Effect of pepper tree (*Schinus molle*) essential oil-loaded chitosan bio-nanocomposites on postharvest control of *Colletotrichum gloeosporioides* and quality evaluations in avocado (*Persea americana*) cv. Hass

Mireya Esbeiddy Chávez-Magdaleno¹ · Ramsés Ramón González-Estrada¹ · Anelsy Ramos-Guerrero¹ · Maribel Plascencia-Jatomea² · Porfirio Gutiérrez-Martínez¹

Received: 16 April 2018 / Revised: 19 May 2018 / Accepted: 25 May 2018 / Published online: 12 June 2018
© The Korean Society of Food Science and Technology and Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract Preventive and curative activity of postharvest treatments with chitosan nanoparticles (CS) and chitosan biocomposites loaded with pepper tree essential oil (CS-PEO) against anthracnose were evaluated on Avocado (*Persea americana*) cv. Hass artificially inoculated in rind wounds. After 10 days of storage significant preventive and curative activity against *Colletotrichum gloeosporioides* was observed with the absence of internal damage by applying CS and CS-PEO. Quality parameters like water losses and firmness changes were assessed on fruit treated. CS and CS-PEO were effective to reduce water losses and firmness losses.

Keywords Anthracnose · Coatings · Postharvest · Disease control · Quality

Introduction

Currently, avocado (*Persea americana* Mill.) production is an important economical activity in Nayarit, Mexico due to the exportation potential to international markets like Canada, Europe and Asia (SAGARPA, 2015). Nevertheless, avocado is susceptible to fungal infection during pre and postharvest handling. The most important pathogen concerned to avocado is *Colletotrichum gloeosporioides*

(Penz.) Penz. and Sacc. (anthracnose) (Campos-Martínez et al., 2016) which, cause severe economical losses in their production. Besides, anthracnose is the most important postharvest disease in many regions around the world (Schaffer et al., 2013). Applying chemical fungicides is a common practice to control postharvest diseases, however these compounds can affect human health and the environment. In this regard, the use essential oils (EOs) instead of chemical fungicides can be a smart choice to control postharvest diseases. The effectiveness of EOs have been demonstrated against pathogens in fruits and vegetables (González-Estrada et al., 2017; Sarkhosh et al., 2018). However, their application is limited due to its volatilization, in this regard, their incorporation into a polymeric matrix can protect them against degradation due to environmental factors and oxidation processes as well as to enhance their bioactivity during food processing and storage (Mohammadi et al., 2015). On the other hand, it is well known that nanomaterials compared to micrometric materials provide greater advantages such as bioavailability, controlled release, protection and better performance of compounds (Acevedo-Fani et al., 2015). In this sense, in a recent study a major performance was obtained with the use of chitosan nanoparticles as matrixes loaded with EOs in comparison with the sole application of EOs for postharvest disease control against *Phytophthora drechsleri* on cucumber (*Cucumis sativus*) (Mohammadi et al., 2015). Chitosan has been used widely to encapsulate different compounds due to it is a biocompatible and biodegradable polymer. In recent studies, chitosan nanoparticles have been applied successfully to control microbial contamination (Brandelli et al., 2017; Madureira et al., 2015; Sotelo-Boyás et al., 2017). The goal of this project was to investigate the potential application of chitosan-pepper tree essential oil biocomposite for preserving

✉ Porfirio Gutiérrez-Martínez
pgutierrez@ittecip.edu.mx

¹ Tecnológico Nacional de México/I.T. Tepic, Tecnológico 2595, 63175 Tepic, Nayarit, Mexico

² Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd. Luis Encinas y Rosales s/n, Col. Centro, 83000 Hermosillo, Sonora, Mexico

postharvest quality of avocado and to evaluate the antifungal activity of the biocomposite against *Colletotrichum gloeosporioides* in inoculated fruit.

Materials and methods

Raw materials

Avocado fruits (*Persea americana*) cv. Has were visually selected on the basis of uniform shape, color, size, firmness and the absence of fungal infection or mechanical injury. Fruits were collected in El Aguacate, Nayarit, Mexico and transported to the laboratory in polystyrene boxes to avoid mechanical damage. Commercial chitosan (Sigma-Aldrich, USA) with a degree of N-acetylation (DA) of 0.178 and a molecular weight of 1.1×10^5 kDa was used. The essential oil from *Schinus molle* leaves were previously extracted following the protocol proposed by Chávez-Magdaleno et al. (2017).

