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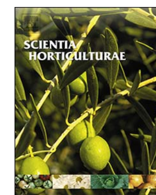

Postharvest ethanolic extract of propolis treatment affects quality and biochemical changes of 'Hindi-Besennara' mangos during shelf life

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ABSTRACT

Quality and biochemical changes of 'Hindi-Besennara' mangos in response to postharvest dipping in ethalonic extract of propolis (EEP) at 2.5, 3.5 or 4.5% were studied during two weeks of shelf life (SL) at 20 ± 2 °C and 60–70% RH. EEP at medium and high rates showed lower weight loss and decay percentages than control during SL. EPP at all rates retained higher membrane stability index (MSI) of fruit peel and higher pulp firmness especially at the high rate than control during SL. Moreover, EPP at all rates maintained lower total soluble solids (TSS) and at the high rate retained higher titratable acidity (TA) concentrations than control during SL. Both total phenols and flavonoids concentrations of fruit peel were higher after one week of SL than initials but decreased thereafter to a level close to initials. In this respect, EPP at high rate retained higher total phenols and flavonoids concentrations than other treatments. Vitamin C concentration of fruit pulp increased during SL in EEP treatments compared to initial but decreased thereafter to a level close to initial. EEP at all rates retained higher vitamin C concentration than control. Compared to initial, free radical scavenging capacity (FRSC) of peel decreased after one week followed by a sharp increase after two weeks and was not affected by the applied treatments. Relations of such biochemical changes with peroxidase (POD) and α-amylase activities were discussed. In conclusion, EEP treatments at 4.5% retained quality of 'Hindi-Besennara' mangos during SL and being suggested as natural alternative to synthetic chemicals.

1. Introduction

Mangos (*Mangifera indica* L.) are climacteric highly perishable fruit with a relatively short SL at ambient conditions (Sivakumar et al., 2011; López-Mora et al., 2013). The high perishability as well as sensitivity to chilling injuries (CI) when stored at temperature below 13 °C limit storage, handling and transport potential of these fruit (Sivakumar et al., 2011). In Saudi Arabia (SA), huge amount of mangos are spoiled due to prevailing high temperature, humidity, pathogens attack and inappropriate post-harvest handling. Several physiological and biochemical changes occurs during SL including the increase in weight loss, chlorophyll and polyphenolic degradation, conversion of starch to sucrose, degradable enzymes and the decrease in pulp firmness, TA and vitamin C (Bibi and Baloch, 2014; Mattiuz et al., 2015; Awad et al., 2017). The use of synthetic chemical preservatives is restricted due to rising consumers concerns on both human health and the environment. Accordingly, natural alternative preservatives to regulate mangos ripening and maintain quality during SL are critically required. Natural edible coatings such as chitosan, carboxymethyl cellulose, candelilla wax, gum Arabic, beeswax and propolis is considered a promising approach to delay ripening, reduce water loss and decay, and extend storage and SL of various fruit and currently attracting much worldwide attention (Bibi and Baloch, 2014; Mattiuz et al., 2015; Awad et al., 2017; Bautista-Banos et al., 2006; Romanazzi et al., 2013; Shi et al., 2013; Passos et al., 2016). Propolis is a natural resin produced by honeybees and is composed of several compounds such as polyphenolics (flavonoids and cinnamic acid derivatives), waxes, vitamins and essential oils that reflect its antioxidant, anti-inflammatory and antimicrobial properties (Burdock, 1998; Sforcin and Bankova, 2011; Pastor et al., 2011). Propolis extracts also contain hydrophobic composites capable of forming a biodegradable film on treated fruit surface that might enhance permeability barriers to gases in various fruit (Zahid et al., 2013; Ali et al., 2014, 2015; Passos et al., 2016). However, the chemical compositions and biological action of propolis varies with the geographic zones, collection time and plant sources (Kujumgiev et al., 1999; Passos et al., 2016). Postharvest application of ethanolic extract of propolis (EEP) alone or combined with other natural edible coatings maintained quality and extended storage and SL of several fruit such as cherries (Candir et al., 2009), apples (Yan et al., 2010), grapes (Ozdemir et al., 2010; Pastor et al., 2011), oranges (El-Badawy

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et al., 2012), dragon (Zahid et al., 2013), pomegranate (Kamel et al., 2015) and banana (Passos et al., 2016). Postharvest dipping of 'Kent' mangos in 1.5% EEP controlled anthracnose better than chitosan during *in vitro* experiment, but was of little efficacy with *in vivo* experiment (Mattiuz et al., 2015). To the best of our knowledge, little available published work on the response of mangos to postharvest treatment with EEP. Therefore, this study aim to evaluate the response of 'Hindi-Besennara'mangos to postharvest dipping in EEP at different concentrations as an attempt to modulate ripening and maintain quality during SL.

