Postharvest effects of 1-mcp and chitosan/oleic acid coating in pitaya (S*tenocereus griseus* H.)

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ABSTRACT

Pitaya (*Stenocereus griseus* H.) is an exotic fruit produced in some regions of Mexico, where it is mainly consumed fresh and valued in local markets. Its pulp has an intense red color, sweet flavor, small and easily chewable seeds, and compounds with antioxidant capacity. The fruit has thorns that make it difficult to handle and cause mechanical damage. However, if the thorns are removed the shelf-life is decreased and its commercialization in distant markets is more difficult. The objective of this study was to evaluate the effects of 1-methylcyclopropene (1-MCP) and a coating prepared with chitosan (Ch) + oleic acid (OA) were evaluated over the quality of pitayas without thorns in refrigeration (11 ± 1 °C; 93 ± 2 % relative humidity (RH)) for 15 d. The quality parameters studied were respiration pattern, ethylene production, weight loss, titratable acidity, skin firmness, and total soluble solids (TSS). The refrigeration conditions minimized notoriously the weight loss of the fruit. In addition, coating of Ch+OA (CO) maintained the quality parameters and delayed the appearance of fungi, extending the shelf-life of without thorns pitayas up to 15 d. However, 1-MCP alone was not capable of preserving the evaluated quality parameters. The best results were observed in (WOT)+CO+1MCP treatment, which delayed the appearance of fungi for 15 d and maintained the quality parameters.

Keywords: *Biodegradable coatings, Stenocereus griseus, shelf-life, quality parameters, ethylene.*

INTRODUCTION

Pitaya (*Stenocereus griseus* H.) is an exotic fruit from the cacti family, native to the arid zones of Mexico (Arreola & Nassar, 2017). The fruit is a slightly oval berry, whose pulp presents an intense, dark red color, is juicy, has a delicate and very sweet flavor, and contains small and numerous seeds that are easily chewable. The epicarp in physiological maturity, acquires tones between green and pale red, and presents prominent thorns grouped in areolae (Martínez-González & Cruz-Hernández, 1995). Currently, Pitayas production in Mexico shows an upward trend, increasing more than 10 times in the last decade, reaching 4,500 t per year, despite presenting a very short seasonality in production; the main producing states of this fruit in Mexico are Oaxaca, Jalisco and Puebla, who planted more than 97% of the total area, highlighting Oaxaca as the main producer with 42.9% of the total (SIAP, 2017). Pitaya fruits are valued and consumed fresh mainly in local markets, being able to be very attractive in selected markets as fruits with high nutraceutical potential, due to their content of betalains (3.5 mg g^{-1} dw) and phenolic compounds (1, 7 mg g^{-1} dw), to which an important antioxidant capacity is attributed (Garcia-Cruz et al., 2012). Pitayas fruits are also rich sources of vitamins C, B (B1 or thiamine, B3 or niacin and B2 or rivoflavin), potassium, iron, calcium and phosphorus. In addition, they are low in calories and help digestion because they are rich in fiber (SIAP, 2017).

Postharvest handling of pitaya is incipient and little technified. Furthermore, this fruit has a short shelf-life of 3-5 d at room temperature; consequently, its commercialization is challenging in markets that are far from the production zones. Moreover, the presence of thorns in pitaya causes a certain rejection by consumers, due to the fear of puncture; makes handling of the fruit difficult, and generates mechanical damages (Sáenz-Hernández et al., 2002). Hence, it is necessary to remove the thorns from pitaya and develop and/or establish postharvest treatment conditions with the objective of extending the shelf-life and promote its consumption.

The application of 1-MCP and biodegradable coatings are alternatives derived from postharvest studies for the quality preservation of several horticultural products (Cheng et al., 2020; Tokatlı & Demirdöven, 2020). 1-MCP acts by binding to ethylene receptors and prevents the activation of the metabolic processes involved in ripening and/or senescence (In et al., 2013). In addition, 1-MCP has been applied as an element that allows extension of the shelf-life of non-climacteric fruits by interfering in the biochemical processes that give rise to the ideal quality characteristics (Dou et al., 2005; Sharma et al., 2010). On the other hand, Ch is a natural, non-toxic, and biodegradable polysaccharide, which has demonstrated to be an effective food antimicrobial (Bourtoom, 2008; Yousuf et al., 2018). However, Ch is highly hydrophilic, for which the addition of hydrophobic compounds has been studied to improve its water vapor permeability properties (Aguirre-Loredo et al., 2014). For instance, OA has been evaluated to modify and favor the barrier properties of films and coatings made from Ch (Etemadipoor et al., 2020; Perdones et al., 2014).

