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Control of blue mold decay on Persian lime: Application of covalently cross-linked arabinoxylans bioactive coatings with antagonistic yeast entrapped

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ABSTRACT

A novel edible film and coating with antagonistic yeast in arabinoxylan (AX) matrix was prepared and its effects on preserving against fungus infection on limes were investigated. The effect of yeast cell incorporation of developed films was evaluated in terms of viability, biocontrol activity and barrier properties. Furthermore, the application of AX as bioactive coatings was investigated in order to evaluate their efficacy to preserve postharvest quality and prevent or cure infection by *Penicillium italicum* during storage. Results revealed AX as a matrix compatible with *D. hansenii* and able to maintain more than 97% viability of the initial inoculum at temperatures tested, films with antagonistic yeast are capable to inhibit the growth of *P. italicum in vitro*. Presence of yeast did not change water vapor permeability on films. Viability of yeasts was preserved on coated limes on 100%. Preventive application of treatments was more effective than curative applications in the control of blue mold decay. Yeast added coatings were effective at reducing weight loss and had no effect on limes color. This study demonstrates the potential application of bioactive AX coatings with *D. hansenii* as an alternative of postharvest disease management.

1. Introduction

Currently, citrus production is an important economical activity in the world, they can be cultivated in tropical and subtropical regions (Lambert et al., 2015). Mexico is in the top list of producers (Palou, Valencia-Chamorro, & Pérez-Gago, 2015). In Mexico the production of Persian lime (*Citrus latifolia* Tanaka) is important due the export potential (SAGARPA, 2015). However, limes are susceptible to mechanical injury, physiological deterioration, water loss and microbiological decay during harvest, transport and storage. The most important postharvest pathogens concerned for citrus are *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer causing important economic losses (Eckert & Eaks, 1989). Mainly, the control of these pathogens is based on the use of synthetic chemical fungicides sprayed on fruits during packinhouses operations and storage. The efficacy of *D. hansenni* in postharvest control diseases has been tested not only in fruits (Hernandez-Montiel et al., 2012; Hernández-Montiel, Ochoa, Troyo-Diéguez, & Larralde-Corona, 2010, 2010) but also Medina-Córdova et al. (2016) evaluated the inhibition of pathogenic fungi on maize. This inhibition is reached through the production of extracellular soluble and volatile compounds, in addition to mechanisms traditionally reported for this yeast. Multiple mechanisms of action have been reported to *D. hansenii* such as competition for space and nutrients, parasitism, cell-wall hydrolytic enzyme production, induction of host resistance, killer toxin production and recently biofilm formation (Strano, Campisano, Renda, Di Silvestro, & Ruberto, 2003; Hernández-Montiel & Ochoa, 2010; Hernández-Montiel & Larralde-Corona, 2010; González-Estrada, Ascencio-Valle, Ragazzo-Sánchez, & Calderón-Santoyo, 2017). The establishment of the antagonist at carposphere fruit is an important trial to facilitate their colonization and efficacy, however they are

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exposed to abiotic stress conditions (contamination, UV, desiccation) that can affect their viability (Ippolito & Nigro, 2000). Antifungal compounds like antagonists can be added as active agents to edible films and coatings formulation (Palou et al., 2015). Entrapment of antagonist in polymeric matrices could be an efficient strategy to protect them (Vemmer & Patel, 2013) and maintain their efficacy against pathogens (Sharma, Singh, & Singh, 2009). Arabinoxylans (AX) are important cereal non-starch polysaccharides constituted of a linear backbone of β-(1 → 4)-linked D-xylopyranosyl units to which α-L arabinofuranosyl substituents are attached through *O*-2 and/or *O*-3 (Dervilly-Pinel, Thibault, & Saulnier, 2001). Some of the arabinose residues are ester-linked on (*O*)-5 to ferulic acid (FA, 3-methoxy-4 hydroxycinnamic acid) (Smith & Hartley, 1983). Previously, AX matrix have been used successfully for probiotics entrapment (Morales-Ortega et al., 2014; Paz-Samaniego et al., 2016). Recently, González-Estrada et al. (2015) entrapped *D. hansenii* in covalently cross-linked arabinoxylans films at a suitable concentration for use in biological control. In the last decade, there are only a few published data on the incorporation of antagonistic yeasts into polymeric matrices with good results (Fan et al., 2009). In addition, recent investigations have reported the improvement of biological control agents entrapped in polymeric matrices for preserving quality of fruits (Aloui, Licciardello, Khwaldia, Hamdi, & Restuccia, 2015; Aloui et al., 2014; Marín et al., 2016; Parafati, Vitale, Restuccia, & Cirvilleri, 2016). To our knowledge, no research has been reported on the incorporation of *D. hansenii* into covalently cross-linked arabinoxylans edible coatings applied on citrus. The objectives of this work were to maintain the *D. hansenii* viability incorporated on films and coatings as well as to evidence its antifungal potential in postharvest limes protection.

