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Impact of carboxymethyl cellulose coating enriched with extract of *Impatiens balsamina* stems on preservation of 'Newhall' navel orange

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ABSTRACT

A new approach to the control of postharvest decay, while maintaining fruit quality, has been implemented by the application of extract of *Impatiens balsamina* L. stems, a commonly used traditional Chinese medicine, amended coatings to 'Newhall' navel orange (*Citrus sinensis* L., Osbeck). After harvest, navel oranges were dipped in amended coating and then the samples were stored at 5 °C and 90–95% RH after being dried naturally. The data suggested that the coating treatment, respectively, reduced the decay rate and weight loss of fruit from 10.2% to 6.1% and from 6.33% to 2.91% after 100 days storage, with none deleterious effects on fruit quality such as soluble solids content (SSC), titratable acidity (TA) and asorbic acid content (AsA). Moreover, the activities of scavenger antioxidant and defense enzymes, including peroxidase (POD), superoxide dismutase (SOD), chitinase (CHI) and β-1,3-glucanase (GLU), were also increased by the coating treatment. It indicates that the film may be an effective and safe alternative preservative for navel orange.

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1. Introduction

Fresh navel orange is one of the consumers' most favorite fruit for its highly nutritional value. However, injuries sustained by navel orange fruits during harvest allow the entry of pathogens, including *Penicillium italicum* and *Penicillium digitatum*, which are the causal agents of blue mold and green mold respectively. These two pathogens are considered the cause of most of the serious postharvest losses of citrus fruit (Porat et al., 2000). Synthetic fungicides such as prochloraz, imazalil and pyrimethanil are generally used in packhouse against postharvest pathogens of fruit and vegetable (Hamed et al., 2012; Sharma et al., 2009). Nevertheless, it has become the global trend to replace synthetic fungicides with safer and biodegradable alternatives to reduce the decay loss (Bosquez-Molina et al., 2010; Cerqueira et al., 2009; Xing et al., 2011). Natural derived antimicrobial agents from plant origin have been reported as a novel and safe alternative and supplement to control postharvest diseases and prolong shelf life (Lee et al., 2007; Mosqueda-Melgar et al., 2008).

In recent years, we have screened 96 herb extracts for antifungal activity against *P. digitatum* and *P. italicum* and demonstrated that the *Impatiens balsamina* extract (IBE) was able to significantly inhibit both molds in vitro. The stems of *I. balsamina* have been widely used in traditional Chinese medicine to treat carbuncle, rheumatism, fractures, traumatic swelling and aches, fingernail inflammation, etc. Moreover, in some regions of China, people ingest this plant as a vegetable or anti-cancer herb, although there are currently no reports confirming the efficacy of this practice (Ding et al., 2008). The objective of this work is to evaluate the influences of IBE amended coating on 'Newhall' navel orange during storage at 5 °C.

2. Materials and methods

2.1. Preparation of amended coating

2.1.1. Preparation of IBE

The stems of *I. balsamina* (origin: Anhui province, China) were purchased from a traditional Chinese medicine store. The sample were grounded into fine powder (less than 20 mesh) after being dried below 50 °C. A mixture of 200 g sample was extracted with 2 L 95% (v/v) ethanol at 30 °C with ultrasonic assisted extraction (40 kHz) for 6 h, and then was filtered. The supernatants were collected and concentrated in a rotary evaporation at 35 °C and stored at 4 °C. Solution of 1 g/ml (raw herb/solvent: w/v) was made in

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sterile water, to which 0.1% Tween80 could be added to enhance the solubility of the extracts.

2.1.2. Preparation of coating

Carboxymethyl cellulose (CMC) solution (1.5%, w/v) was prepared by dissolving 1.5 g of CMC powder in 100 mL of 0.05% citric acid, 0.5% sucrose esters, 0.5% glycerin and 2% calcium propionate in distilled water, with agitation for 8 h. All the film reagents are food grade. Put 5 ml IBE solution into the coating, with agitation for 1 h.

2.2. Fruit preparation

‘Newhall’ navel oranges were harvested at commercially mature stage (19th December, 2011) from Chang-an Orchard in Jiangxi province, China. Fruits were uniformed in terms of shape, size, color and free of any damage or visual defects. They were washed with tap water and air-dried, then dipped by hand into IBE amended coating while the control group was treated without coating. All fruits were air-dried, packed into perforated, low-density polyethylene bags (d = 0.04 mm) and stored at 5 °C. Each treatment comprised six replicate bags, each containing 50 fruits. Samples were taken initially and at 10-day intervals during storage for quality parameters and other analysis.

2.3. Evaluation of quality and physiological parameters

2.3.1. Quality measurement

Decay incidence was expressed by the percentage of fruits indicating fungal infection. Weight loss was calculated on the basis of a comparison of initial and final weights.

Flesh tissues from 10 navel oranges were homogenized in a waring blender and centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant phase was collected to analyze soluble solids content (SSC), titratable acidity (TA) and asorbic acid content (AsA). SSC expressed in °Brix was assayed by a RA-250WE digital brix-meter (KYOTO, Tokyo, Japan) at a temperature of 20 ± 0.5 °C. TA expressed as percentage of citric acid was determined by the titration using a standard solution of sodium hydroxide (0.1 M). AsA was assayed by the titration using a solution of 2,6-dichlorophenolindo-phenol and the value was expressed in mg/100 ml pulp juice.