In the above context, the effects of 1-MCP and Ch coatings have not been evaluated yet in pitaya fruits. The objective of the present study was to evaluate the impact of the postharvest application of 1-MCP and/or a Ch+OA coating (CO) over the quality of pitaya without thorns, stored under controlled conditions of refrigeration and relative humidity (RH).

MATERIALS AND METHODS

Materials

Ch of low molecular weight and 85 % deacetylation grade was purchased from Merck KGaA (St. Louis, Missouri, USA). Acetic acid, glycerol and OA were aquired from J.T. Baker, Ferandelh, S.A. de C.V. (Texcoco, Estado de México, México). Tween® 80 was bought from Sigma-Aldrich (Toluca, Estado de México, México). 1-MCP was obtained from a commercial powder formulation with 0.014 % w/w of the active ingredient (EthylBloc™ Sachet, Floralife Inc., Walterboro, SC, USA).

Harvest and conditioning of biological material

Pitaya fruits (*Stenocereus griseus* H.) of horticultural maturity were harvested in Santa Clara Huitziltepec, Puebla (18º 51' 06'' north latitude, 97º 55' 00'' west longitude; 1920 masl). After cooling in a cold room at 12 °C for 24 h, undamaged pitayas of 130 ± 20 g were selected, thorns were manually removed (except the fruits of the control treatment), disinfected by submerging in a NaClO solution (250 ppm) for 5 minutes, drained, and dried at room temperature.

Optimization for development of CO coating

The experiment was designed by response surface methodology (RSM) (Montgomery, 2002) to develop a coating that minimized weight loss of refrigerated and without thorns pitaya. A rotatable central composite design (RCCD) (Box, Hunter & Hunter, 1978) was used, where the factors were Ch and OA with five levels each. The proportions of Ch and AO were established based on previous studies (Aguirre-Loredo et al., 2014; Ochoa-Velasco & Guerrero-Beltrán, 2014) and in the previous experimental phase. Table 1 shows the experimental points (factorial, central, and axial points) for each

Table 1. Rotatable central composite design of chitosan/oleic acid (Ch/OA) emulsion and their codified and uncodified levels.

Factor	Factorial point		Axial point		Central point
	Low (-1)	High (+1)	Low (-1.414)	High (+1.414)	(0)
Ch ($g\ L^{-1}$)	5.0	15.0	2.9	17.1	10
OA ($mL\ L^{-1}$)	5.0	9.0	4.0	10.0	7.0

factor x $(X_i = (i = 1; 2))$. The response variable of the process was weight loss of pitayas, which was determined according to Equation (1)

$$\%\ WL = \frac{W_i - W_f}{W_i} x100 \qquad (1)$$

Where, % WL is the percentage weight loss, W_i the initial weight (g) and W_f the final weight (g).

The emulsions were prepared by first dispersing Ch (Table 1) in an acetic acid (1 % v/v) in distilled water solution at a temperature of 23 ± 2 °C and constant magnetic stirring (1100 rpm). Afterwards, glycerol (0.5 % v/v), Tween® 80 (0.1 % w/v), and finally the corresponding amount of OA were added (Table 1). The final mixture was homogenized with the equipment ULTRA-TURRAX® (model T 50 digital, IKA Labortechnik, Staufen, Germany) at 2200 rpm for 15 min. The prepared emulsions were used as coating in pitaya by submerging the fruit during 30 s. Once the coating was dry in refrigerated conditions for 2 h, the fruits were stored for 12 d at medium refrigeration temperature (at 11 ± 1 ° C) to reduce the metabolic activity in pitaya fruit and a high relative humidity (93 ± 2% RH) to avoid the loss of weight of the fruit due to the difference in vapor pressure of water (Armella et al., 2003).

Studies of parameters during refrigeration

The optimal CO emulsion developed during the first experimental phase was applied in the second experimental phase related to shelf-life studies. Five treatments were assessed in pitayas with the following characteristics or conditions: 1) Fruits with thorns (WT), 2) Fruits without thorns (WOT), 3) Fruits without thorns with CO coating (WOT+CO), 4) Fruits without thorns treated with 1-MCP (WOT+1-MCP), and 5) Fruits without thorns with CO coating and treated with 1-MCP (WOT+CO+1-MCP). Refrigeration conditions for all treatments were established as 11 ± 1 °C and 93 ± 2 % RH for 15 d following the methodology proposed by Armella et al. (2003).

