

Ethylene and 1-MCP affect the postharvest behavior of yellow pitahaya fruits (*Selenicereus megalanthus* Haw.)

Etileno y 1-MCP afectan el comportamiento poscosecha de frutos de pitahaya amarilla (*Selenicereus megalanthus* Haw.)

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ABSTRACT

The pitahaya or dragon fruit is one of the most representative exotic fruits that Colombia has, with an important, growing international market, but the cultivation and postharvest of this fruit lack sufficient technological support to be more competitive. Therefore, alternatives that provide good-quality products that meet market requirements are very important. Therefore, the objective of this research was to determine the effect of a ripening retardant and ethylene application on the conservation and quality of pitahaya fruits and the possible changes during ripening associated with ethylene, for which a completely randomized design with three treatments was used, corresponding to the application of ethylene (ethephon, 3 mL L⁻¹), 1-methylcyclopropene (1-MCP, 600 mg L⁻¹) and a control, with four replications, for a total of 12 experimental units. The fruits were stored at 18°C with 75% relative humidity. The 1-MCP application significantly decreased the loss of firmness, total soluble solids, loss of fresh mass and respiratory rate. Fruits from the control and ethylene treatment tended toward a climacteric respiratory behavior. The total carotenoid content of the fruits was significantly higher in the ethylene application and the control treatment, which was consistent with the color change of the fruits. It can be concluded that the 1-MCP application reduced the ethylene action, slowing the ripening of the dragon fruits.

Key words: 1-methylcyclopropene, quality, ripening, climacteric, cactus.

RESUMEN

La pitahaya es uno de los frutos exóticos más representativos que tiene Colombia, con un importante y creciente mercado internacional, sin embargo el cultivo de este frutal carece del suficiente respaldo tecnológico para ser más competitivo. Por lo tanto, se requiere buscar alternativas que permitan ofrecer un producto de buena calidad con el fin de cumplir con las exigencias comerciales. Debido a lo anterior, esta investigación buscó determinar el efecto de la aplicación de un retardante de madurez y etileno en la conservación y calidad de frutos de pitahaya y los posibles cambios durante la maduración asociados a etileno, para lo cual se utilizó un diseño experimental completamente al azar con tres tratamientos, que correspondieron a la aplicación de etileno (ethephon, 3 mL L⁻¹), 1-metilciclopropeno (1-MCP) (600 mg L⁻¹) y un testigo, con cuatro repeticiones, para un total de 12 unidades experimentales. Los frutos fueron almacenados a 18°C con una humedad relativa del 75%. La aplicación de 1-MCP disminuyó significativamente la pérdida de firmeza, los sólidos solubles totales, la pérdida de masa fresca y la tasa respiratoria. Los frutos control y los tratados con etileno tendieron a un comportamiento respiratorio climaterico. El contenido en los carotenoides totales en los frutos fue significativamente para la aplicación de etileno y el tratamiento control lo que es consistente con el cambio en la coloración de los frutos. Se puede concluir que la aplicación de 1-MCP reduce la acción de etileno, lo cual reduce el proceso de maduración en los frutos de pitahaya.

Palabras clave: 1-metilciclopropeno, calidad, maduración, climaterio, cactus.

Introduction

The yellow pitahaya or dragon fruit (*Selenicereus megalanthus* Haw.; syn. *Hylocereus megalanthus* [K. Schum. ex Vaupel] Ralf Bauer; Corredor, 2012) is a cactus that is cultivated in countries such as Colombia, Ecuador, Israel (Corredor, 2012), Vietnam, Malaysia, Mexico, Costa Rica

and Nicaragua, which, 15 years ago, was virtually unknown in the international markets (Le Bellec and Vaillant, 2011).

The fruit is an ovoid berry with scales or helically distributed clusters of deciduous spines, weighs between 120 and 250 g, and has epidermis that in immature states, is green and, when ripe, is yellow, while the pulp is white with

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black seeds (Corredor, 2012; Le Bellec and Vaillant, 2011). The pitahaya is an exotic fruit with high demand due to its nutritional value, flavor, aroma and medicinal properties (Dueñas *et al.*, 2008). The fruit is consumed fresh due to its refreshing pulp, which has a texture close to that of kiwi (Le Bellec and Vaillant, 2011). Colombia has 478 ha planted, with the Boyaca department being the largest producer with 221 ha and an annual production of 1,688 t (ICA, 2010).