Colletotrichum gloeosporioides inoculum

The pathogen used in this investigation was obtained from infected avocado fruits. The fungus was grown on potato dextrose agar (PDA) for 5–7 days at 27 °C. The conidial suspension was prepared using 1 week old fungal cultures in a PDA Petri dish. Ten mL of a sterile distilled water containing 0.05% (v/v) of Tween 80 were added to the cultures and Petri dishes were scraped using a sterile loop. Then, the suspensions were filtered on sterile gauze and recovered in a dilution flask. Spore concentration was adjusted to 1×10^6 spores mL^{-1} by microscopic counting in a hemocytometer.

Preparation of chitosan solutions

The solution was prepared as follows: 0.5 g of dry flakes were dissolved in 100 mL of acetic acid solution at 2% (v/v), then, chitosan solution was maintained in constant magnetic stirring for 24 h at room temperature until the biopolymer was completely dissolved.

Fig. 1 Efficacy of treatments on internal severity of fruits: (A) preventive and (B) curative applications. Mean values and LSD intervals ($n = 10$). For each treatment, mean values not followed by the same lowercase letter are significantly different ($P < 0.05$). Control (1)

(treated with sterile distilled water); pathogen (2); CS (nanoparticles of chitosan) (3) and CS-PEO (chitosan-pepper tree essential oil biocomposites) (4)

Preparation and application of chitosan nanoparticles (CS) and chitosan-pepper tree essential oil (CS-PEO) biocomposites

The treatments of nanoparticles and biocomposites were prepared following the protocol proposed by Chávez-Magdaleno et al. (2017). Before the application of treatments, avocado fruits were surface-sterilized applying 2% sodium hypochlorite solution (v/v) for 1 min and then washed with distilled water. Finally, were left to dry in a biosafety hood. For treatments application, avocados were dipped for 1 min in the treatment's solution. After treatments application, fruits were left to dry for 60 min at 25 °C and 38% RH in a biosafety hood. Treatments for postharvest protection were separated to evaluate the effect in preventive and curative way, firmness and weight loss evaluation were performed independently. Avocado fruits were stored during 10 day for evaluation of postharvest protection and the evaluation of quality.

Evaluation of treatments applied on fruit: postharvest protection (preventive and curative)

Avocado were wounded with a sterile needle (2.5 mm deep and 3 mm wide), and inoculated with 40 µL of a spore

suspension of the fungus (10^6 spores mL⁻¹), the inoculation was done using a disposable pipette tip. Curative treatments were performed by first inoculating the fruit with the spore suspension and leaving to dry 30 min to allow the pathogen establishment before applying the appropriate treatment. Preventive treatments were done by first applying the treatment to wounded fruit, leaving the fruit to dry 30 min at ambient temperature (25 °C) and then exposing treated fruit to the pathogen by inoculating with the spore suspension. Control treatments consisted of inoculating the wounded fruit with sterile water only, before or after inoculation as relevant. Fruits were stored for 10 days in plastic boxes at 25 °C. Internal damage was evaluated using a visual scale, the following percentages represents the internal severity in fruit: 0% no symptoms showed; 25% quarter of the fruit showing symptoms; 50% half of the fruit; 75% three quarters of the fruit; and 100% entire fruit decayed. The following treatments were applied: (1) control (treated with sterile distilled water) (2) pathogen; (3) CS (nanoparticles of chitosan) and (4) CS-PEO (chitosan-pepper tree essential oil biocomposites). Each treatment consisted of 10 fruits and was carried out in duplicate.

Assessments of firmness and water losses on avocado

In order to determine weight loss, fruit for each treatment were weighed at initial storage and at the end of each storage period. The difference between initial and final fruit weights was considered as total weight loss during storage and was expressed as a percentage loss of initial weight. Firmness determinations were performed as follows: the penetration test was applied on 3 points along the fruit husk (ends and middle) using a texture analyzer (Shimpo model FGE-50), with a punch of 5 × 10 mm diameter. The results were expressed in Newtons (N). Three fruits were used per treatment.