The 1-MCP treatments were done by first placing the pitaya without thorns fruit in 0.029 m^3 containers. Then bags of the 1-MCP commercial formulation were submerged in 5 mL of distilled water for 2 s to release 1-MCP gas, and immediately 3 of these pretreated bags were placed in each container, each of which was tightly sealed. Pitayas were exposed to 1-MCP for 24 h at 23 °C and 85 % RH.

The quality parameters evaluated during the shelf-life study were weight loss, respiration rate, ethylene production rate, skin firmness, total soluble solids (TSS), titratable acidity, and rotting incidence. All the response variables were measured at the beginning of the experiment and then every 3 d, except rotting incidence which was recorded the day at which the first signs of decay were detected.

Weight loss was determined as described in section 2.3. The respiration (mL CO_2 Kg^{-1} h^{-1}) and ethylene production (µL Kg^{-1} h^{-1}) rates were measured using the static method proposed by Tovar et al. (2001), for which two fruits per treatment were taken and placed in hermetic containers for 1 h. Then 1 mL of the headspace was taken with a syringe and was analyzed by using a gas chromatography system (Agilent Technologies-7890B®) equipped with a capillary column 19094P-QO4 (30 m long, 0.530 mm diameter and 40 µm thickness), a thermal conductivity detector (TCD) to determine CO_2 and a flame ionization detector (FID) for ethylene. The temperature of injection and the detectors was 150 °C and 180 °C, respectively. Nitrogen was used as carrier gas. To determine the concentrations of CO_2 and ethylene of each sample, a calibration curve was previously constructed.

Furthermore, skin firmness was obtained with a texture analyzer TA-XT2i (Stable Micro Systems, UK) using a conical probe and a penetration distance of 5 mm. Results of this variable were expressed in Newtons (N). Moreover, TSS were determined by macerating and filtering the pulp to obtain juice, which was then analyzed with a refractometer (Master-M Atago®, Japan), and the results were reported as °Brix. In addition, titratable acidity was estimated by titrating with a 0.01 N NaOH solution to pH 8.2 and was quantified in malic acid equivalents (% w/w) based on Equation (2)

$$\text{Malic acid (\%)}=\frac{\text{mL NaOH consumed}\times\text{N}\times 0.067}{\text{aliquot (g)}}\times 100 \qquad (2)$$

Where, N is the normality of NaOH.

Finally, during fruit refrigeration, rotting incidence was determined by direct observation of the fruits, and the day at which the first signs of decay appeared was recorded. Samples were taken of the damaged tissue with a disinfected scalpel for pathogen identification, which was done by incubation in a humid chamber, isolation in culture medium of potato dextrose agar, the identification and classification was made through the use of taxonomic keys.

Statistical analysis

A mixed model analysis of data from repeated measures design was carried out (Loughin, 2006). The treatments were randomly assigned to the experimental units with four repetitions per treatment and measurements of response variables were registered every 3 d for 15 d. Analysis of variance (ANOVA) and mean comparison tests (Tukey; $p < 0.05$) were done by using the software Statistical Analysis System 9.0, (SAS Institute Inc., NC, USA).

RESULTS AND DISCUSSION

Optimization process of CO coating

Figure 1 illustrates the effect of factors Ch and OA over the weight loss of pitaya fruit without thorns, stored under refrigeration for 12 d. Based on the results of the RSM, the optimal concentrations to achieve the minimum weight loss (2.26 %) in pitaya fruit without thorns were 9.1 g L^{-1} of Ch and 6.4 mL L^{-1} of OA. This constitutes the optimal treatment applied in the second experimental phase.