To maintain the market position of the fruit, it is vital that techniques of storage and ripening retardant applications be used, which reduce the effect of ethylene (Watkins, 2008) and conserve a longer postharvest quality (Magaña *et al.*, 2004; Andrade *et al.*, 2006). Ethylene is a natural gaseous hormone (Martínez-Romero *et al.*, 2007) that regulates the processes related to fruit ripening and senescence (Binder, 2008). The action of ethylene results from the binding of molecules to receptors located in the cell membrane of the endoplasmic reticulum (Serek *et al.*, 2006). This binding activates the receptors, which send transduction signals, generating a gene expression and physiological response (Pereira *et al.*, 2008). For this reason, the endogenous and/or exogenous presence of this hormone accelerates ripening and creates desirable senescence effects (fast, uniform ripening) or undesirable effects, such as reductions in the fruit's life (Giovannoni, 2004).

There is a group of compounds called analogues of ethylene that competes for membrane receptors and inhibits the effect of this hormone, within which 1-methylcyclopropene (1-MCP) is notable, which has been widely used in various fruits and vegetables (Nanthachai *et al.*, 2007; Watkins, 2008), generating changes in multiple metabolic processes, such as decreases in respiratory rates, ethylene production and volatile degradation of chlorophylls, changes of color, sugars, acidity and softening, which vary depending on the fruit, concentration and exposure time (Watkins, 2006). Serna *et al.* (2011) applied aqueous solutions of 1-MCP to yellow dragon fruits, reducing changes in acidity, respiratory rate and color.

In this sense, the employment of technologies such as 1-MCP applications has a potential use in the reduction of changes associated with quality losses during postharvest, which would extend the shelf-life of fruits and vegetables (Vilas-Boas and Kader, 2007); so the greater exposure time of the product, the lower the needed concentration will be in order to obtain the desired effect (Bassetto, 2002).

In the dragon fruit, changes during ripening associated with the presence of ethylene are not well-known and this knowledge can be a useful parameter for increasing the postharvest life of this fruit with the use of ethylene action inhibitors. Therefore, the objective of this study was to evaluate the effect of applications of ethylene and 1-MCP on the ripening and postharvest behavior of yellow dragon fruits.

Materials and methods

The study was conducted in the postharvest laboratory of the Faculty of Agriculture Sciences of the Universidad Nacional de Colombia, Bogota. The fruits were collected from a commercial orchard (Piedras Verdes farm) in the municipality of Miraflores (Boyaca, Colombia), located at 1,600 m a.s.l., latitude 5°11' and longitude 73°08', with an average annual rainfall of 2,500 mm, a temperature ranging between 18 and 24°C and an average relative humidity of 87%.

The plant material used was yellow dragon (or pitahaya) fruits (*Selenicereus megalanthus* Haw.), which were collected in maturity grade 3 (4580 norm of Icontec, 1996). The fruits presented uniform size, no mechanical damage and good phytosanitary condition. 1-MCP (powdered form from Rohm and Haas, Bogota) and ethylene (Ethrel® 48 SL from Bayer CropScience, Bogota) were used.

The experimental design was completely randomized with three treatments that corresponded to the application of ethylene (3 mL L⁻¹), 1-MCP (600 mg L⁻¹) and a control with four replications, for a total of 12 experimental units (EU). Each EU was composed of six fruits. The fruits were stored at an average temperature of 18°C and a relative humidity of 75%. The fruits were disinfected with a solution of 2% NaCl and washed with distilled water, then subjected to the treatment applications.

The 1-MCP was vaporized according to the methodology of Herrera (2007), where 600 mg L⁻¹ of 1-MCP was weighed in a 10 mL glass tube, which was sealed with a rubber stopper, through which the hot water (45-50°C), provided by the manufacturer, was injected. The dissolution of 1-MCP in the hot water generated the release of gaseous 1-MCP in the headspace of the tube. This was introduced into a 2 L sealed chamber containing the fruits; then the chamber was opened to release the compound and immediately sealed for 24 h. The Ethrel® was dissolved in 10 L of water, in which the fruits were submerged for 15 min. Measurements were taken every 3 days after storage (das) for the variables of

fresh mass loss and respiration rate and every 6 das for the other evaluated variables, described below. The fruits were stored until they lost organoleptic quality.

