Research article

Effect of the immersion in 1-Metylciclopropene on the physico-chemical and physiological properties of yellow pitahaya fruit (Selenicereus megalanthus How) with minimum processing

Efecto de la aplicación de 1-Metilciclopropeno por inmersión sobre las características físicas, químicas y fisiológicas de pitahaya amarilla (Selenicereus megalanthus How) mínimamente procesada

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Abstract

Yellow pitahaya (Selenicereus megalanthus How), is an exotic fruit with great commercial potential, it has been commercialized without using methods that add value and/or prolong its shelf-life. The effect the application of 200 mg/lt 1-MCP on the respiratory rate and quality properties of vacuum sealed minimally processed yellow pitahaya slices during shelf-life under refrigeration was evaluated. Soluble solids, total titratable acidity, weight loss, total sugars, firmness and color were measured in fruit slices with and without skin. The application of 1-MCP before storage increased the respiratory rate and consequently increased soluble solids and total sugars, and did not show any detrimental on weight loss, total titratable acidity, color changes and firmness. However, the application of 1-MCP reduced loss of vitamin C in yellow pitahaya slices with and without skin during storage time.

Key words: Color, firmness, respiratory rate, soluble solids, tropical fruit, vitamin C.

Resumen

La pitahaya amarilla (Selenicereus megalanthus How) es una fruta exótica con gran potencial comercial. No obstante sólo ha sido aprovechada comercialmente como fruta entera, sin procedimientos que le den valor agregado y mayor tiempo de vida de anaquel. En este trabajo se evaluó la aplicación de 200 mg/lt de 1-MCP (Metylciclopropene) en pitahaya amarilla mínimamente procesada (rodajas con y sin cáscara), empaquetada a vacío y almacenada bajo refrigeración, sobre la intensidad respiratoria y parámetros de calidad como: sólidos solubles, acidez total titulable, pérdida de peso, azúcares totales, firmeza y color. La aplicación de 1-MCP aumentó la producción de CO2 lo cual se manifestó en mayores contenidos de sólidos solubles y azúcares totales, pero no incidó en la pérdida de peso, variación de la acidez total titulable, cambios de color y retención de la firmeza. En ambos tipos de procesamientos se logró reducir durante el almacenamiento la pérdida de vitamina C.

Palabras clave: Color, firmeza, fruta tropical, sólidos soluble, tasa respiratoria, vitamina C.
Introduction

Global changes in food habits have generated a consumer sector demanding healthier, additives–free, and fresh-like food products. Minimally processed food products have experienced growing demand (Rico et al., 2007) since consumers recognize their benefit and quality (Djioua et al., 2009). This tendency has stimulated the research and development of new technologies focused on conservation and storage of minimally processed fruits and vegetables (Oms-Oliu et al., 2010; Soliva-Fortuny and Martin-Belloso, 2003).

In minimal processing, fruits and vegetables are subjected to one or more unit operations, including peeling, cutting and slicing (Hong et al., 2000). These processing operations cause the rupture of cell walls and tissues (Watada and Qi, 1999) which in turn accelerate physiological and biochemical processes such as respiration and ethylene production (Ahvenainen, 1996; Soliva-Fortuny and Martin-Belloso, 2003). Such reactions reduce fruit shelf-life due to appearance, texture, flavor, and/or aroma degradation (Ahvenainen, 1996; Rico et al., 2007).

1-methylcyclopropene (1-MCP), an ethylene inhibitor, is used to extend the shelf-life and maintain the quality of plant products (Dong et al., 2002). 1-MCP reduces maturation by partially blocking ethylene receptors in plant cells, thus increasing available time for proper fruit storage (Osuna et al., 2005). 1-MCP at various concentrations was used to effectively extend the shelf-life of fruits such as bananas (50 nL.L−1; Jiang et al., 1999), strawberries (2µL.L−1; Tian et al., 2000), kiwis (0.5 µL.L−1; Koukounaras and Sfakiotakis, 2007), pears (300 nL.L−1; Spotts et al., 2007), plums and apricots (500 µL.L−1 concentrated stock in a 1L sealed bottle; Dong et al., 2002), and vegetables such as broccoli (12 µL.L−1; Able et al., 2002), avocados (30-70 nL.L−1; Feng et al., 2000) and tomatoes (250 nL.L−1; Mostofi et al., 2003). There are also reports combining 1-MCP and minimal processing in pears (300 nL.L−1 and 1µL.L−1; Arias et al., 2009; Lu et al., 2009), pineapples (1µL.L−1; Rocculi et al., 2009) and kiwis (1µL.L−1; Mao et al., 2007). However, these combined methods had not been previously reported to extend the shelf-life of yellow pitahaya (Selenicereus megalanthus How).