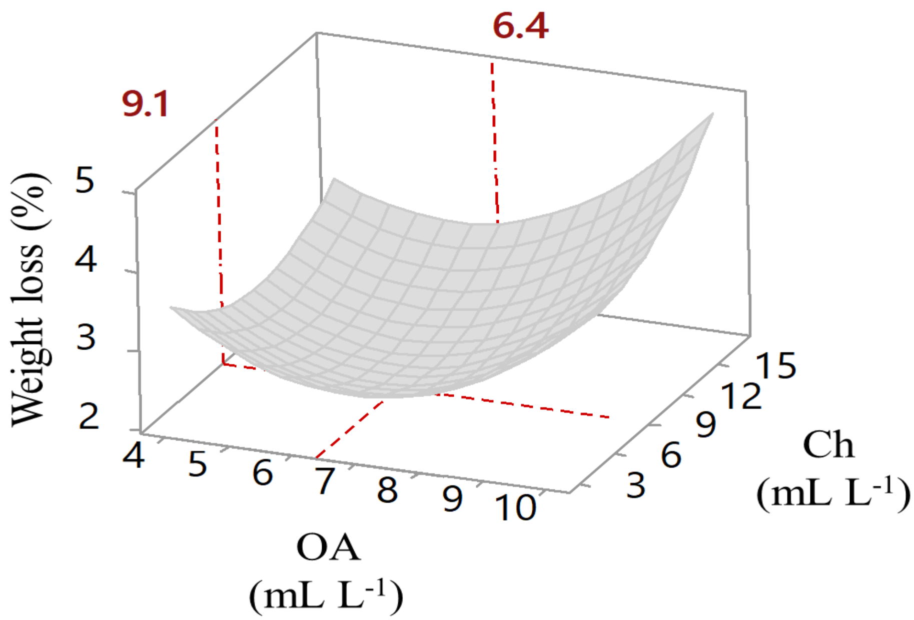

Figure 1. Response surface that shows the effect of chitosan (Ch) and oleic acid (OA) over the weight loss of pitaya fruit without thorns (*S. griseus* H.), stored at 11 ± 1 °C and 93 ± 2 % RH for 12 d.

Studies of response variables during refrigeration

Weight loss

Fruits of all treatments showed the characteristic upward trend of weight loss. However, there were some differences among treatments for this variable (Figure 2). At the end of the storage period, weight loss ranged from 2.2 to 3.1 %, which represents a relevant decrease compared with Rosas-Benítez et al. (2016). The authors reported a weight reduction of 5.8 and 10.0 % for pitaya fruits without thorns and with thorns, respectively, after the same storage period used in this study, but at 12 °C and 76 % RH. The different results could have been due to a smaller deficit in water vapor pressure (WVP) caused by the higher RH used in this study. It is known that weight loss is associated with water loss, which is the result of the transpiration phenomenon. The speed of this process is related to WVP, which is generated between the air vapor pressure in intercellular spaces of the vegetable tissue and the atmospheric vapor pressure that surrounds the tissue (Imsabai et al., 2006).

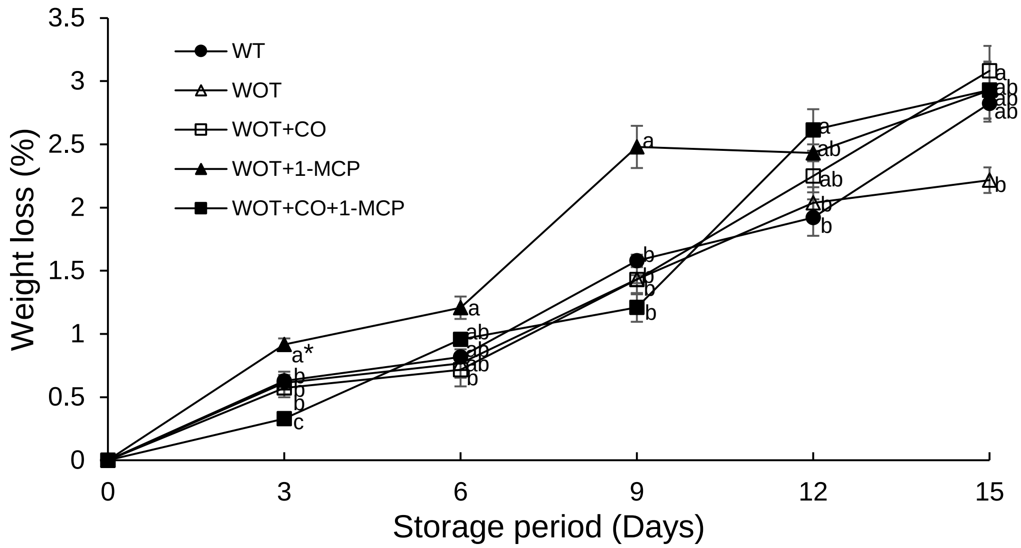

Figure 2. Effect of different treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid (CO) coating over the weight loss in pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d). *Means with different letters between days of storage are significantly different (Tukey, $p \leq 0.05$). Bars represent the mean ± standard error (n = 4). Treatments: WT (Fruit with thorns), WOT (Fruit without thorns), WOT+CO (Fruit without thorns and with CO), WOT+1-MCP (Fruit without thorns and treated with 1-MCP), and WOT+CO+1-MCP (Fruit without thorns, with CO and treated with 1-MCP).

