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Extending the shelf-life of pomegranate arils with chitosan-ascorbic acid coating

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ABSTRACT

The aim of this study was to evaluate the mixture of chitosan and ascorbic acid as an edible coating to extend the shelf-life of pomegranate arils. Pomegranate arils coated with varying concentrations of chitosan and ascorbic acid were stored at 5 ± 1 °C for 28 days. Physical, chemical, microbiological and sensory quality attributes of the arils were determined during storage. There were no significant differences in the contents of anthocyanins, organic acids and sugars for coated and control (uncoated) samples during storage. Chitosan-ascorbic coating helped keeping the visual quality of arils during storage as confirmed by their surface color measurement. Chitosan-ascorbic coating inhibited bacterial and fungal growth on arils. Furthermore, the chitosan-ascorbic acid solution inhibited the mesophilic aerobic bacteria immediately after coating and coated arils presented no growth during storage. The bacterial and fungal growth were analyzed by using the Gompertz model to estimate the microbiological shelf-life of samples. The results revealed that chitosan-ascorbic coating can prolong the lag time of microorganisms and extend the shelf-life of arils up to 21 days during storage at 5 °C. Sensory scores (color, taste, aroma) were also higher in chitosan-ascorbic acid coated arils that were quite acceptable even after 25 days of refrigerated storage.

1. Introduction

Pomegranate is an important source of anthocyanins, phenolic compounds, vitamins and minerals (Gündoğdu & Yılmaz, 2012; Melgarejo, Salazar, & Artés, 2000; O'Grady, Sigge, Caleb, & Opara, 2014). It has been reported to have many positive health benefits due to its anti-inflammatory and anti-atherosclerotic properties as well as other benefits such as chemoprevention (Aviram & Dornfeld, 2001; Faria & Calhau, 2011; Malik et al., 2005). The edible part of the fruit is arils and constitutes 52% of total fruit (w/w), comprising 78% juice and 22% seeds (Elnemr, Ismail, & Ragab, 1990). However the difficulty in peeling the fruit and separation of arils limit its consumption. Therefore, ready-to-eat fresh arils could be an alternative to increase consumption of pomegranates. Ready-to-eat fresh arils have an important commercial value due to their healthiness and convenience. Nevertheless, the arils are highly perishable and quickly deteriorate during storage.

Most of the studies in literature about pomegranates focus on chemical composition, chemical and physical changes during ripening of whole fruit and postharvest treatments for improving quality and shelf-life of fruit (Artés, Villaescusa, & Tudela, 2000; Fawole & Opara, 2013; Varasteh, Arzani, Barzegar, & Zamani, 2012). However, there are few studies on the preservation of pomegranate arils. Shelf-life of pomegranate arils could be extended by the application of edible coatings instead of using chemical preservatives or modified atmosphere packaging. Edible coatings maintain a semi-permeable membrane on coated fruits so this membrane decreases the exchange of O_2 and CO_2 between coated fruit and environment (Olivas & Barbosa-Canovas, 2005; Park, 1999). Moreover, some edible coatings improve the

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appearance of food and have potential to delay or even inhibit the growth of pathogenic and spoilage microorganisms (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Quintavalla & Vicini, 2002).

Chitosan is one of the edible coating materials which is a natural carbohydrate polymer obtained by the deacetylation of chitin [poly-β-(1–4)-*N*-acetyl-D-glucosamine] that has been generally recognized as safe. Chitosan possesses excellent film-forming properties and can be applied as an edible surface coating to fruits and vegetables. Chitosan also possess antimicrobial properties that depend on several factors like deacetylation degree, molecular weight, pH and temperature (Devlieghere, Vermeulen, & Debevere, 2004; Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). It has been successfully used to prolong shelf-life of longan fruit, fresh cut broccoli, raspberry and many other fruits and vegetables (Jiang & Li, 2001; Moreira, Roura, & Ponce, 2011; Tezotto-Uliana, Fargoni, Geerdink, & Kluge, 2014). The application of chitosan is restricted to some extent because it is insoluble at neutral pH (Ge & Luo, 2005). However chitosan is soluble in acidic environment so acetic, formic and hydrochloric acids were used to prepare chitosan solutions in several publications (Chien, Sheu, & Yang, 2007; Jiang & Li, 2001; Tezotto-Uliana et al., 2014). Acetic acid has strong unpleasant and pungent smell with sour vinegar taste, and it adversely affects the sensory properties of coated fruits. Similarly, hydrochloric and formic acids have a highly pungent and penetrating odor.

In this study, ascorbic acid was used as an alternative organic acid to aid dissolving chitosan for the preparation of chitosan film coatings. Ascorbic acid is essential for human health and it is found naturally in many fruits. Application of chitosan and ascorbic acid combinations in varying concentrations were investigated for the first time in order to improve the shelf-life of pomegranate arils during cold storage. For this purpose, microbial growth, weight loss, anthocyanins, sugar and organic acids, pH, titratable acidity, total soluble solids (TSS) content and CIE $L^* a^* b^*$ color parameters and sensory quality were determined during storage time. Moreover, microbial growth was also modelled as a way of predicting the shelf-life of pomegranate arils.

