Postharvest Biology and Technology 101 (2015) 10–14

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Preservation of mango quality by using functional chitosan-lactoperoxidase systems coatings

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ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form 25 October 2014
Accepted 3 November 2014

Keywords:
Chitosan
Lactoperoxidase system
Edible coatings
Mango

ABSTRACT

Influence of chitosan coating with or without the active antimicrobial lactoperoxidase system was studied on postharvest mangoes. Mangoes were treated with three concentrations of chitosan (0.5; 1; 1.5%) containing or not lactoperoxidase with or without iodine as a second electron donor. Coatings containing 1 and 1.5% chitosan incorporated with lactoperoxidase system efficiently inhibited fungal proliferation and delayed mango ripening. Iodine did not influence antifungal activity. Ripening parameters (firmness, respiration, weight loss and color) were not influenced by the lactoperoxidase system, but were more influenced by chitosan concentration. Chitosan coating alone reduced weight loss, and delayed the decline in firmness and respiration rate. It exhibited a beneficial effect on the contents of total soluble solids (TSS), ascorbic acid, total acidity (TA) and pH.

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1. Introduction

Developing countries experience significant postharvest losses of fruit and vegetables, and among these agricultural products, mango is a dominant tropical fruit variety (FAO, 2003). However, mangoes face problems in storage due to various diseases caused by fungi and bacteria. The control of these diseases has become difficult because of strain resistance to fungicides and increasingly rigorous regulations. These regulations on the use of fungicide have reduced the ability to develop control strategies based on chemicals (Johnson and Sangchote, 1994). An alternative to this problem could be the use of natural compounds that have a broad antimicrobial spectrum.

The lactoperoxidase system (LPOS) has been described as an excellent system for fighting pathogenic microorganisms as it has a broad antimicrobial spectrum. This enzyme system has shown a bactericidal effect on Gram-negative bacteria and a bacteriostatic effect on Gram-positive bacteria (Seacheol et al., 2005). In addition, it has antifungal (Jacob et al., 2000) and antiviral activity (Pakkanen and Aalto, 1997; Seifu et al., 2005). This system generates intermediate antimicrobial products such as hypothiocyanite ($OSCN^-$) and hypothiocyanate acid (HOSCN). These highly reactive products inhibit microorganisms by oxidation of the sulfhydryl groups of microbial enzymes (Martínez-Camacho et al., 2010). Presence of iodine in addition to thiocyanate increases the fungicidal and bactericidal effect against microbes such as *Candida albicans*,*Escherichia coli* and *Staphylococcus aureus* (Bosch et al., 2000). LPOS incorporated into matrix polymers by immobilization, absorption, or trapping has been operating in the pharmaceutical and food areas. The effectiveness of incorporation of the LPOS into whey proteins (Min et al., 2005; Min and Krochta, 2005) and alginate films (Fatih et al., 2009) has been demonstrated. Chitosan has multiple biological and chemical properties. Amino and hydroxyl groups of the linear polyglucosamine chain are very reactive, and consequently it is amenable to chemical modification. Dissolved in an acid solution, chitosan has a high positive charge on $NH3^+$ groups which can form an aggregate with polyanions. This characteristic provides excellent ionic properties to chitosan gels which give them remarkable affinity to proteins. In addition, chitosan has antimicrobial properties and can protect fruit against fungal deterioration (Atia et al., 2005; Photchanachai et al., 2006). Chitosan could be used to stabilize LPOS antimicrobial activity for a long time, and it may also delay the ripening of fruit.

The objective of this study, therefore was to enhance the effectiveness of LPOS linked to chitosan and thereby extend the postharvest preservation of mangoes.

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http://dx.doi.org/10.1016/j.postharvbio.2014.11.003
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2. Materials and methods

Experiments were performed on mangoes (*Mangifera indica* L. cv. Kent) imported from Brazil, and purchased in a local supermarket in Montpellier France. Selected mature green fruit were uniform in size (591 ± 30 g), with good quality and were free from injury or disease.

LPOS was composed of lactoperoxidase (LPO; 140 U/mg, Bioserae, France), glucose oxidase (GO; 158.9 U/mg, Sigma–Aldrich); D (+) glucose (Glu Sigma–Aldrich), potassium thiocyanate (KSCN, Bioserae, France), with or without potassium iodide (KI, Fluka). Chitosan (>90% DDA viscosity 500–2000 cps) was obtained from France Chitin (Marseille, France). Glycerol, used as a plasticizer to improve coating flexibility, was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). Strains of *Colletotrichum gloeosporioides*, *Phomopsis* sp. RP257, *Pestalotiopsis* sp. and *Lasiodiplodia Theobromae* ngr 05 A were isolated and identified by CIRAD (Montpellier-France).

2.1. Preparation of solution of LPOS

The weight ratios of the LPOS components were 0.35, 1.00, 1.09, 2.17 and 108.70 respectively for LPO, GO, Glu KSCN, and KI. The composition was adapted from Min and Krochta (2005). The components were dissolved separately in 50 mL phosphate buffer (pH 6.2) and 15.5 mg of LPO was added. LPOS solution was incubated at 23 ± 2 °C for 24 h with shaking at 160 RPM using a water bath shaker (Julabo SW 20 Silab, France) to increase the antimicrobial activity of LPOS (Bosch et al., 2000; Min et al., 2007). Two solutions of the enzyme system were prepared, one with iodine (LPOSI) and one without (LPOS).