Respiration and ethylene production rates

There were no significant differences (Tukey, p=0.05) among treatments during the days of storage. However, during the storage period, three stages were distinguished: pre-climatic (low values of ethylene emission), climacteric maximum (exponential increase in ethylene emission) and post-climatic (very pronounced reduction of ethylene emission) (Hiwasa-Tanase & Ezura, 2014). The Figures 3 and 4 show that from day 3 there were relevant increases in the respiration and ethylene production rates, respectively. At day six of refrigeration, maximum levels were reached in both variables for almost all treatments, except for fruits of the WOT+CO+1-MCP treatment, which presented maximum respiration rates until day 9. Further, application of 1-MCP alone did not manage to delay the ripening process with respect to the control as expected. The observed patterns for most treatments suggest that the climacteric maximum could have taken place between days 7 and 8. In general, from day nine a reduction of both variables occurred until the end of the refrigeration period. The obtained results confirm that the increase in ethylene production coincides with the elevation of the respiration rate, this process is necessary to achieve an accumulation of pigments, synthesis of aromatic compounds and general softening of the fruit that influence the quality attributes of the fruit (Martínez-González et al., 2017).

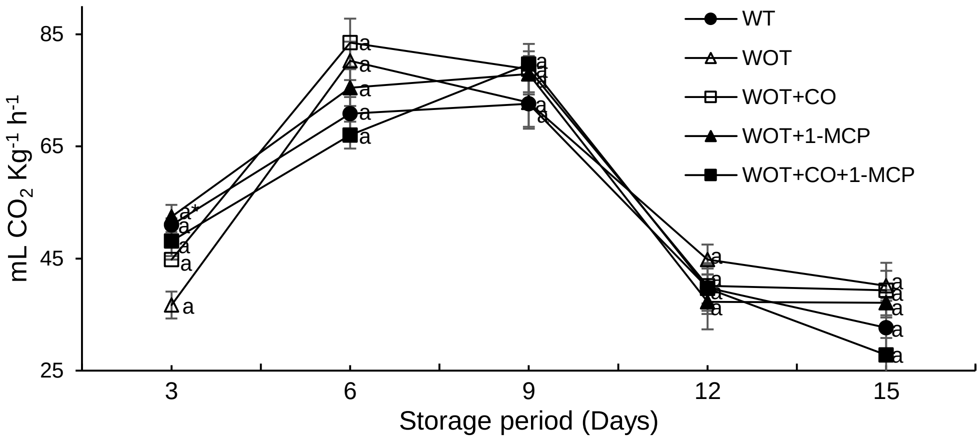

Figure 3. Respiration rate pattern in pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d), after diverse treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid (CO) coating. *Means with different letters among days of storage are significantly different (Tukey, $p \leq 0.05$). Bars represent the mean ± standard error (n = 4). Treatments: WT (Fruit with thorns), WOT (Fruit without thorns), WOT+CO (Fruit without thorns and with CO), WOT+1-MCP (Fruit without thorns and treated with 1-MCP), and WOT+CO+1-MCP (Fruit without thorns, with CO and treated with 1-MCP).

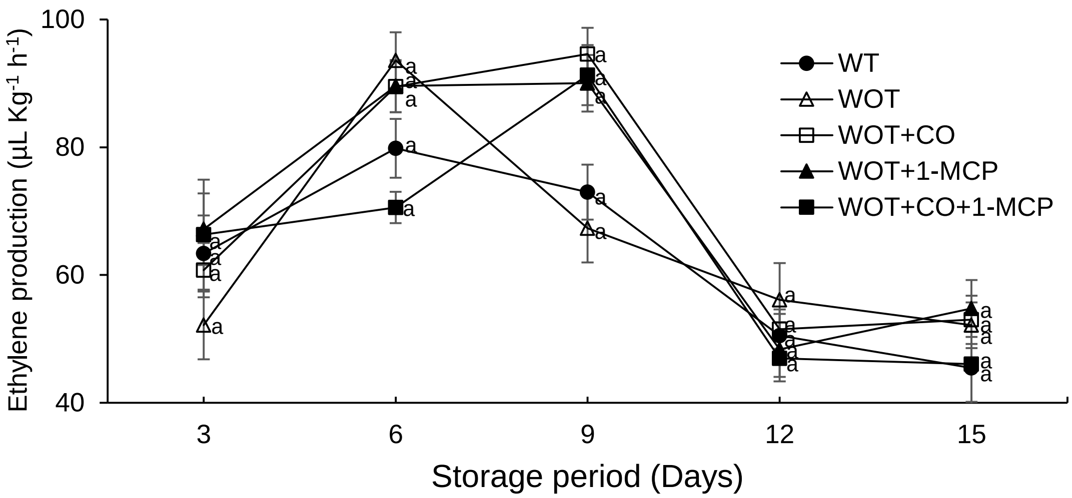

Figure 4. Ethylene production pattern in pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d), after varying treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid (CO) coating. *Means with different letters among days of storage are significantly different (Tukey, $p \leq 0.05$). Bars represent the mean ± standard error (n = 4). Treatments: WT (Fruit with

thorns), WOT (Fruit without thorns), WOT+CO (Fruit without thorns and with CO), WOT+1-MCP (Fruit without thorns and treated with 1-MCP), and WOT+CO+1-MCP (Fruit without thorns, with CO and treated with 1-MCP

