness. Obtaining good quality plum fruits can be achieved with the application of a dose of 100 mg L\(^{-1}\) ethylene. As for the immersion time, there was no difference in any of the variables, so a minimum of 5 min is recommended for treatments to be effective.