The evaluated variables were: fruit firmness (N), determined with a digital PCE-PTR200 penetrometer (PEC Ibérica SL, Albacete, Spain); fresh mass loss (%), by measuring the fresh weight of the fruits with a 0.0001 g precision balance (Ohaus, Ohio, OH); total titratable acidity (TTA; % citric acid), using the formula: % Acidity = $(A * B * C) * 100/D$, where: A = volume of NaOH used, B = normality of NaOH (0.097), C = equivalent weight in g of predominant acid in the fruit (citric acid 0.064 meq g⁻¹), D = weight in grams of the sample used (5 g); total soluble solids (TSS), using a digital Hanna refractometer (Hanna Instruments, Woonsocket, RI), 0 to 85% range at 20°C. The maturity index (MI) was calculated with the TSS/TTA ratio.

For the extraction and quantification of chlorophyll and carotenoids, approximately 1 g of pulp was weighed, 5 mL of acetone was added, vortexed for 1 min and then centrifuged for 10 min at 4,000 rpm. The supernatant was poured into a 25 mL flask; acetone was added to the pellet, vortexed and then centrifuged. This procedure was repeated three times. Acetone was added to the obtained supernatant to obtain a volume of 25 mL. The absorbance was measured in a spectrophotometer at 450, 663 and 645 nm.

For the determination of the respiratory rate, approximately 300 g of fruit were placed in an airtight 2 L chamber, in which an infrared CO₂ sensor was located, which was connected to a LabQuest (equipment for data capture). Every 4 sec for 5 min, CO₂ values were recorded. With these

values, the slope was calculated, which corresponded to the respiration rate. Also, the fruit weight and volume of the chamber were taken into account to convert the data to mg CO₂ kg⁻¹ h⁻¹.

Normality tests were carried out to determine the distribution of the data; afterwards, an analysis of variance (ANOVA) was conducted to determine statistical differences between the treatments and to classify them with a least significant difference (LSD) test ($P \leq 0.05$). Analyses were performed using SAS statistical software v. 9.1e (SAS Institute Inc., Cary, NC).

Results and discussion

Firmness

There were significant differences between the 1-MCP and the ethylene treatment but not with the control treatment (Fig. 1A). Lower values of firmness were measured in the fruits treated with ethylene (8.13 N at 15 das). In the loss of firmness behavior, apparently, the action of hydrolase enzyme is involved, which is induced by ethylene (Sañudo *et al.*, 2008); enzymes such as polygalacturonase (PG), pectin methyl esterase (PME), β -galactosidase and pectate lyase (PL) degrade the polymeric carbon hydrates, especially those of pectic and hemicellulosic substances (Giovannoni, 2004; Goulao *et al.*, 2007), through which the cell walls and the tensile force, which hold the cells together, are weakened (Wills *et al.*, 2007) and the softening of the dragon fruit may have been accelerated. Similar cases of decreasing firmness were presented in *Hylocereus undatus* fruits stored at 20°C when they reached consuming maturity (To *et al.*, 2002). Also in avocado fruits exposed to ethylene, firmness was