2.3.2. Physiological parameters evaluation

Activities of peroxidase (POD) and superoxide dismutase (SOD) were determined by following the methods of Prochazkova et al. (2001). Pulp tissue (1 g) was homogenized in 10 ml PBS (25 mmol/L, pH 7.8), containing 0.8 g/L PVPP and 1 mmol/L EDTA, then centrifuged at 12 000 rpm for 15 min at 4 °C. The resulting supernatants were used directly for POD and SOD assays.

For POD determination, 0.5 ml of enzyme extract was incubated in 2 ml buffered substrate (100 mmol/L sodium phosphate, pH6.4 and 25 mmol/L guaiacol) for 5 min at 30 °C and the increasing absorbance measured at 470 nm every 30 s for 180 s after adding 0.2 ml H_2O_2 (0.5 mol/L). POD activity was expressed as unit/g FW, one unit was defined as an increase of 1 absorbance at OD 470 nm per min.

For SOD determination, the reaction mixture (3 ml) contained 50 mmol/L sodium phosphate buffer (pH 7.8), 130 mmol/L methionine, 750 μmol/L nitro-blue tetrazolium, 100 μmol/L EDTA-Na_2, 20 μmol/L riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by light (4000 lx) for 20 min at 30 °C and the absorbance was then determined at 560 nm. Identical solutions held in the dark served as blanks. One unit of SOD was defined as the amount of enzyme with gave half-maximal inhibition and the SOD activity was expressed as unit/g FW.

A weighed portion of pericarp tissue was grounded in liquid nitrogen and homogenized in 0.1 M sodium citrate buffer, pH 5.0, at a ratio of 1:2 (w/v) with a pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 5 min at 4 °C, then the supernatant was desalted by dialysis overnight at 4 °C and used as crude enzyme preparation.

Chitinase (CHI, EC 3.2.1.14) activity was colorimetric assayed following Mauch et al. (1984) with some modifications. An aliquot (0.5 ml) of the crude enzyme preparation was mixed with 0.5 ml 10 g/L colloidal chitin (Sigma), 0.5 ml 50 mmol/L sodium phosphate buffer (pH 6.4) and incubated in a water bath at 37 °C for 1 h. Then, 0.1 mL 20 g/L desalted snailase (Sigma) was added, and the mixture was incubated at 37 °C for another 1 h. The reaction was stopped by addition of 0.2 ml of 0.6 M potassium tetraborate and boiling for 5 min. After rapid cooling, 2 ml of 100 g/L 4-(dimethylamino) benzaldehyde reagent diluted with glacial acetic acid (1:5 v/v) was added and the mixture was incubated at 37 °C for 20 min, and then absorbance was measured at 585 nm. CHI activity was calculated for an enzyme concentration approaching zero using standard curves and expressed as unit/g FW. The amount of enzyme producing 1 nmol N-acetyl-D-glucosamine per hour under these assay conditions was defined as one unit.

β-1,3-glucanase (Gülçin et al., 2004) activity was assayed by measuring the reducing release of sugar from laminarin as the substrate as described by (Zheng et al., 2011) with minor modifications. Desalted enzyme extract (400 μl) was mixed with 100 μl laminarin (0.4 g/L), and the mixture was incubated in a shaking water bath at 37 °C for 1 h. The reaction was stopped by adding 1.5 ml DNS reagent and boiling for 5 min. The mixture was diluted to 25 ml and absorbance at 540 nm was determined. Enzyme activity is expressed as unit/g FW, where a unit is defined as the formation of 1 nmol glucose equivalents released from laminarin per hour under these assay conditions.

2.4. Statistical analysis

All statistical analysis was performed using SPSS 11.0 and Excel (2007). The statistical significance was applied at the level $P < 0.05$. When the analysis was statistically significant, Ducan's multiple range test was applied to the separate mean values. Each experiment had three replicates and whenever similar results were obtained, the experiments were conducted a fourth time.

3. Results

3.1. Decay rate and weight loss

Fruits of control treatment began to become infected with pathogens 50 days after storage while clear signs of pathogens stasis being found 70 days after storage on those with amended coating treatment (Fig. 1A). After been stored for 100 days, fruits treated by amended coating exhibited significantly lower decay incidence than the control group at the level of $P < 0.05$ (6.1% and 10.2%, respectively).

Meanwhile, the amended coating group displayed less moisture loss than the control treatment, but the values did not differ significantly until 40 days after storage (Fig. 1B). The weight loss of the coating navel oranges and control group reached 2.91% and 6.33% respectively after 100 days storage.

3.2. Fruit quality

As illustrated in Fig. 2A, the SSC increased continuously during the early stage of storage and declined slightly during the later storage days of both treatments. The SSC in the control navel oranges reached peak value (14.93 ± 0.12 °Brix) 30 days after storage, which

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