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Antimicrobial soy protein based coatings: Application to Persian lime (*Citrus latifolia* Tanaka) for protection and preservation

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ABSTRACT

Preventive antifungal activity of postharvest treatments with SPI (soy protein isolate) −coatings forming solutions against blue mould decay were evaluated on Persian lime (*Citrus latifolia* Tanaka) artificially inoculated in rind wounds with *Penicillium italicum*. Stimulatory effects were observed with the use of citral, however with the use of limonene an inhibitory effect was obtained. After 13 d of storage, significant preventive activity against blue mould was observed with a 20% of disease incidence applying SPI-coating forming solutions with limonene added. SEM micrographs showed that limonene could act as an inhibitor of germ tube elongation, delaying the process of infection. Quality parameters like water losses, change in colour and aroma compounds release were assessed on fruit coated. Depending to storage condition, SPI-coating forming solutions were effective to reduce water losses, maintain colour and controlling the liberation of active agent.

1. Introduction

Postharvest diseases represent a major factor of losses during storage and shelf-life of produce, due to the deterioration of quality, contamination and reduction to the market value (Mari et al., 2016). *Penicillium italicum* (blue mould), is one of the most important pathogen of postharvest diseases of citrus fruit during storage under refrigerated conditions (Erasmus et al., 2015). Traditionally, postharvest decay control is obtained using chemical fungicides, but currently consumers are concerned about food safety, quality and environmental issues. In citrus packing houses fruit coating is a common practice (Palou et al., 2015). Antifungal edible coatings can be an interesting alternative to replace the use of chemical fungicides for postharvest disease control. Any type of material used for enrobing (coating or wrapping) various food to extend shelf life of the product, that may be eaten together with food with or without further removal, is considered as an edible film or coating. The most important properties of coatings are edibility and biodegradability, migration, permeation, and barrier functions, physical and mechanical protection, allowing the quality preservation and shelf-life extension (Palou et al., 2015). For fruit and vegetables, a good packaging film must enable a slow but controlled respiration reducing O_2 absorption, be a selective barrier to gases (CO_2) and water vapour, improve mechanical handling and serve as a vehicle to incorporate food additives (flavour, colours, antioxidants, antimicrobial agents) preventing or reducing microbial spoilage during extended storage (Tharanathan, 2003; Van Long et al., 2016). Their functionality can be better expressed by using in combination with other ingredients such as plasticizers and additives (Tharanathan, 2003). The efficacy of pads based of soy protein-1-MCP-releasing was evaluated to extend the shelf-life of tomato fruit, in this work the pads were effective to delay ripening and prevent tomato postharvest deterioration (Ortiz et al., 2013). A coating with SPI with tea polyphenols was shown to be effective for maintaining the quality of Red Fuji apples during storage (Liu et al., 2012). During harvesting, transport and storage, limes are susceptible to injury and colonization by moulds of the genus *Penicillium* causing severe economic losses (Moscoso-Ramírez et al., 2013; Aloui et al., 2015). To avoid this deterioration, it is possible to formulate an antimicrobial coating directly linked to the fruit, which can release, under specific conditions, an active compound such as aroma compound during storage. The aroma compound choice is crucial because it needs to be effective without disrupting the fruit quality. Management of limes losses by applying a self-supported coating enriched with an active agent already present in lime appears to be an efficient solution to avoid food waste and control limes losses. Antimicrobial properties of essential oils (EOs) from various plant species have been demonstrated to have antimicrobial action against fungus

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concerned in fruit and vegetables spoilage (Bosquez-Molina et al., 2010). The major advantage of using essential oils into the polymeric matrices of coatings compared to direct spraying, is that they help to slow down the diffusion rate of antimicrobial agents, leaving higher concentrations of active compounds in contact with the fruit surface, where contamination has occurred for a longer period (Du Plooy et al., 2009; Sánchez-González et al., 2011). The goal of this project was to investigate the potential application of SPI-coatings forming solutions incorporated with citral and limonene for preserving postharvest quality of Persian lime and to evaluate the antifungal activity of these coatings against *P. italicum* in inoculated limes.

2. Materials and methods

2.1. Raw materials

Persian limes (*Citrus latifolia* Tanaka) were visually selected on the basis of uniform shape, colour, size, firmness and the absence of mechanical injury or fungal infection. Limes were purchased from a wholesale distributor located in Nayarit (Mexico) at commercial maturity and transported to the laboratory in polystyrene boxes to avoid mechanical damage. SPI used in *in vivo* trials were provided from Seah International (SAMPROSOY 90 NB, Wimille, France). Glycerol was purchased from Fluka (Germany) and added as plasticizer to all coating-forming solutions. Citral, limonene, and 2-heptanol (Fluka, USA) (used as internal standard) were purchased from Sigma Aldrich (St Quentin Fallavier, France).

2.2. Penicillium italicum inoculum

The pathogen used in this study was obtained from infected Persian limes collected in packinghouse in San Pedro Lagunillas, Nayarit, Mexico. The fungus was grown on potato dextrose agar (PDA) for 7 d at 28 °C. Spore suspensions were prepared by adding 10 mL of sterile saline containing Tween 80 (0.05%) to *P. italicum* cultures. Spores suspensions were scraped from the agar using a sterile inoculation loop, and then the suspensions were filtered on sterile gauze and recovered in a dilution flask. Spore concentration was adjusted to 1×10^5 spores mL^{-1} by microscopic counting in a hemocytometer.