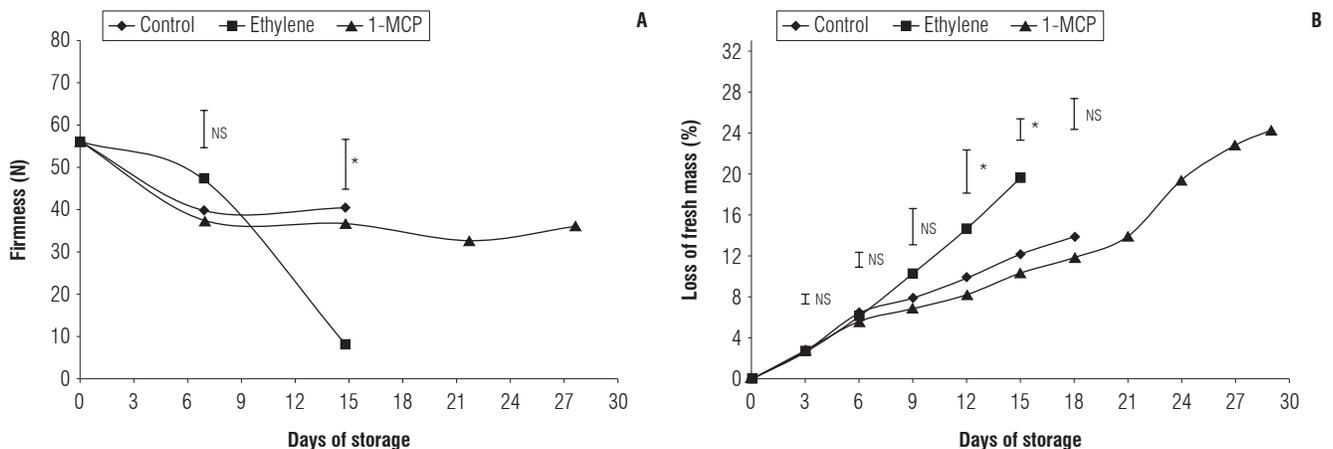


FIGURE 1. A, firmness behavior; B, loss behavior in the fresh mass of the yellow dragon fruits subjected to applications of ethylene (3 ml L⁻¹) and 1-MCP (600 mg L⁻¹). The bar indicates the least significant difference test, LSD ($P \leq 0.05$). If the difference between the two averages at each sampling point is greater than the LSD, then there is a difference $P \leq 0.05$. *, 5% statistical differences; NS, no differences.

quickly lost in the first 5 d of storage (Feng *et al.*, 2000). Similarly, ethylene concentrations between 0.5 and 100 mL L⁻¹ significantly reduced the firmness of strawberry fruits after 3 d at 20°C (Tian *et al.*, 2000). Contrary to ethylene, the 1-MCP application delayed softening in apple, pear and melon fruits (Hiwasa *et al.*, 2004). Also, in yellow dragon fruits, applications of 400 mg L⁻¹ of 1-MCP slowed the loss of firmness due to a decreased activity of the enzymes: exo-PG, endo-PG and PME (Serna *et al.*, 2011).

Loss of fresh mass

The postharvest life of the yellow dragon fruits with and without ethylene treatments (control) was 15 das, as compared to fruits treated with 1-MCP, which showed a 28 das postharvest life. The cumulative loss of fresh weight presented significant differences at 15 das between the ethylene (19.60%) and 1-MCP treatments and the control, however, the latter two did not differ from each other (10.33 and 12.16%, respectively, Fig. 1B).

Increased exogenous ethylene may decrease fruit quality (Wills and Warton, 2004), caused by the degradation of the cell wall, which accelerates the physiological processes of transpiration and respiration, causing major losses of water, substrates (Kader, 2002) and antioxidants (Rodríguez *et al.*, 2006) and finally affecting the organoleptic characteristics of the fruit, so that a loss of fresh mass greater than 5% may be sufficient to reduce the quality of fruits (Wills *et al.*, 2007). In this experiment, fresh weight loss was slightly higher than that found by Nerd *et al.* (1999) for pitahaya fruits, with 4.2% after 1 week at 20°C.

According to Dueñas *et al.* (2009), the loss of fresh mass during fruit development is a normal response to increased transpiration and respiration, so it is important to minimize these losses. The 1-MCP application decreased mass losses in the dragon fruits, which agrees with Mata-Montes *et al.* (2007) for *Artocarpus heterophyllus*, *Allium tuberosum* (Wu *et al.*, 2009), tomato (Guillén *et al.*, 2007) and plum fruits, during storage (Valero *et al.*, 2003); contrary to Serna *et al.* (2011), who found that 1-MCP applications showed the highest mass loss of yellow dragon fruit.

Total soluble solids (TSS)

There were significant differences between the ethylene and other treatments. The 1-MCP treatment showed the highest values (19.30, 19.29 and 19.48 °Brix) for all the measurements during the fruit storage (Fig. 2A). The amount of TSS in the pitahaya progressively increased for the 1-MCP treatment and the control; while at 15 das, the TSS in the ethylene treated fruits decreased (17.65°Brix).