The observed behavior for both variables was similar to that of climacteric fruits. However, previous studies reported that the respiration rates of pitaya presented a non-climacteric behavior (Magaña-Benítez et al., 1999; Armella et al., 2003; García-Cruz et al., 2016). Moreover, it has been reported that some non-climacteric fruits can present respiration and ethylene production patterns that are comparable to those of climacteric fruits during ripening (Barry & Giovannoni, 2007). Detailed analyses about ripening and associated changes (specially, CO_2 and ethylene levels) in some non-climacteric fruits, such as strawberry and grape, have revealed that the classification of fruits according to their ethylene production is not conclusive (Paul et al., 2012), for which more research would be needed to confirm the respiratory nature of pitaya.

Skin firmness

The skin firmness of pitayas declined in all treatments, but at the end of the storage period, significant differences (Tukey, p=0.05) among them were recorded (Figure 5). The fruits that presented greater firmness up to the end of the storage period were those of the WT and WOT+CO+1-MCP treatments, followed by the fruits of the WOT+CO treatment. However, the 1-MCP treatment alone did not manage to prevent firmness loss, in contrast with the results reported for yellow pitahaya fruits (*Selenicerus megalanthus* Haw), in which 1-MCP reduced firmness loss (Serna et al., 2012; Deaquiz et al., 2014).

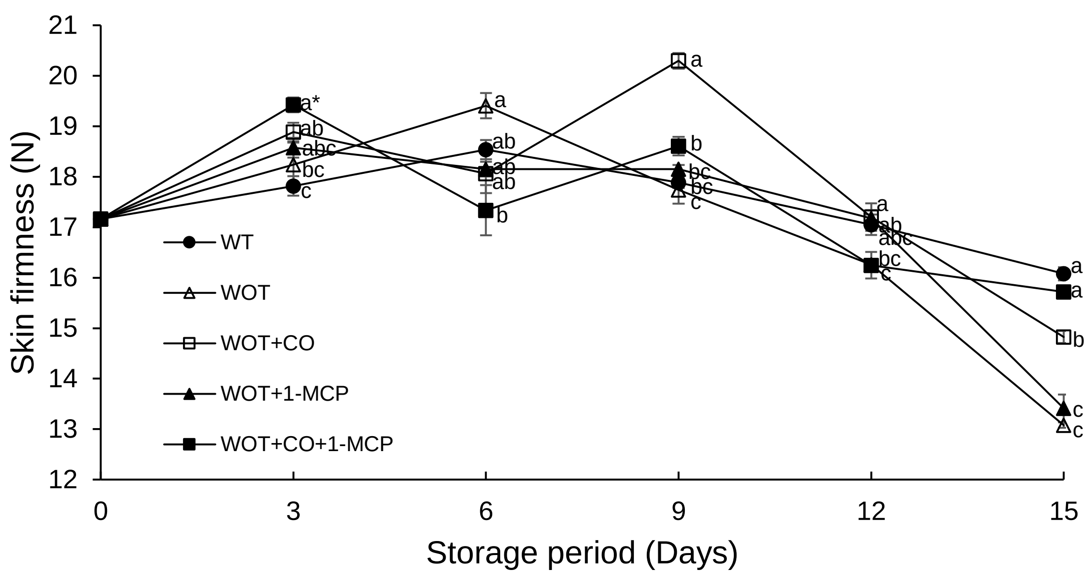

Figure 5. Changes in skin firmness of pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d), after diverse treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid (CO) coating. *Means with different letters among days

of storage are significantly different (Tukey, $p \leq 0.05$). Bars represent the mean ± standard error (n = 4). Treatments: fruits WT (With thorns), WOT (without thorns), WOT+CO (without thorns and with CO), WOT+1-MCP (without thorns and treated with 1-MCP), and WOT+CO+1-MCP (without thorns, CO and treated with 1-MCP).

Total soluble solids

The treatments showed significant differences (Tukey, p=0.05) in TSS during the storage period (Figure 6). The fruit of the treatments WOT+CO, WOT+1-MCP and WOT+CO+1-MCP had a significantly higher TSS content than those of treatments WOT and WT. TSS remained without major changes during the storage period, because the fruits were harvested when they had already reached the maturity of consumption.