2. Materials and methods

2.1. Chemicals and consumables

Fresh pomegranates (*Punica granatum* L.) were purchased from a local wholesale distributor. Cyanidin-3,5-diglucoside (Cy-3,5-dG, ≥90%), cyanidin-3-glucoside (Cy-3-G ≥95%), pelargonidin-3,5-diglucoside (Pg-3,5-dG, ≥90%), pelargonidin-3-glucoside (Pg-3-G, ≥97%) and acetonitrile (HPLC grade), ascorbic acid (AA, analytical grade), chitosan (CH) (crab shells, degree of deacetylation 75–85% and medium molecular weight) were purchased from Sigma-Aldrich (Steinheim, Germany). Formic acid (98%), plate count agar (PCA) and potato dextrose agar (PDA) were obtained from Merck (Darmstadt, Germany).

A Phenomenex Polyphenol C12 (Synergi Max-RP 80 Å, 250 × 4.6 mm, i.d., 4 μm, Waldbronn, Germany) LC Column was used for the analysis of anthocyanins. Nylon syringe filters (pore size 0.45 μm) and OASIS HLB cartridges were supplied by Waters (Milford, MA, USA).

2.2. Preparation of chitosan-ascorbic acid film formulations and treatments

Aqueous solutions (w/v) of 1% chitosan and 1% ascorbic acid (1% CH-1%AA), 2% chitosan and 2% ascorbic acid (2%CH-2%AA), 1% ascorbic acid were prepared and distilled water was used as control. To achieve complete dissolution of chitosan, ultrasonification was applied for 1 h and a translucent solution was obtained. Pomegranates were washed in tap water then arils were separated and the peel was carefully removed manually. Arils were randomly distributed into four groups and immersed in the coating solutions for 5 min. Arils (100 g) were immersed in 200 mL of solution for each time. After the immersion they were left to dry at air conditioned laboratory at 25 °C for 2 h. For analyses, 35 packages (10 packages for microbiological analyses, 3 packages for weight loss, 2 packages for color analyses, 10 packages for sensory analysis and 10 packages for chemical analyses) were prepared for each group. Packaging material was sterile polypropylene jars (30 mL) covered with BOPP (biaxially oriented polypropylene) film (thickness of 30 μm and O_2 permeability of 1600 $cm^3\ m^{-2}$ per day). Each package consists of 10 g of sample. Packaged samples were stored at 5 ± 1 °C for 28 days and sampling was carried on 0, 7, 14, 21 and 28th days of storage.

Microbial quality, visual quality, sensory analysis and weight loss were analyzed in control group, 1% AA treated arils, 1%CH-1%AA and 2%CH-2%CH coated arils. Analyses of pH, titratable acidity, total soluble solid content, sugar, organic acids and anthocyanins were only tested on control group and 1%CH-1%AA treated arils.

2.3. Weight loss

Pomegranate arils were weighed at the end of each sampling day. Weight loss was calculated as the percentage difference between the initial weight and the final weight of the pomegranate arils.

2.4. Color measurement

Color analyses were performed by using a computer vision based image analysis technique (Gökmen & Süğüt, 2007). A color image obtained by a digital camera, under controlled and defined illumination conditions. Illumination was achieved with 2 Philips, Natural Daylight 18 W fluorescent lamps with color temperature of 6500 K. The images were analyzed using a software developed for this purpose by using Matlab R2011a (The MathWorks Inc., USA). Images were captured at a resolution of 20.2 megapixels and stored in a personal computer in JPEG format.

2.5. Determination of pH, titratable acidity and total soluble solid content

The aril juice was obtained using a kitchen type press. The pH, titratable acidity (TA) and total soluble solid (TSS) of the juices were determined. The pH value of the juice was measured by using a pH-meter (Hanna instruments, USA). TA was measured on 5 g juice by adjusting the pH to 8.2 with 0.1 M NaOH and it was expressed as citric acid equivalent. TSS content of pomegranate arils was determined by using a digital refractometer (Pal-1, Atago, Tokyo, Japan).

2.6. Analyses of sugars and organic acids

Five grams of pomegranate arils were extracted with 50 mL of the mixture of acetonitrile: water in 1% formic acid (20:80, v/v) by homogenization at 10 000 rpm for 2 min (Heidolph Silent Crusher M, Schwabach, Germany). After centrifugation at 7500×*g* for 5 min, 1 mL of the supernatant was passed through a preconditioned Oasis HLB cartridge. The first eight drops of the eluent were discarded, and the rest was collected into an HPLC vial.

Concentrations of sugars and organic acids were determined by using an Agilent Technologies (Waldbronn, Germany) 1100 HPLC system equipped with a refractive index (RI) detector, diode array detector (DAD), quaternary pump, auto-sampler and column oven.