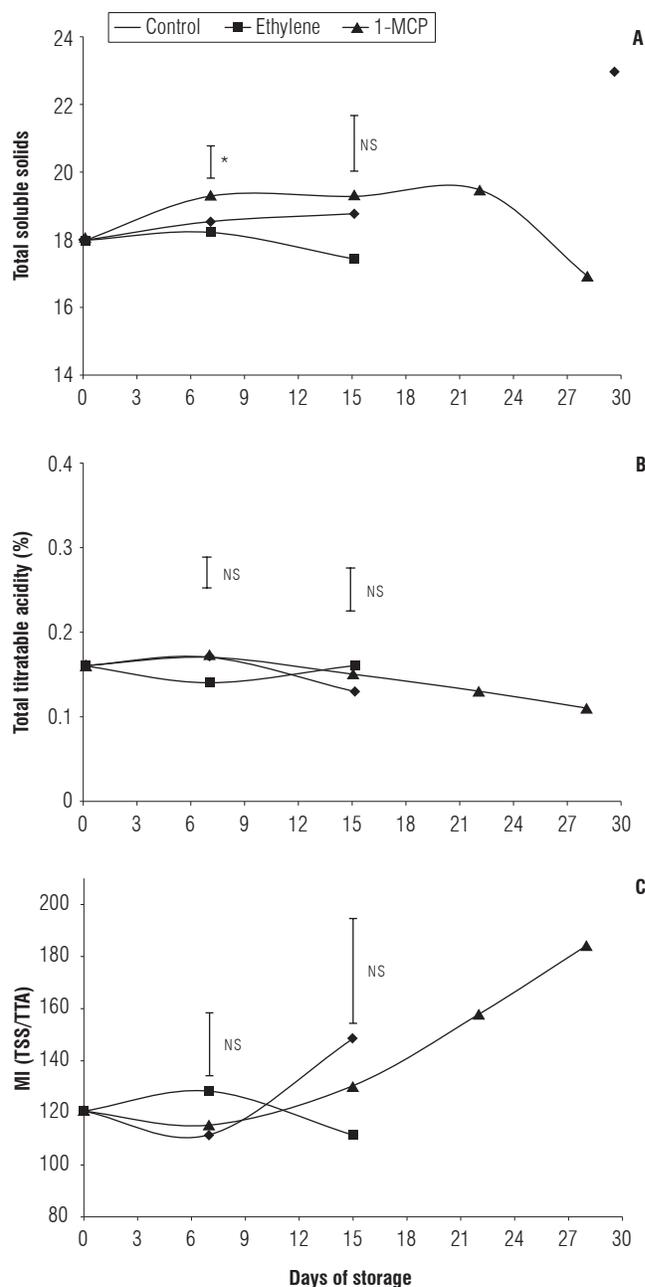


FIGURE 2. A, performance of total soluble solids (TSS); B, behavior of total titratable acidity (TTA); C, behavior of maturity index (MI) in the yellow dragon fruits subjected to applications of ethylene (3 mL L⁻¹) and 1-MCP (600 mg L⁻¹). The bar indicates the least significant difference test, LSD ($P \leq 0.05$). If the difference between the two averages at each sampling point is greater than the LSD, then there is a difference $P \leq 0.05$. *, 5% statistical differences; ns, no differences.

This increase of TSS in the dragon fruits can be explained by the hydrolysis of various structural polysaccharides, such as starch, pectins and other oligosaccharides in the cell wall, which, when solubilized in the aqueous phase, become part of the cellular juice (Menéndez *et al.*, 2006). Similarly, starch accumulation during fruit maturation will be degraded in sugars through the enzymatic action of

α -amylase, β -amylase and starch phosphorylase, increasing TSS (Kays, 1997).

The obtained results agree with Rodríguez *et al.* (2006), who reported an increase in the TSS of pineapple guava, followed by a decrease that may be associated with high respiratory rates. Similarly, as the storage became more prolonged, the TSS decreased in guava (Mercado *et al.*, 1998) and mango fruits (Jha *et al.*, 2006); this decrease may be associated with a loss of taste. However, the application of 1-MCP on the dragon fruits increased the TSS at 22 daa, contrary to that found with guava fruit cv. Safed (Singh and Pal, 2008) and with kiwi (Mao *et al.*, 2007).

Total titratable acidity (TTA)

No significant differences were seen for the TTA content of the dragon fruits during storage (Fig. 2B); however, the TTA declined throughout the post-harvest period. This occurs because organic acids are respiratory substrates (Rodríguez *et al.*, 2005) and, at this time, the fruit is transpiring rapidly by increasing metabolic processes and, thus, the respiratory rate (Wills *et al.*, 2007). Marin *et al.* (2009) mentioned that 1-MCP may affect the metabolism of carbohydrates. The above mentioned behavior is similar to that found in tomato (Beckles, 2012) and strawberry (Hernández-Muñoz *et al.*, 2006). Although the effect of 1-MCP was not significant, the tendency was for a reduction in the TTA decline, which coincides with that observed in guava (Bassetto *et al.*, 2005), pineapple (Selvarajah *et al.*, 2001) and plum fruits (Dong *et al.*, 2002).