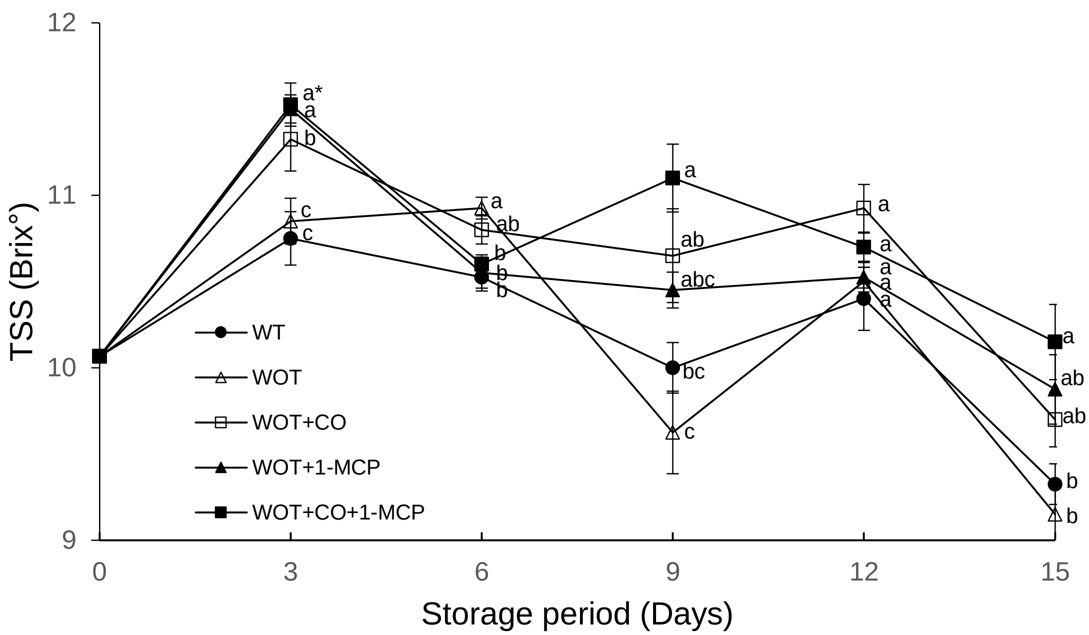

Figure 6. Effects of different treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid (CO) coating over the total soluble solids (TSS) in pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d). *Means with different letters among days of storage are significantly different (Tukey, $p \leq 0.05$). Bars represent the mean ± standard error (n = 4). Treatments: fruits WT (With thorns), WOT (without thorns), WOT+CO (without thorns and with CO), WOT+1-MCP (without thorns and treated with 1-MCP), and WOT+CO+1-MCP (without thorns, CO and treated with 1-MCP).

Titratable acidity

The treatments caused significant differences (Tukey, p=0.05) in titratable acidity during the storage period of pitaya fruits with the exception of day 6 (Figure 7). For all treatments, the pattern was similar, where an increase in acidity was observed at the beginning; then, from day 6 to the end of the storage period (day 15), a general decrease was registered. However, at the end of the storage period, the titratable acidity of fruits of the WOT+1-MCP treatment was significantly lower than fruits of the WOT+CO treatment, which suggests that the CO coating inhibited to a certain degree the decrease of acidity in the fruits, whereas 1-MCP did not have this effect. About this, Guillén, (2009) mentioned that the effect of 1-MCP over TSS and acidity varies greatly according to the product. The value interval found (0.08 to 0.15 % of malic acid) agrees with that reported by Beltrán-Orozco et al. (2009) and García-Cruz et al. (2012), who concluded that the titratable acidity values of pitaya are low (0.04 to 0.18 % of malic acid).

