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Effects of chitosan coating and modified atmosphere packaging on postharvest quality and bioactive compounds of pomegranate fruit cv. 'Hicaznar'

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ABSTRACT

The objective of this study is to determine effects of chitosan (CH) coating and modified atmosphere packaging (MAP) on postharvest quality and bioactive compounds of 'Hicaznar' pomegranate fruit. Pomegranates were subjected to CH treatment (0% or 1%) and packaged with or without MAP bags. Following treatments, pomegranates were kept at 6 ± 0.5 °C and 90 ± 5% relative humidity for 6 months. After 2, 4 or 6 months of storage, fruit were removed from cold storage and kept at 20 °C for 7 days to simulate a shelf life period. The untreated fruit was served as a control treatment. CH, MAP and CH + MAP treatments maintained better husk color, titratable acidity (TA) and ascorbic acid (AsA) content, compared to control treatment. CH + MAP and MAP treatments significantly reduced weight loss and husk scald. CH coating alone was the most effective treatment to control fungal decay during cold storage and its effect continued during the shelf life period. The arils of CH-coated fruit were deep red and had highest antioxidant activity, total monomeric anthocyanin (TMA) and total phenolic (TP) content. After 6 months of storage plus shelf life period, control and CH coated fruit became unmarketable while MAP and CH + MAP treated fruit were still marketable. The best results were obtained from CH + MAP treatment for controlling husk scald, decay and weight loss of 'Hicaznar' pomegranate fruits with maintaining visual quality and initial red aril color intensity for 6 months of cold storage plus shelf life.

1. Introduction

Pomegranate (*Punica granatum* L.) fruit is a very rich source of antioxidant phenolics and anthocyanins (Gil et al., 2000). The production and marketing of pomegranates have been increased recent years due to consumer demand for fresh pomegranate fruit and juices. The antioxidant properties of pomegranates possess health-promoting benefits in the prevention of various chronic diseases, such as cancer, cardiovascular disorders, diabetes, male infertility and Alzheimer's disease (Sreekumar et al., 2014).

Pomegranate fruits are very susceptible to chilling injury if they are stored longer than one month below 5 °C, or longer than two months at 5 °C (Kader et al., 1984). The recommended conditions for storage of pomegranates are 5 °C for up to 2 months and 7 °C for longer durations with 90 − 95% relative humidity to avoid chilling injury (Erkan and Kader, 2011). The goal of commercial storage of the pomegranates is prolonged at least until late March when prices are the highest in European and domestic markets (Selcuk and Erkan, 2015). However, long term storage of pomegranate fruit is often limited by several factors including weight loss, decay development, husk scald, and impaired internal quality and taste (Elyatem and Kader, 1984; Ben-Arie and Or, 1986).

Modified atmosphere packaging (MAP) has been demonstrated to reduce water loss, visible shriveling symptoms, husk scald and decay and extending storage life up to three months or more 'Mollar de Elche' (Artés et al., 2000), 'Ganesh' (Nanda et al., 2001), 'Primosole' (D'Aquino et al., 2010), 'Wonderful' (Porat et al. 2016), 'Hicrannar' and 'Hicaznar' (Selcuk and Erkan, 2014; 2015) pomegranates. MAP bags have become widely used for pomegranate storage and shipping. However, storage potential of pomegranate fruit in MAP bags may be limited due to the enhanced decay development for longer periods than 4 months (Porat et al., 2016). Chitosan (CH), a high molecular weight cationic polysaccharide, produced by the deacetylation of chitin and has been suggested as an ideal edible coating because of its selective permeability to gases (O_2 and CO_2), good mechanical properties, and antimicrobial effects on several pathogens; however, its use may be limited mainly because of their high water vapor permeability (Bautista-Baños et al., 2006; Vargas et al., 2008). Preharvest and