Yellow pitahaya is an exotic fruit with increasing worldwide acceptance due to its pleasant taste and attractive shape and color (Baquero et al., 2005). The application of 1-MCP prior to minimal processing, could be an alternative for preserving nutritional and organoleptic quality of fruits (Arias et al., 2009; Mao et al., 2007).

The objective of this study was to assess the effect of minimal processing (slicing with and without skin) and the application of 200 µg.L−1 of 1-MCP on the respiratory rate (RR), weight loss, color change (∆E), soluble solids (SS), total sugars, total titratable acidity (TTA), vitamin C (ascorbic acid) and firmness of yellow pitahaya fruits.

Materials and methods

Plant material

Yellow pitahaya fruits with a maturity stage three, according to the NTC 3554 standard classification, were selected (ICONTEC, 1996). The fruit plantation is located in Roldadillo, Valle del Cauca, Colombia (4°24'37'' N and 75°93'72” W, 1500 m.a.s.l.). Harvested fruits were selected, scrubbed using soft-bristle brushes to remove spine residues and organic matter, washed and sanitized using chlorine–treated water (200 µg.L−1), and rinsed with distilled water. Finally, the fruits were classified and grouped based on specific treatment, dried using a paper towel, and immersed in the 1-MCP solution.

1-MCP preparation

A powder 1-MCP formulation (3.8% w/w) obtained from Rohm and Haas (Philadelphia, Pennsylvania) was used. The 1-MCP application was made by immersion following manufacturer’s instructions. Forty liter solutions were prepared in a plastic container with a 200 µg.L−1 concentration at 25 ± 1°C. The immersion time for each treatment was 10 minutes. The selected 1-MCP concentration and immersion time were based on previous studies in pitahaya where the commercial life of the whole fruit was extended (Serna-Cock
et al., 2011). After each immersion, the fruits were rinsed in distilled water (5 min) and dried using absorbent paper.

**Minimal processing and packaging**

After treatments, fruits were subjected to two cutting protocols: slicing with skin (S) and slicing without skin (NS). Slices (1 cm thick and 4±0.2 cm diameter) were done using a Javar (model GE 250, Bogotá, Colombia) cutter.

The minimally processed pitahaya fruits were vacuum packaged (EGAR Vac. S.C.P basic -B, Spain), using a flexible polyamide and low-density polyethylene coextruded with 70 μm thickness, 39 cm³ m⁻² 24 h⁻¹, atm⁻¹ y 23°C⁻¹, permeability to O₂, 107 cm³ m⁻² 24 h⁻¹, atm⁻¹ y 23 °C⁻¹, permeability to CO₂, and 10.2 gm⁻² 24 h⁻¹ atm⁻¹ y 38°C, water vapor permeability. The bags were sealed with a 1.5 kg.cm⁻² pressure at 160°C for 3 seconds. The pitahaya fruits were then stored in an environmentally controlled chamber (1000 L Dies, Colombia) at 8 ± 2°C and 85-95% relative humidity.

The following nomenclature was used: S-200 and NS-200 to identify pitahaya fruits pre-treated with 200 μg/lt of 1-MCP and then cut in slices with skin (S) and without skin (NS); S-0 and NS-0 to identify control (untreated) fruit slices with and without skin (S and NS, respectively).