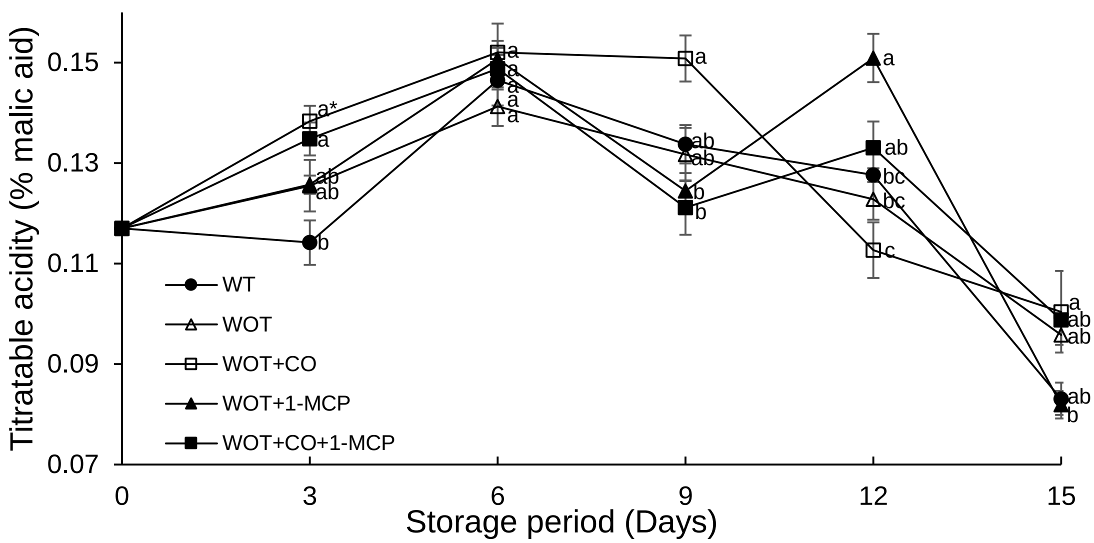

Figure 7. Effects of varying treatments of 1-methylcyclopropene (1-MCP) and chitosan/oleic acid coating over the titratable acidity of pitaya fruits during refrigeration (11 ± 1 °C and 93 ± 2 % RH for 15 d). Bars represent the mean ± standard error (n = 4). Same letters at day 15 indicate non-significant difference (Tukey-Kramer, $p<0.05$). Treatments: fruits WT (With thorns), WOT (without thorns), WOT+CO (without thorns and with CO), WOT+1-MCP (without thorns and treated with 1-MCP), and WOT+CO+1-MCP (without thorns, CO and treated with 1-MCP).

Rotting incidence and pathogen identification

From day 12 of storage, the presence of fungi was firstly observed in pitaya fruits of the treatments without CO (WT, WOT Y WOT+1-MCP), whereas in fruits treated with CO (WOT+CO and WOT+CO+1-MCP), the fungi appeared at 15 d of storage. It seems that CO had antifungal properties by delaying the incidence of fungi, which agrees with Ochoa-Velasco & Guerrero-Beltrán, (2014) who observed that Ch coatings retarded microbial growth in red and white prickly pear varieties. Likewise, Özdemir & Gökmen, (2017) reported that treatments of Ch and acetic acid (in 1%/1% and 2%/2% proportions, respectively) applied to red pomegranate arils were useful to reduce the fungal load for 26 d. Other studies concluded that the polycationic structure of Ch is a relevant factor for its antimicrobial activity (Bautista-Baños et al., 2006; Kong et al., 2010). Moreover, the fruits of the WOT+CO treatment had higher acidity and skin firmness than fruits of the WOT treatment. These conditions could have also influenced in the delay of fungi incidence.

The fungus that developed in pitaya fruits was identified as *Alternaria alternate*. It is known that the Alternaria genus attacks a wide number of plants of agricultural relevance, which causes significant damages to fruits and vegetables in pre- and postharvest stages (Sánchez et al., 2007). For instance, this fungus rots yellow pitahaya (*Selenicereus megalanthus*), and consequently, causes most losses during the postharvest period (Vilaplana et al., 2017).

CONCLUSIONS

An optimal coating was developed, based on Ch and OA (CO), that minimized the weight loss of pitaya fruit without thorns under refrigeration. The fruits of practically all treatments presented weight losses considerably smaller than those reported by other studies. The respiration and ethylene production patterns evidenced a behavior similar to that presented by climacteric fruits. The factor that determined the end of the pitaya's shelf-life was rotting incidence, which was 12 d for pitayas fruit without thorns. However, by applying the CO coating, the shelf-life of the pitaya fruit without thorns was extended up to 15 d by delaying the appearance of fungi. Meanwhile, 1-MCP alone was not capable of preserving the evaluated quality parameters, but by combining with CO coating, inhibited loss of skin firmness and TSS of the fruit and delayed the appearance of fungi until day 15. The results obtained herein are a progress in the limited knowledge of the postharvest physiology of pitaya fruit, which is of great horticultural and nutraceutical relevance. More research is needed to deepen this knowledge and to optimize the postharvest handling of pitaya, in order to develop its market at global level for the benefit of producers and consumers.

AUTHOR CONTRIBUTIONS

All authors contributed jointly to all aspects of the work reported in the manuscript. All authors have read and approved the final manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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