2.3. Preparation and application of coating-forming solutions

The coating-forming solutions were carried out using SPI (10% w/v), glycerol (20% w/w of SPI) and both aroma compounds; citral and limonene (5 and 10% w/w of dry SPI). First, the SPI was dissolved in distilled water (80 mL) heated to 50 °C and the solution was continuously stirred at 500 rpm for 30 min at 50 °C. Glycerol (2 g) was added to the suspension which was mixed using magnetic stirring at 500 rpm for 30 min. In a last step, the coating-forming solution was cooled at 25 °C and 1 or 0.5 g of pure limonene or citral was added and mixed again at 8000 rpm for 20 min using high shear lab mixing (Silverson L4RT, England). Finally, an ultrasonic treatment was applied for 15 min to eliminate bubbles. Fruit selected for coating were disease-free and without visible damaged. They were individually immersed for 10 s in a 100 mL volume of the respective SPI-coatings forming solutions. After applied the treatments, the fruit were dried in air for 1 h, before being stored for 15 d at 25 °C (75% RH) and 13 °C (95% RH), simulate marketing and storage conditions to evaluate postharvest protection and quality parameters.

2.4. In vivo inoculation (preventive)

Persian limes fruit were surface-sterilized by dipping in 1% sodium hypochlorite solution (v/v) for 2 min, and then washed with distilled water. Thereafter, fruit were wounded (2 holes per fruit) with a sterile needle (2.5 mm deep and 3 mm wide), and inoculated with 10 µL of a spore suspension of *P. italicum* (1×10^5 spores mL^{-1}), and left to air-dry. Each treatment consisted of 20 fruit and was carried out in duplicate. First, fruit were wounded and thereafter the specific treatment was applied, leaving the fruit to dry 12 h at ambient temperature (25 °C) and then inoculating the fruit with the spore suspension. Control treatments consisted of inoculating the wounded fruit with sterile water only (Du Plooy et al., 2009). Fruit were stored for 13 d at 13 °C in a camera (Novatech model CA-550) with humidifiers (Vick™ Model V420-LA) to ensure high relative humidity (95%), at which time disease incidence (percentage of infected fruit), infected wounds (percentage) and severity (lesion diameter) were evaluated. A lime was considered decayed when at least one of the inoculated wounds was infected. The results of the incidence and infected wounds were expressed as percentage and severity in mm.

2.4.1. Microstructural analysis of coatings on Persian lime surface

The microstructural analysis of limes surfaces coated with SPI-coating forming solutions was carried out after 13 d post application, following the protocol proposed by Usall et al. (2001) with some modifications, using a Scanning Electron Microscope MINI-SEM (SNE–3200 M, South Korea). Flavedo tissues samples were immersed in 2.5% glutaraldehyde in phosphate buffer at pH 7.0 for 24 h rinsed with phosphate buffer and dehydrated through ethanol (30, 50, 70 and 100%), 2 h in each stage and two changes in 100% ethanol at room temperature. Then, the samples were put in a desiccator to drying 72 h. Samples were observed, after gold coating, using an accelerating voltage of 20 kV. Images of the coated limes surface were obtained to analyse the effectiveness of treatments on *P. italicum*.

2.5. Assessment of Persian lime quality

Persian lime external colour with coatings was evaluated with a colorimeter (Dr Lange model Luci 100). The Chroma (C^*_{ab}) and hue angle (h^*_{ab}) were calculated using the following equations:

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}}$$

$$h^*_{ab} = arctg\left(\frac{b^*}{a^*}\right)$$

Three readings were taken at different locations on each lime, using a total of 10 fruit from each coating-forming solution. 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. Changes in weight and colour were assessed at different storage times (0, 3, 6, 9, 12 and 15 d for 13 °C and 0, 3, 7 and 9 d for 25 °C). Measurements were done by duplicate.

2.6. Determination of citral and limonene retention/release in coatings on fruit

Aroma compound content was determined by the following extraction method, fruit were individually immersed in a 10 mL volume of distilled water and a spatula was used to carefully remove the coating on the surface. Then, the water containing the coating (10 mL) were mixed with *n*-pentane (50:50 v/v). One hundred microliters of an internal standard solution (10 g L^{-1} of 2-heptanol) was added, and the mixture was shaken for 16 h under magnetic agitation (500 rpm). The organic phase containing citral or limonene and 2-heptanol was removed, dried over ammonium sulphate ($(NH_4)_2SO_4$), and analyzed by gas chromatography. The analysis was carried out on a Varian 3800 GC-FID (Les Ulis, France) equipped with a DBWAX column (Varian) (30 m × 0.32 mm, film thickness 0.25 µm) and a flame ionization detector (FID; hydrogen, 30 mL min^{-1}; air, 300 mL min^{-1}; nitrogen; 30 mL min^{-1}). The storage times of the evaluation were 9 and 14 d at

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