Maturity index (MI)

This variable was not significantly different (Fig. 2C); however, at 15 das, the ethylene treatment had a significant

decrease of 13.15% in the MI, just as the point of greatest fruit ripening was observed with 1-MCP at 28 das. This was probably due to an increase in the concentration of TSS and a diminishing of the organic acid content (Kays, 2004; Taiz and Zeiger, 2006).

According to Kays (2004), increased MI occurs when fruits have reached their maximum respiratory rate, which increases metabolism by stimulating enzyme activity that quickly degrades organic acids, similar to what happened in the ethylene treatment. Therefore, this index can be used to measure the organoleptic quality (Rodríguez *et al.*, 2006) of dragon fruits because of the very low acid content. The organoleptic quality was obtained with an MI of 157.80, where the best expression of the taste quality of the fruit is presented (Campana, 2007).

The behavior of the MI during the postharvest phase showed a progressive increase during fruit storage, which is consistent with the findings of Rodríguez *et al.* (2005), who obtained yellow dragon fruits with a high IM in ripening stage 3. Also, in passion fruits, a high TSS/TTA ratio tends to generate a pleasant taste (Télez *et al.*, 2007).

Pigment content

The total chlorophyll content showed significant differences at 7 das (Fig. 3A). The control and ethylene treatments generated the highest values of total carotenoids during storage (Fig. 3B), which may have occurred because the ethylene produced by both, endogenously as well as exogenously applied, accelerated the degradation of chlorophyll and the appearance of red, yellow and orange pigments, which are involved in the expression of gene coding for the synthesis of carotenoids (Kazokas and Burns, 1998;

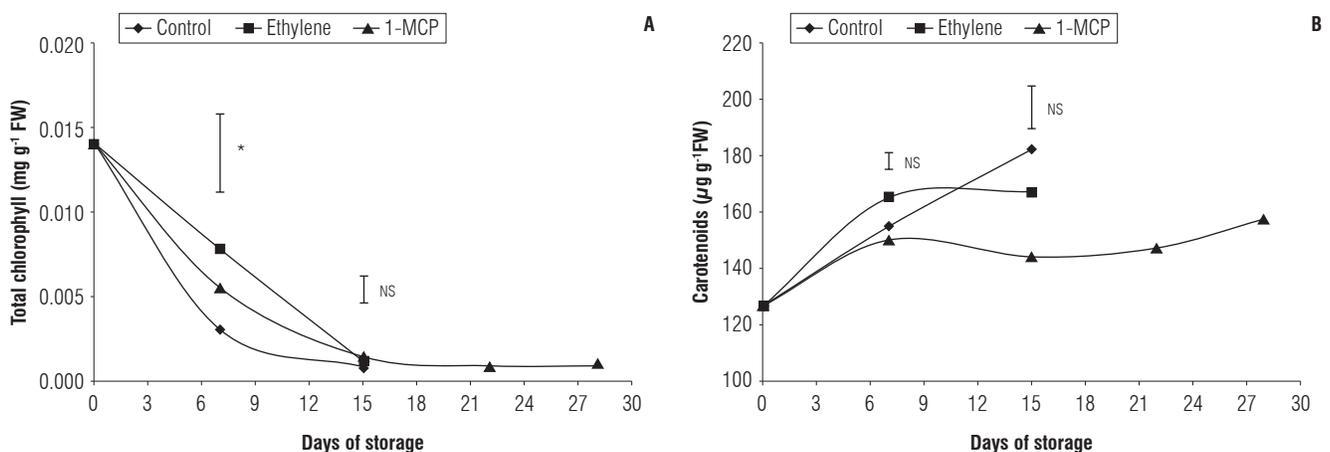


FIGURE 3. A, behavior of the total chlorophyll; B, behavior of carotenoids content in the yellow dragon fruits subjected to applications of ethylene (3 mL L⁻¹) and 1-MCP (600 mg L⁻¹). The bar indicates the least significant difference test, LSD ($P \leq 0.05$). If the difference between the two averages at each sampling point is greater than the LSD, then there is a difference $P \leq 0.05$. *, 5% statistical differences; ns, no differences.