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postharvest CH treatments have been considered as safe for the consumer and the environment and suitable alternative treatment to replace the use of synthetic fungicides (Romanazzi et al., 2017). CH based coating has been demonstrated to control fungal decay, reduce weight loss and respiration rate, maintain postharvest quality in several fruits, including pomegranates (El Ghaouth et al., 1991; 1992; Zhang and Quantick, 1997; Romanazzi et al., 2002; Han et al., 2004; Jiang et al., 2005; Varasteh et al. 2012; 2017; Meighani et al., 2015). Therefore, we investigated the effects of the combination of CH coating and MAP on the postharvest quality, decay, physiological disorders and antioxidant properties of 'Hicaznar' pomegranate cultivar during storage at 6 °C for 6 months and subsequent shelf life for 7 days at 20 °C.

2. Materials and methods

2.1. Plant material and postharvest treatments

Pomegranates (cv. Hicaznar) were obtained from 9-year- old pomegranate trees planted at 5 m × 5 m spacing under drip irrigation system with fertilizer regime (160 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 140 kg K_2O ha^{-1}) in the commercial orchard (36°12′59′′ N latitude, 36°25′43′′E longitude, at an elevation of 88 m above sea level) located in the Eastern Mediterranean region of Turkey (Antakya-Hatay). The soil texture of the orchard was loamy-clayey and slightly alkaline. This location has a typical Mediterranean climate; annual average temperatures of 8.2 °C (min) and 27.7 °C (max), with 1126 mm precipitation which primarily falls during winter and spring, and 69% average annual relative air humidity.

Fruits were harvested at commercial maturity (< 1.85% of titratable acidity and > 17% of total soluble solid) during the 2015 and 2016 season. After harvest, pomegranates were immediately transported within 20 min via a ventilated truck to cold storage facilities located at the Department of Horticulture at Mustafa Kemal University (Antakya, Hatay). After sorting for uniform size and maturity and freedom from defects and blemishes, pomegranates were randomly divided into four lots for control, CH, MAP or CH + MAP treatments. Untreated fruit was served as a control treatment. Control fruit was stored in open plastic boxes. For CH treatment, ChitoPlant® is a water-soluble commercial CH formulation containing 99.9% of crab shell CH. It was obtained from ChiPro GmbH (Bremen, Germany). ChitoPlant® was found to be as effective as the practical grade CH solutions for the control of postharvest diseases (Romanazzi et al., 2013). CH solution was prepared by dissolving the powder (1%, w/v) directly in distilled water 2 h before use (Romanazzi et al., 2013). Fruits were dipped in 1% of CH solution for 1 min at 20 ± 1 °C and then the treated fruits were allowed to dry on a paper towel at room temperature for 1 h before packaging and storage. CH coated fruit was stored in open plastic boxes. For the MAP and CH + MAP treatments, fruits with or without CH coating were packaged with 5-kg of commercial MAP bag for pomegranate (Code: 815-PG28/m, Patent No: 6190710, Xtend®, Serpak Co., Antalya, Turkey). Pomegranates were cooled to 6 °C for 24 h before sealing the bags and then stored together with fruit from other treatments at 6 ± 0.5 °C and 90 ± 5% relative humidity for 6 months. The fruit was removed from cold storage at a 2 month-interval and kept at 20 ± 1 °C for 7 days to simulate a shelf life period.