2. Materials and methods

2.1. Raw materials

Healthy and size homogeneous Persian limes (*Citrus latifolia* Tanaka) were selected without mechanical injury. Limes were purchased from a wholesale distributor located in Nayarit (Mexico) and transported to the Food Microbiology Laboratory at Instituto Tecnológico de Tepic in polystyrene boxes to avoid mechanical damage. AX (molecular weight 440 kD) used as coating material were extracted from wheat flour provided by wheat milling industry in Northern Mexico (Molino La Fama) following the protocol proposed by Carvajal-Millan et al. (2005). Commercial laccase (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) from *Trametes versicolor* was purchased from Sigma Chemical Co. (St Louis, MO, USA) and used as a cross-linking agent. Glycerol (99.5% purity; Sigma-Aldrich) was used as a plasticizer.

2.2. Microorganisms

D. hansenii DhhBCS03 originally was obtained from seawater samples collected at a depth of 100 m at the Cortés Sea (Baja California, Mexico) and belonging to the Yeast Collection of the CIBNOR A.C. The yeast was maintained in culture medium yeast extract peptone dextrose (YPD) agar at 4 °C (yeast extract 10 g, peptone 20 g, dextrose 20 g, agar 20 g, dis-solved in 1 L of distilled water). *P. italicum* was isolated from decayed Persian lime fruits harvested from San Pedro lagunillas in Mexico and stored onto PDA medium at 4 °C. The conidial suspension was prepared using one week old fungal cultures in a PDA Petri dish, 10 ml of a sterile distilled water containing 0.1% (v/v) Tween 80 and NaCl 0.9% (w/v) were added to the cultures and Petri dishes were scraped using a sterile loop. The liquid was filtered in sterile gauze and recovered in a dilution flask. Spore concentration was determined by microscopic counting in a hemocytometer.

2.3. Preparation of films and application of coating-forming solutions

AX solution was prepared with the protocol proposed by González-Estrada et al. (2015). The casting method was used to obtain films. Before the application of coating-forming solutions, limes were surface-sterilized by dipping in 1% sodium hypochlorite solution (v/v) for 2 min, and then washed with distilled water. For coatings application, limes were dipped for 1 min in the coating-forming solution. After coating treatments, fruits were left to dry for 30 min at 25 °C and 45% RH in a biosafety hood. Treatments for postharvest protection, color and weight loss evaluation were performed independently. Fruits were stored during 13 days for evaluation of postharvest protection and 15 days for the evaluation of quality.

2.4. Evaluation of properties of AX films

2.4.1. Viability of D. hansenii during storage of films

The viability of the antagonistic yeast incorporated into films was assessed at 13 and 25 °C as follows: films were aseptically cut and placed into a sterile flask with 20 ml of sterile saline solution (0.85%). Then, the solution containing the film was stirred at 500 rpm at 25 °C for 12 h to dissolve the film and allow microorganisms to be released into the solution. Serial dilutions were prepared and 100 μL of sample was spread on YPD. Counts for *D. hansenii* were performed after incubation for 24 h at 28 °C. Analyses were performed in triplicate.

2.4.2. In vitro antifungal properties of AX films

Prior to the experiment, *P. italicum* was pre-cultured on PDA for 7 days at 28 °C. Ten mL of sterile saline containing Tween 80 (0.05%) was added to each plate to harvest the fungal lawn and spores suspensions were made by rubbing the agar surface with a sterile inoculation loop. Then, the suspension was filtered on sterile gauze and recovered in a dilution flask. Spore concentration was adjusted to 1×10^5 spores/mL by microscopic counting in a hemocytometer. Fifty μL of fungal spores (1×10^5 spores/mL) were spread onto PDA disks (1.4 cm) and then let to dry for 5 min in a biosafety hood, after that the disks were covered with films containing or not the antagonistic yeast. Subsequently, the disks were incubated at 13 °C and 28 °C. After incubation time, the AX films were aseptically removed to determine the germination rate of *P. italicum* by microscopy. The percentage of germinated spores was measured after 12 h (28 °C) and 48 h (13 °C) in samples of approximately 200 spores. Spores were considered germinated when the germ tube length was equal to or longer than the spore diameter (Yao, Tian, & Wang, 2004). The determination of antifungal performance of films against *P. italicum* growth was made on PDA plugs (6 mm) with mycelium; they were aseptically placed onto PDA medium without fungus. Then, the inoculated medium was covered with films containing or not yeasts. Finally, the inoculated medium covered by films was incubated at 13 °C and 28 °C. Plates were covered with parafilm to avoid dehydration. Growth diameter was registered during 6 days (28 °C) and 12 days (13 °C). Inoculated but uncoated PDA Petri dishes were used as control. The percentage of inhibition of mycelial growth was calculated according to the following formula:

$$\text{Inhibition } (\%) = [(dc - dt)/dc] \times 100$$

where *dc* (cm) is the mean of colony diameter for the control sets