**Respiratory rate (RR)**

The respiratory rate was measured by titration and expressed as mg.CO₂.kg⁻¹.h⁻¹, following a modification to the methodology described by Parra-Coronado et al. (2006). A refrigerated temperature controlled chamber was used, externally equipped with a compressor (Electromec Shulz and Fiat, Colombia), CO₂ traps placed at the beginning and end of airflow, and a moisture trap saturated with silica grain gel columns. The CO₂ traps included 2 bottles containing 50 mL of 2 N KOH each and 6 bottles containing 50 mL of 0.1 N NaOH each. Internally, the chamber contained six desiccators (Bel-Art, Pequannock, New Jersey, USA) equipped with input and output hoses (Figure 1).

The compressor (1) allowed external air to flow for 30 minutes into the initial CO₂ traps (2) to eliminate the CO₂ present in the air. Then, the CO₂-free air was blown first through the moisture trap (3) to remove the water vapor and second through the desiccators (4) containing the previously weighed minimally processed pitahaya fruits. The desiccators were hermetically sealed allowing the resulting air from fruit respiration to be collected in the secondary CO₂ traps (5). After 30 minutes, the airflow was stopped and the secondary CO₂ traps were removed from the system and immediately sealed. A 20 mL aliquot from each CO₂ trap was mixed with 15 mL of 10% w/v BaCl₂ and 4 drops of phenolphthalein. The solution was immediately titrated with 0.1 N HCl.

The respiratory rate was determined using equations 1 and 2.

\[ RR = \frac{mg CO₂ \times h}{kg \times h} = \frac{(V_b - V_m) \times N_{HCl} \times 22 \times f}{w \times t} \]  

(Eq. 1)

\[ f = \frac{V_{NaOH}}{V_{aliquot}} \]  

(Eq. 2)

where:

- RR = Respiratory rate expressed in mg.CO₂.kg⁻¹.h⁻¹
- \( V_b \) = Volume (mL) of HCl used in blank titration
- \( V_m \) = Volume (mL) of HCl used to titrate the sample
- \( N_{HCl} \) = Normality of HCl used in the titration
- \( w \) = sample weight (kg)
- \( t \) = test time (hours) (elapsed time were the compressor is turned on)
- 22 = milliequivalent weight of CO₂ (g-meq)
- \( f \) = sample factor = volume of NaOH used in the respiration meter/total solution volume (BaCl₂ and phenolphthalein)

**Chemical Analysis**

Soluble solids were estimated by extracting the juice from the homogenized flesh and using a refractometer (Attago Hand Held 500 HRS, Washington, USA) following AOAC method 932.12 (AOAC, 2000a). Total soluble solids, total titratable acidity, and vitamin C content were determined by mashing the flesh...
from three pitahaya packages. The total titratable acidity was determined at 20 ± 2 °C by using AOAC official method 942.15A (AOAC, 2000b), and was reached and expressed as equivalents gram of citric acid. Total sugars were determined by spectrophotometry (Genesis UV10 Thermo Spectronic, Boston, USA) following the Antrona method (DuBois et al., 1956). Vitamin C content was estimated with a reflectometer (RQflex 10 plus, Merck, Darmstadt, Germany) with a 25-450 mg.L⁻¹ measuring range for ascorbic acid. The method consists of reducing yellow molybdophosphoric acid to molybdenum blue by the action of ascorbic acid. The results were expressed in mg.100 g⁻¹ of flesh.

**Physical Analysis**

Weight loss was determined by measuring the wet weight from three minimally processed pitahaya fruits slices per treatment, using a three-decimal precision scale (Metler Toledo 1200, Columbus, Ohio, USA). Fruits were weighted at days 0 and 12, and weight loss percentage was estimated.

Firmness was determined by uniaxial compression (force vs. distance) using a texture analyzer (Shimadzu, EZ-Test, Somerset, New Jersey, USA). Firmness was determined as the highest peak after plotting force vs. distance. A cylindrical geometry (40 mm diameter), penetrating at 10 mm.min⁻¹ and maximum deformation distance of 8 mm was used. Pitahaya fruit slices (1 cm thickness and 4 cm diameter) were used to perform the tests.