2.2. Postharvest quality evaluation

Fruits were numbered and individually weighted to determine weight loss. Weight loss was calculated as percentage loss of initial weight. Headspace O_2 and CO_2 concentration of the bags were monitored using a Check Point model O_2/CO_2 analyzer (PBI-Dansensor America Inc., NJ). External husk color (three different measurements at three equidistant points on the equatorial region of each individual fruit) and external arils color (three different measurements on 20 g of arils disposed in a Petri dish) were assessed (Artés et al., 1998) for each replication of treatments. Husk and aril color were measured using the CIE L*a*b* color space with a Minolta Chroma Meter CR-300 (Osaka, Japan). From these values, Chroma (C*) and hue angle (h°) values were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$and $h^\circ = \tan^{-1}(b^*/a^*)$. The arils of five fruit per replicate were squeezed through cheesecloth by a hand press, and the juice obtained was analyzed for total soluble solid (TSS) content and titratable acidity (TA). The TSS content was determined with a refractometer (Atago Model ATC-1E). TA was measured by titration of 5 mL of juice with 0.1 N NaOH to a pH of 8.1, and it was expressed as percent of citric acid equivalents. Ascorbic acid (AsA) was extracted according to the method described previously (Lee and Coates, 1999). Five mL of pomegranate juice were mixed with 5 mL 2.5% of metaphosphoric acid and homogenized using the Ultra-Turrax homogenizer for 2 min. After centrifugation at 9418 × *g* for 5 min at 5 °C, the supernatant was recovered and kept at −20 °C until analysis. Twenty µL of sample was filtered using a Millex-HV 0.45 µm filter and injected directly into a Shimadzu HPLC. HPLC analysis were performed on LC-10A equipment consisting of LC-10AD pumps, an in-line degasser, a CTO-10A column oven, an SCL-10A system controller, an SPD 10AVP, a photodiode array detector, a refractive index detector and operated by LC solution software (Shimadzu, Japan). AsA was separated on a TransgenomicTM ICSep ION300 300 mm × 7.8 mm i.d. column (Transgenomic, San Jose, CA) at 65 °C. The mobile phase used was 0.0085 N H_2SO_4 at a flow rate of 0.4 mL min^{-1} and detected using a photodiode array detector at 244 nm. Quantification was performed according to the external standard method by the comparison of retention times to L-Ascorbic acid standard. The results were expressed as mg 100 mL^{-1}. Total monomeric anthocyanin (TMA) content was determined using the pH-differential method described by Lee et al. (2005) using a UV-1208 model UV–vis spectrophotometer (Shimadzu, Japan). TMA content was expressed as mg cyanidin-3-glucoside equivalents (molecular weight = 493.5 g mol^{-1}; molar extinction coefficient = 26,900 in L × mol^{-1} × cm^{-1}) per L of juice. Total phenolic (TP) content was determined in 1:5 of diluted pomegranate juices (Gil et al., 2000) by the Folin-Ciocalteu method adapted from Singleton et al. (1999) at 765 nm on a UV–vis spectrophotometer and expressed as gallic acid equivalents (mg GAE L^{-1}). Antioxidant capacity was estimated in 1:5 of diluted pomegranate juices (Gil et al., 2000) by two standard procedures; the ferric reducing antioxidant power (FRAP) and the trolox equivalent antioxidant capacity (TEAC) assay according to a modified procedure recommended by Ozgen et al. (2006) and expressed as Trolox equivalents (TE) (mmol TE L^{-1}). The overall visual quality was assessed on a 5 pointscale, where: 1 = very poor; 2 = poor (limit of marketability); 3 = good; 4 = very good; 5 = excellent (Selcuk and Erkan, 2015). The incidence of fungal decay was determined by counting the number of decayed fruit in each replicate of treatments. The fruit was examined visually for physiological disorders such as chilling injury and husk scald as described by Elyatem and Kader (1984) and Defilippi et al. (2006). Scald incidence was expressed as a percentage of the fruit affected by scald. Severity of scald was evaluated following the scale: 1 = no scald, 2 ≤ 10%, 3 = 11 − 250%, 4 = 25 − 50%, 5 = 50 − 75 and 6 = 75 − 100% of the surface affected.

2.3. Statistical analysis

The data were analyzed as a factorial experiment in a completely randomized block design by analysis of variance (ANOVA) using SAS software (SAS, 2017). Each treatment was repeated three times using 5 kg of fruit per replication. For MAP and CH treatments, each treatment contained three 5 kg of MAP bags. Mean separation was performed by Fisher's least significant difference (LSD) test at a $P < 0.05$ level using the SAS Proc GLM procedure.