Rodrigo and Zacarias, 2007). This shows that only a small amount of exogenous ethylene is necessary to begin the process of chlorophyll degradation (Vendrell *et al.*, 2001), in accordance with Grierson and Tucker (1983) who reported, for the tomato, that an exogenous application of ethylene generated an increase in the synthesis of pigments, but Mostolfi *et al.* (2003) found the opposite effect from 1-MCP applied to tomato fruits.

The total carotenoids had no significant differences between the treatments; however, the 1-MCP treatment, at 7 das, generated the lowest levels of total carotenoids in the dragon fruits, which were stable over the rest of the storage time, contrary to the control, in which the total carotenoids increased throughout the postharvest phase, reaching b-carotene values of 182.03 mg g⁻¹ fresh weight. The ethylene treatment increased the total carotenoid content of the yellow pitahaya, which is consistent with reports for the orange and papaya 'Golden' (Fabi *et al.*, 2007).

Respiration rate

This variable was not significantly affected by the treatments; however, it is worth noting that the 1-MCP application presented the lowest respiration rates relative to the ethylene and the control treatments up to 15 das, which subsequently increased. This behavior probably occurred because the cells synthesized more ethylene receptors and the fruits continued their maturation processes. Similarly, the delay in ripening was associated with a decrease in ethylene production and climacteric respiration peak (Hershkovitz *et al.*, 2005).

According to this respiration behavior, especially in fruits of the control and ethylene treatments (Fig. 4), the yellow dragon tended to be a climacteric fruit or one with an intermediate climacteric behavior, which is contrary to the findings of Nerd *et al.* (1999) and To *et al.* (2002), who affirmed that the respiration rate varies between 50 and 120 mg CO₂ kg⁻¹ h⁻¹ in *Hylocereus*. The climacteric peak of yellow pitahaya is reported by Dueñas *et al.* (2009) and Serna *et al.* (2011). This behavior would indicate that the fruit continues to mature even after being harvested (Narváez and Restrepo, 2002). On this point, Kader and Saltveit (2003) affirmed that fleshy fruits release ethylene to reach maturity, with a proportional increase in respiration to meet the energy requirements of enzymes, which contribute to senescence. Therefore, the 1-MCP application was important because the application of this acid in storage has been found to decrease the ethylene production and respiration rate in bananas (Lohani *et al.*, 2004) and plums (Khan and Singh, 2007).

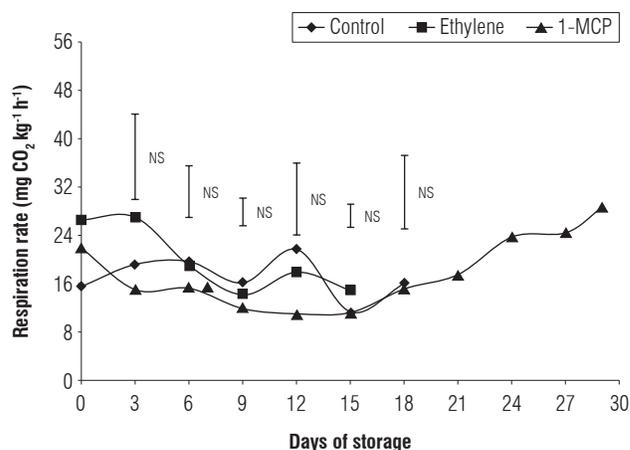


FIGURE 4. Behavior of respiration rate of the yellow dragon fruits subjected to applications of ethylene (3 ml L⁻¹) and 1-MCP (600 mg L⁻¹). The bar indicates the least significant difference test, LSD ($P \leq 0.05$). If the difference between the two averages at each sampling point is greater than the LSD, then there is a difference $P \leq 0.05$. *, 5% statistical differences; NS, no differences.

Conclusions

Exposure of yellow dragon fruits to exogenous ethylene (ethephon) accelerates maturation, which generates metabolic processes that reduce postharvest fruit life. The 1-MCP treatment extended the postharvest life of the dragon fruits, slowing the metabolic processes of pigment degradation and loss of firmness and likewise decreased the respiration rate and carotenoid content.

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