The surface color of minimally processed pitahaya fruit slices was estimated by measuring the CIE-L*, a*, b* coordinates, with a D65 standard illuminant and 10° observer, using a Color flex colorimeter (Hunter Lab., Reston, Virginia, USA). Thirty six measurements were made for each storage time corresponding to three measurements per slice, three slices per treatment, and four treatments. Differences in color (ΔE) were estimated comparing a fresh sample as shown in equation 3 (Mendoza et al., 2006).

\[ \Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \]  
(Eq. 3)

where:
- \( L^* \): Luminosity
- \( a^* \): red to green color
- \( b^* \): blue to yellow color

**Experimental design and statistical analysis**

To determine the effect of minimal processing and 1-MCP pretreatment on physicochemical
and physiological characteristics of yellow pitahaya fruits, a complete randomized design with a 2^3 factorial arrangement was used. The three factors were: (1) 1-MCP concentration with two levels (0 and 200 µg.L^-1), (2) product presentation with two levels (with skin, S; without skin, NS) and (3) storage time at two levels (0 and 12 days). Polyamide bags containing 6 slices of pitahaya were considered as an experimental unit.

Total sugar, weight loss, and color change, and firmness determinations were done at day 0 and day 12 of storage. Respiratory rate and vitamin C content were measured at days 0, 1, 2, 3, 6, 9, 12 and 15. Total soluble solids and total titratable acidity were measured at day 0, 4, 8, 12 and 15. Experiments were done in triplicates and a statistical analysis by using the SAS version 9.13 (SAS Institute, Inc., Cary, NC, USA, 2008) GLM procedure (General Linear Models) was carried out. Comparison among means was done using the multiple range Duncan test (α=0.05).

**Results and discussion**

The respiratory rate during storage ranged from 0.54 to 24.1 mg CO₂.kg⁻¹.h⁻¹, with the lowest values (0.9 to 8.2 mg CO₂.kg⁻¹.h⁻¹) observed between the second and sixth day of storage (Figure 2). The application of 1-MCP significantly increased the respiratory rate of pitahaya fruit slices. While the NS-0 treatment control exhibited an oscillating reduction in respiratory rate until the 15th day, the S-0, NS-200 and S-200 treatments showed respiratory rate peaks in the 12th, 11th and 9th days, respectively. These results agree with reports from Bower *et al.*, 2003, where CO₂ production was higher in strawberries treated with 1mL.L⁻¹ 1-MCP than in control fruits. Moreover, these authors found that 1-MCP applications at low concentrations were not statistically significant when compared with untreated controls. In studies with limes (Win *et al.*, 2006) and pears (Lu *et al.*, 2009), the application of 1-MCP did not affect the production of CO₂. Product presentation (S and NS) resulted in significant differences in respiratory rate in pitahaya fruits (p<0.0007). Agar *et al.* (1999) noted that the CO₂ and ethylene production as a result of processing cuts was higher in kiwi slices with skin than in those without skin.

![Figure 2](image_url)

*Figure 2.* Effect of pretreatment with 1-MCP on respiratory rate of minimally processed yellow pitahaya fruits during storage (8±2 °C, HR 85-90%). Vertical bars represent the mean ± SD (n = 3).
Respiratory activity and ethylene production of plant tissues are associated with an increase in physiological and biochemical activity (Wiley, 1994). In the case of minimally processed products, the respiratory activity and ethylene production increase depending on processing, degree of cutting and temperature (Ahvenainen, 1996), suggesting that fruit slices without skin would have a shorter shelf-life compared with those with skin. The treatment application time (e.g., 1-MCP application before or after minimal processing) (Rico et al., 2007) and concentration (Mangnaris et al., 2008) may also alter CO₂ production. Fruits treated with 1-MCP exhibited a climacteric respiratory curve, in agreement with reports from Rodriguez et al. (2005) and in disagreement with Nerd and Mizrahi (1997, 1999), who reported that yellow pitahaya did not show a climacteric peak when stored at 20 °C.

Fruits sliced with skin (S) showed lower soluble solids values than fruits sliced without skin (NS). However, only NS fruit kept soluble solids close to initial values (17.9 °Brix) during the storage time (Figure 3A). Control treatments, S-0 and NS-0, showed a similar soluble solids pattern during storage.