2.2. Preparation of chitosan film-forming solutions

Chitosan solutions were prepared by dissolving chitosan flakes (0.5, 1 and 1.5 g) in distilled water (80 mL) containing 0.7 mL of lactic acid (Sigma) under agitation using a magnetic stirrer, incubated over night at room temperature (22 °C). The pH of the solution was adjusted to 5.5 with 0.46 M K_2HPO_4 (Sigma–Aldrich) and the solution was made up to 100 mL with distilled water. Glycerol (25% p/p of chitosan) was added and the solution was stirred at ambient temperature for 30 min.

2.3. Antimicrobial tests

Fruit were sterilized by washing with chlorinated water (1%) and rinsing with distilled water. Identical lesions (diameter 1 mm and depth 3 mm) were performed on the two opposite sides of the fruit with sterile nails. Fruit were then inoculated individually by immersion for 1 min in the microbial solution (10^5 spore/mL of selected mould) and left overnight at room temperature. They were dipped into the coating solution and then stored at 18 °C and 60% RH. Percentages of inhibition of microorganisms by the different coatings were calculated by comparing with the control (uncoated mango), when the diameter of the lesions of the latter exceeded 1 cm. Percentages of inhibition (%) = $1 - (D_S/D_C) \times 100$, where D_S is the diameter of the lesion zone in the coated mango and D_C is the diameter of lesion in the control (uncoated mango) (Martínez-Camacho et al., 2010).

2.4. Respiration rate

Respiratory rate (RR) was determined by individually placing each fruit in a 3 L glass jar hermetically closed for 3 h. Then 0.5 mL of gas was withdrawn with a syringe and analyzed to determine the % of CO_2 and O_2 by gas chromatography (GC 800, CE instrument, Italy) for oxygen and GC 1000, Dani, Italy, for carbon dioxide). The respiratory rate was expressed in mmol kg^{-1} h^{-1} in normal conditions of temperature and pressure.

2.5. Evaluation of the quality of mangoes

Weight loss was determined by daily weighing mangoes with a balance (Precisa, Switzerland). Weight loss was expressed as a percentage of initial weight.

Firmness was determined using a TA XT2 texture analyzer (Instron Co., USA), calibrated at 5 kg and equipped with a 2 mm diameter probe. Initial grip separation was 30 mm with a stroke speed of 1 mm/s.

The color of the fruit skin was measured using a Minolta chromameter (Chroma meter CR 400, Japan). Three determinations were performed on different sides of each fruit and the average represented the color value. The results were determined in the color space L*, a* and b*.

Total soluble solids (TSS) concentration were measured with a digital refractometer Atago PR-101 (Atago Co., Ltd., Tokyo, Japan) at 20 °C and expressed as % of dry matter.

Thirty (30) grams of mango pulp were homogenized in 150 mL of distilled water using a blender for 2 min and then filtered. The pH was determined with a pH meter (Kyle, USA). Total acidity (TA) was determined on 10 mL of homogenate pulp by automatic titration with 0.1 N NaOH up to pH 8.1. The results were expressed as g citric acid equivalent per 100 g fresh weight. Ascorbic acid content was determined by colorimetry using 2,6-dichloropheno-lindorhenol titration (AOAC, 1984).

2.6. Statistical analysis

Experimental data were subjected to ANOVA analysis using Statistica 7. The overall least significant differences (Student's procedure, $p < 0.05$) were calculated and used to detect significant differences among treatments. Each trial contained three replicates of 126 fruit.

3. Results and discussion

3.1. Antifungal activity of different coatings

Uncoated mangoes contaminated with *Phomopsis* RP257 showed signs of fungal decay (>1 cm) after seven days of storage whereas those treated with *Colletotrichum gloeosporiodes* strains and *L. diplodia* showed signs of fungal decay after fourteen days.

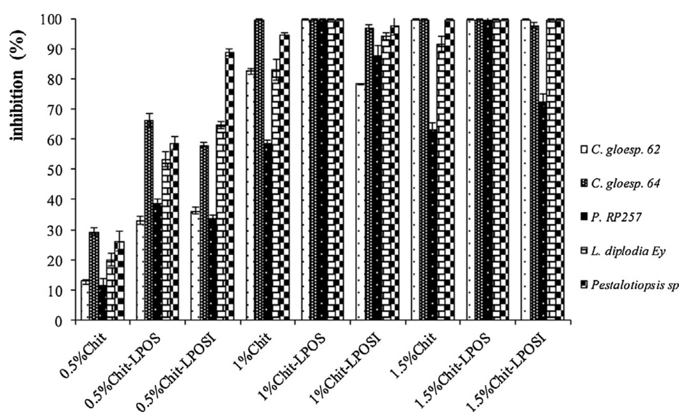

Fig. 1. Antifungal activity of different coatings against pathogenic strains on 'Kent' mango fruit. Coatings are defined in Section 2.

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