2. Materials and methods

2.1. Preparation of ethanolic extracted propolis (EEP)

Crude propolis were collected from beehives of apiary located in the experimental station of the Faculty of Meteorology, Environment and Arid land Agriculture at Hada Al-Sham (110 km north east of Jeddah 21° 48′ 3″ N, 39° 43′ 25″ E), SA and were then kept and air dried in the dark until extraction. The propolis exudates collected by a hybrid bees strain (*Apis mellifera lamarkii* x *A. m. carnica*) were primarily from a mixture of plant species mainly *Cinnamomum camphora, Acacia spp, and Ziziphus spina-christi*. The collected propolis was frozen at −20 °C and ground into a fine powder in a chilled mortar and mixed with 70% ethanol (v/v) at a ratio of 1: 20 with continuous shaking (Orbital shaker No. 321, Hangzhou Allsheng Instruments Co.,Ltd, China) at 150 rpm for 6 d in dark. The mixture was filtered with cheesecloth and Whatman No. 1 paper, respectively and evaporated in a Rotary Evaporator (Julabo, Model F12, Germany). The filtrates (EEP) were frozen at −80 °C in ultra-low temperature freezer (Sanyo, Japan) and lyophilized in a lyophilizator (Labconco, Freezone, No. 26570/D, Japan) under 5 mm-Hg pressure at −50 °C. Then, different concentrations of EEP solution 2.5, 3.5 and 4.5% were prepared by dissolving the corresponding weight of lyophilized propolis in 25% ethanol.

2.2. Plant materials and experimental procedure

This experiment was performed on mature hard-green 'Hindi-Besennara' mangos collected from a commercial orchard located in Jizan region (17.4751° N, 42.7076° E), SA. Fruit were packed in perforated cardbox (12 fruit of each box, about 3.0–3.5 kg) and transported to the postharvest laboratory at King Abdulaziz University in Jeddah within about 8 h at 15 °C. Fruit of uniform size, weight (200–250 g/fruit) and appearance and free of visual defects were selected for this experiment.

2.3. Fruit treatment

A completely randomized experimental design with three replicates (20 fruit of each) was established. Fruit of each treatment/replicate were soaked either into water (control), 2.5, 3.5 or 4.5% of EEP for 5 min. A surfactant (Tween 20 at 0.5 ml/l) was added to all treatments. Following air draying of about 1 h, all treatments/replicates were weighted and stored at 20 ± 2 °C and 60–70% (RH) in perforated cardboard cartons for 2 weeks. Before applying the treatments, additional three samples (5 fruit of each) were randomly collected for initial quality and biochemical analyses as described below. After one and two weeks of SL, weight loss and decay incidence were recorded for each treatment/replicate as described below. Also, samples (5 fruit of each) from each treatment/replicate were randomly collected for quality and biochemical analyses. Then, these fruit samples were peeled and the peel tissue was sliced and mixed. Random part of this peel was used for electrolyte leakage measurement and the remaining peel was kept at −80 °C for later total phenols, flavonoids, enzymes and antioxidant activity analysis. Pulp firmness was measured in each sample directly following peeling then, the pulp tissue was sliced and mixed. Random portion of this pulp tissue was directly used for TSS, TA, pH, and vitamin C determinations.

2.4. Weight loss determination

The total fruit weight loss was calculated on initial weight basis and expressed in percentage.

2.5. Decay incidence

Decay incidence, due to skin browning, shriveling and diseases, was recorded and calculated on initial fruit number basis for each samples and expressed in percentage.

2.6. Firmness, TSS, TA, pH and vitamin C measurements in fruit pulp

Pulp firmness of fruit was measured independently in 5 fruit (two opposite measurements in the middle of each fruit) per replicate by a digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter and the results were expressed as Newton. A homogeneous sample was prepared from these 5 fruit per replicate for measuring TSS content, TA, pH and vitamin C concentration. TSS content was measured in fruit pulp juice with a digital refractometer (Pocket Refractometer PAL 3, ATAGO, Japan) and expressed in percentage. TA was determined in distilled water diluted fruit juice (1: 2) by titrating with 0.1N sodium hydroxide up to pH 8.2, using automatic titrator (HI 902, HANNA Instrument, USA) and the results expressed as a percentage of citric acid. Fruit juice pH was measured by a pH meter (WTW 82382, Weilheim, Germany). Vitamin C was measured by titrating juice sample with freshly prepared dye solution of 2,6-dichlorophenol-indophenol until pink color and the results expressed as g Kg^{-1} on a fresh weight (FW) basis (Ranganna, 1979).

2.7. Leakage of ions from fruit peel

Leakage of ions was measured in peel disks according to Awad et al. (2017) with some modifications and was expressed as membrane stability index percentage (MSI%). Three grams of peel disks per replicate/treatment was randomly taken and placed in 30 ml of deionized water at ambient temperature for 4 h in a shaker. Conductivity before boiling (C1) was measured with an electrical conductivity digital meter (Orion 150A+, Thermo Electron Corporation, USA). The same disks were kept in a boiling water bath (100 °C) for 30 min to release all electrolytes, cooled to 22 ± 2 °C with running water, and conductivity after boiling was recorded (C2). MSI was expressed in percentage using the formula: $[1-(C1/C2)] \times 100$.

2.8. Preparation of methanol extract of fruit peel

Two grams of fruit peel (randomly collected from 5 fruit/replicate) were extracted by shaking at 150 rpm for 12 h with 20 ml methanol (80%) and filtered with Whatman No. 1 paper. The filtrate designated as methanol extract that was used for total phenols, total flavonoids and antioxidant activity estimations.

2.8.1. Estimation of total phenols

Total phenols concentration was measured according to Hoff and Singleton (1977). Fifty µl of the methanol extract was mixed with 100 µl Folin-Ciocalteu reagent, 850 µl of methanol and allowed to stand for 5 min at ambient temperature. A 500 µl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as g Kg^{-1} FW gallic acid equivalent.

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