**Figure 3.** Effect of pretreatment with 1-MCP on (A) Soluble Solids and (B) Total Titratable Acidity of minimally processed yellow pitahaya fruits during storage (8±2 °C, HR 85-90%). Vertical bars represent the mean ± SD (n = 3).
but fruit slices pre-treated with 1-MCP exhibited higher soluble solids content than their respective control. Studies done with golden berries (Gutierrez et al., 2008), kiwis (Koukounaras and Sfakiotakis, 2007), apples (PreAymard et al., 2005) and oranges (Porat et al., 1999), showed no significant differences in soluble solids content between 1-MCP and control treatments. The product presentation (S and NS) showed significant differences in soluble solids content (p <0.001). The high variation on soluble solids observed during storage time (14.8 to 19.20 °Brix) was similar to results found by Rodriguez et al. (2005) working with pitahaya fruits with a maturity degree number three and stored at 8 ºC. Soluble solids content in fruits and vegetables treated with 1-MCP may show different patterns, depending on product conditions and storage. Watkins (2006) noted that starch degradations were not always delayed by 1-MCP applications. Low temperature damage causes alterations in normal metabolic processes such as respiratory rate and ethylene production (Wang, 2000), which could be related to the observed oscillation phenomenon. A possible cause for the °Brix decline may be sugar fermentation promoted by anaerobic conditions inside packages.

An increase in acidity during storage, from 1.21 to 1.58% and from 1.21 to 1.69%, was observed in S-200 and S-0 fruit slices, respectively (Figure 3B). Fruit slices with and without skin (S and NS) did not show significant differences in total titratable acidity due to the pre-treatment with 1-MCP. Similar results were observed in apricots (Dong et al., 2002), kiwis (Koukounaras and Sfakiotakis, 2007), plums (Menniti et al., 2004) and oranges (Porat et al., 1999). On the other hand, product presentation (S and NS) significantly affected the total titratable acidity during the entire storage time (p < 0.0001).

A reduction of total sugars for all treatments was observed during storage (p < 0.05, Table 1). The application of 1-MCP had higher effect on pitahaya fruit slices with skin (S-200; p < 0.047). Golding et al. (1998) found no differences in total sugars content in bananas treated with 1-MCP vs. controls. Similarly, Bregoli et al. (2005) found no significant differences between treated and control nectarines stored at 4 ºC for 3 days. In addition, they reported higher sugars content in control fruits that in those treated with 1-MCP when fruits were stored at 25 ºC for 3 days. Total sugars content in control samples with and without skin (S-0 and NS-0) followed the same

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatments</th>
<th>S-0</th>
<th>S-200</th>
<th>NS-0</th>
<th>NS-200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sugars (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>5.77 Aa*</td>
<td>5.77 Aa</td>
<td>7.79 Ab</td>
<td>7.79 Ab</td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>3.41 Bc</td>
<td>4.30 Bb</td>
<td>4.86 Ba</td>
<td>5.44 Ba</td>
<td></td>
</tr>
<tr>
<td>Weightloss (%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>0.46 Aa</td>
<td>0.50 Aa</td>
<td>0.86 Aa</td>
<td>0.61 Aa</td>
<td></td>
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<tr>
<td>Color (∆E)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td>0.00 Ba</td>
<td></td>
</tr>
<tr>
<td>Day 12</td>
<td>6.42 Ac</td>
<td>5.68 Ac</td>
<td>23.22 Aa</td>
<td>23.99 Aa</td>
<td></td>
</tr>
</tbody>
</table>

*Values are average of three replications. Different upper and lower case letters denote significant differences (P ≤0.05) due to storage time and treatments, respectively.
pattern as observed for soluble solids.

Fruit slices without skin (NS-0 and NS-200) exhibited higher weight loss compared with slices that were not peeled before storage (S-0 and S-200) (P < 0.0001, Table 1). Vargas and Vargas et al. (2005) reported similar weight losses in sliced pitahaya fruits (Hylocereus undatus) stored at 8 ºC. No significant effects on percent weight loss was observed when 1-MCP was applied before storage (P =0.68), as reported in oranges (Manganaris et al., 2008) and plums (Porat et al., 1999). However, Bassetto et al. (2005) found higher weight losses in guava treated with 1-MCP.