Statistical analysis

Uni-factorial statistical designs were applied to internal severity, firmness and weight loss determinations. Ten fruits were used per replicate for internal severity, firmness and weight loss. Analysis of variance (ANOVA) of data was performed using STATISTICA version 8.0 High Performance Analytical Software Solutions. Differences between means of data were compared by least significant differences (LSD). Differences at $P < 0.05$ were considered to be significant. All the experiments were repeated twice.

Fig. 2 Weight loss of avocado fruits during storage at 25 °C after treatment applications: (A) Preventive and (B) curative effect. Mean values and LSD intervals (n = 10). For each treatment, mean values not followed by the same lowercase letter are significantly different ($P < 0.05$). Control (1) (treated with sterile distilled water); pathogen (2); CS (nanoparticles of chitosan) (3) and CS-PEO (chitosan-pepper tree essential oil biocomposites) (4)

Fig. 3 Firmness determinations of avocado fruits during storage at 25 °C after treatment applications: (A) Preventive and (B) curative effect. Mean values and LSD intervals ($n = 10$). For each treatment, mean values not followed by the same lowercase letter are significantly different ($P < 0.05$). Control (1) (treated with sterile distilled water); pathogen (2); CS (nanoparticles of chitosan) (3) and CS-PEO (chitosan-pepper tree essential oil biocomposites) (4)

Results and discussion

Postharvest protection (preventive and curative)

The efficacy of treatments on treated fruit against *C. gloeosporioides* infection during storage at 25 °C is presented in Fig. 1. At day ten of storage, fruits inoculated with the pathogen showed total internal damage (Fig. 1). In general, preventive and curative applications of treatments were effective to avoid the fungus establishment in fruit tissues compared to control ($P < 0.05$). In this study, similar results were obtained in both types of treatments evaluations. These results are important due to the effectiveness of the treatments to cure or prevent the fungal infection by *C. gloeosporioides* on avocado fruits. Control fruits showed an important internal damage, evidencing the presence of the fungus from the field as a quiescent infection. No significant differences between CS and CS-PEO were obtained. The effectiveness of treatments for controlling fungal decay on avocado could be related to the damage of spore's membrane, leading to the loss of its integrity, creating an homeostatic disequilibrium and cell death, as previously reported (Chávez-Magdaleno et al.,

2017). In agreement with our results, 96% of inhibition on spore development was obtained with the application of CS and CS-PEO against *C. gloeosporioides* spores isolated from infected avocado fruits in vitro tests (Chávez-Magdaleno et al., 2017).

Assessments of firmness and water losses on avocado

Control as well as fruits inoculated with the pathogen differed ($P < 0.05$) in weight during storage time, however fruits treated with CS and CS-PEO avoided water losses efficiently (Fig. 2). The maximum weight loss was observed in fruits inoculated with the pathogen and compared to CS and CS-PEO treatments, which only lost 1% in curative and preventive applications. The weight loss of avocado fruit increased gradually during the storage time, probably due to different metabolic processes such as transpiration and respiration. In a recent study, the application of chitosan nanoparticles loaded with essential oils was effective to reduce water losses on coated cucumbers (Mohammadi et al., 2015). The edible coatings can prevent fruit weight loss due to their barrier properties by reducing water vapor transfer between the fruit and the environment

(de Oliveira et al., 2014). Fruits treated with CS and CS-PEO exhibited higher firmness values ($P < 0.05$) than control and inoculated fruits (Fig. 3), after 9 days of storage. The loss of firmness was higher in fruits inoculated with pathogen (96%) than control (88%) in both curative and preventive applications. Conversely, fruits treated with CS and CS-PEO only showed 80% of firmness loss in preventive and curative applications. This result could be due to the formation of a modified atmosphere created by CS and CS-PEO coatings. Changes in gas concentrations (low levels of O_2 , high levels of CO_2) in fruits reduce enzyme activities of polygalacturonase and pectin-esterase, associated with pectin depolymerization and softening of fruit, as previously reported (Maftoonazad and Ramaswamy, 2005). Similarly, other studies have reported that the application of nanochitosan and essential oils alone or in combination as a coating material improved the firmness of fruits (Abdollahi et al., 2010; Eshghi et al., 2014). The use of CS particles can be a suitable alternative to chemical methods to preserve avocado fruits of quiescent infections and control fungal decay as well as the preservation of fruit quality on postharvest stage favoring their commercialization and exportations.