Fruit slices without skin (NS-0 and NS-200) showed the highest color differences during storage (P < 0.0001; Table 1) and no significant differences were observed due to the 1-MCP application. Dong et al. (2002) reported similar results in apricot when applying 10, 100 and 1000 nL.L⁻¹ 1-MCP. These results are in contradiction with those reported by Feng et al. (2000), who found that the pretreatment with 30, 50 and 70 nL.L⁻¹ 1-MCP delayed color changes in avocados and results by Arias et al. (2009), who observed a slowdown of browning in pears treated with 300 nL.L⁻¹ 1-MCP.

A reduction in vitamin C content was observed during the first 3 days of storage for all treatments (Figure 4). However, pretreatment with 1-MCP significantly reduced (P<0.0001) the loss of vitamin C in sliced pitahaya fruits and a faster vitamin C loss was observed in untreated fruit slices (from 16.7 to 7.1 mg.100 g⁻¹ in S-0 and from 16.7 to 9.3 mg.100 g⁻¹ in NS-0). From day 3 until day 15 of storage, the fruit slices exhibited little variability in their vitamin C content. Moreover, no significant differences were observed when storing fruit slices with and without skin (S vs. NS; P=0.63). Similar loss of vitamin C content was reported by Selvarajah et al. (2001) in pineapples treated with 4.5 nmol.L⁻¹ 1-MCP and by Win et al. (2006) in limes treated with 250 nL.L⁻¹ 1-MCP. Singh and Pal (2008) found that a 300 and 600 nL.L⁻¹ 1-MCP application helped maintaining high vitamin C content in Psidium guajava (Guavas) stored at 10°C for 25 days.

Fruit slices with skin and treated with 1-MCP (S-200) exhibited greater firmness than those without skin (NS) after 8 days of storage (Figure 5). Fruit slices stored without skin showed a gradual reduction in firmness regardless of receiving 1-MCP pre-treatments. Although the pre-treatment with 1-MCP did not significantly affect firmness retention,
product presentation had a significant effect on firmness (p<.0001). These results are similar to those obtained by Rocculi et al. (2009), who studying the effect of 1mL.L⁻¹ 1-MCP application in minimally processed pineapples, did not observe improvements in firmness preservation during storage. Koukounaras and Sfakiotakis (2007) obtained comparable results working with kiwis treated with 0.5 µL.L⁻¹ 1-MCP. On the other hand, Mao et al. (2007) found a delay in firmness loss in whole and minimally processed kiwis treated with 1 mL.L⁻¹ 1-MCP and stored for 8 days. Arias et al. (2009) also reported a softening reduction in minimally processed pears treated with 300 nL.L⁻¹ 1-MCP.

Unlike whole fruits and vegetables, minimally processed plant products suffer tissue damage that accelerates deterioration. Mechanical operations destroy subcellular compartments and bring together substrates and enzymes that are normally separated, and accelerate reactions that are naturally slower on whole fruits, e.g., enzymatic cell wall degradation which is the main cause of fruit softening (Oms-Oliu et al., 2010).

Conclusions

- The results from this study show that the application of 200 µg/lt 1-MCP did not reduce the respiratory rate in minimally processed pitahaya fruit slices and increased the production of CO₂. Furthermore, soluble solids content and total sugars increased in pre-treated samples, confirming that 1-MCP did not delay the maturation process. Fruit total titratable acidity, weight loss, firmness, and color, did not show significant effects due to 1-MCP pre-treatments. However, the 1-MCP application reduced the loss of vitamin C content in pitahaya fruit slices stored with and without skin.
- The application of 1-MCP (200 µg/lt), did not extend the shelf-life in minimally processed pitahaya fruits. It is recommended to evaluate additional concentrations and exposure times to elucidate whether 1-MCP treatments can potentially extend the shelf-life of minimally processed pitahaya fruits.

Figure 5. Effect of pretreatment with 1-MCP on firmness (N) of minimally processed yellow pitahaya fruits during storage (8±2 °C, HR 85-90%). Vertical bars represent the mean ± SD (n = 3).
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