Acknowledgements The authors thank CONACYT (Mexico) for the scholarship granted to Mireya Esbeiddy Chávez-Magdaleno.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Abdollahi A, Hassani A, Ghosta Y, Bernousi I, Meshkatsadat M. Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape. *J. Plant Prot. Res.* 50: 45–52 (2010)
- Acevedo-Fani A, Salvia-Trujillo L, Rojas-Graü M, Martín-Belloso O. Edible films from essential-oil-loaded nanoemulsions: physicochemical characterization and antimicrobial properties. *Food Hydrocoll.* 47: 168–177 (2015)
- Brandelli A, Brum W, dos Santos J. Nanostructured bioactive compounds for ecological food packaging. *Environ. Chem. Lett.* 15: 193–204 (2017)
- Campos-Martínez A, Velázquez-del Valle G, Flores-Moctezuma H, Suárez-Rodríguez R, Ramírez-Trujillo J, Hernández-Lauzardo A. Antagonistic yeasts with potential to control *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletotrichum acutatum* JH Simmonds on avocado fruits. *Crop Prot.* 89: 101–104 (2016)
- Chávez-Magdaleno M, Luque-Alcaraz A, Gutiérrez-Martínez P, Cortez-Rocha M, Burgos-Hernández A, Lizardi-Mendoza J, Plascencia-Jatomea M. Effect of chitosan-pepper tree (*Schinus molle*) essential oil biocomposites on the growth kinetics, viability and membrane integrity of *Colletotrichum gloeosporioides*. *Rev. Mex. Ing. Quim.* 17: 29–45 (2017)
- de Oliveira C, Magnani M, de Sales C, de Souza Pontes A, Campos-Takaki G, Stamford T, de Souza E. Effects of chitosan from *Cunninghamella elegans* on virulence of post-harvest pathogenic fungi in table grapes (*Vitis labrusca* L.). *Int. J. Food Microbiol.* 171: 54–61 (2014)
- Eshghi S, Hashemi M, Mohammadi A, Badii F, Mohammadhoseini Z, Ahmadi K. Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fruit (*Fragaria × ananassa* Duchesne) during storage. *Food Bioprocess Technol.* 7: 2397–2409 (2014)
- González-Estrada R, Chalier P, Ragazzo-Sánchez J, Konuk D, Calderón-Santoyo M. Antimicrobial soy protein based coatings: application to Persian lime (*Citrus latifolia* Tanaka) for protection and preservation. *Postharvest Biol. Technol.* 132: 138–144 (2017)
- Madureira A, Pereira A, Castro P, Pintado M. Production of antimicrobial chitosan nanoparticles against food pathogens. *J. Food Eng.* 167: 210–216 (2015)
- Maftoonazad N, Ramaswamy H. Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *LWT-Food Sci. Technol.* 38: 617–624 (2005)
- Mohammadi A, Hashemi M, Hosseini S. Chitosan nanoparticles loaded with *Cinnamomum zeylanicum* essential oil enhance the shelf life of cucumber during cold storage. *Postharvest Biol. Technol.* 110: 203–213 (2015)
- SAGARPA (Secretaría de agricultura, ganadería, desarrollo rural, pesca y alimentación). Nayarit uno de los principales exportadores de aguacate. 2015. Available from: <http://www.sagarpa.gob.mx/Delegaciones/nayarit/boletines/Paginas/BN032015.aspx>. Accessed April 04, 2018.
- Sarkhosh A, Schaffer B, Vargas A, Palmateer A, Lopez P, Soleymani A, Farzaneh M. Antifungal activity of five plant-extracted essential oils against anthracnose in papaya fruit. *Biol. Agric. Hortic.* 34: 18–26 (2018)
- Schaffer B, Wolstenholme B, Whiley A. The avocado: botany, production and uses. CABI, Wallingford (2013)
- Sotelo-Boyás M, Correa-Pacheco Z, Bautista-Baños S, Corona-Rangel M. Physicochemical characterization of chitosan nanoparticles and nanocapsules incorporated with lime essential oil and their antibacterial activity against food-borne pathogens. *LWT-Food Sci. Technol.* 77: 15–20 (2017)