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Aloe vera gel coating maintains quality and safety of ready-to-eat pomegranate arils

Domingo Martínez-Romero[a,*], Salvador Castillo[a], Fabián Guillén[a], Huertas M. Díaz-Mula[a], Pedro J. Zapata[a], Daniel Valero[a], María Serrano[b]

[a] Department of Food Technology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain
[b] Department of Applied Biology, EPSO, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain

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ABSTRACT

Several postharvest treatments were performed on pomegranate arils prior to storage in rigid polypropylene boxes for 12 days at 3 °C: water (control), ascorbic + citric acids (at 0.5 or 1%), *Aloe vera* gel (at 50 or 100%), 50% *A. vera* gel + 0.5% ascorbic and 0.5% citric acid, and 100% *A. vera* gel + 1% ascorbic and 1% citric acid. *A. vera* (alone or in combination with acids) led to lower CO_2 and higher O_2 concentrations inside the packages compared with arils treated with water (control). With respect to quality attributes, *A. vera* coatings led to firmness retention and increased levels of total anthocyanins and total phenolics. In addition, *A. vera* treatments led to significantly lower counts for both mesophilic aerobics and yeast and moulds. Sensory analysis scores for flavour, texture, aroma, colour and purchase decision were higher in arils treated with *A. vera*, especially in those arils treated with 100% *A. vera* + 1% ascorbic and citric acids. Finally, no off-flavours in pomegranate arils were perceived by judges as a consequence of *A. vera* gel treatment.

1. Introduction

Pomegranate fruit (*Punica granatum* L.) is one of the oldest of edible fruit, cultivated extensively in Mediterranean countries including Spain, and generally consumed as fresh (arils) or juice. Over recent years there has been a great increase in pomegranate commercial farming, due to the high quality attributes of pomegranate arils and their potential health benefits, such as anti-mutagenic, anti-hypertension, antioxidant activities and even antitumor properties *in vivo* and *in vitro* (Heber and Bowerman, 2009).

Pomegranates have a non-climacteric ripening pattern, but are characterized by a reduced shelf-life due to acceleration of loss of quality loss. The growing demand for minimally processed fruit has led to increasing research on designing and implementing methods for maintaining quality of these highly perishable fruit. In this sense, minimally processed "ready-to-eat" pomegranate arils in modified atmosphere packaging (MAP) have become very popular due to their convenience, sensory attributes and health benefits (Ayhan and Eştürk, 2009). However, maintaining the nutritional and microbial quality of pomegranate arils is a major challenge, since minimally processed arils easily loose quality attributes such as texture and colour, together with an increase in microbial spoilage (Gil et al., 1996; Caleb et al., 2012). Recently, the postharvest shelf-life of MA-packaged arils was shown to be limited to 10 days due to fungal growth, and only 7 days when taking into account data for flavour and aroma (Caleb et al., 2013). Moreover, the application of UV-C before MA-packaging could not increase the shelf-life beyond 10 days since the microbial limit was reached (López-Rubira et al., 2005).

Thus, new alternatives are needed to reduce the microbial population on pomegranate arils under MA conditions and to delay quality loss. In this sense, *Aloe vera* gel applied as an edible coating has been found to be effective in quality retention and reduction of microbial spoilage of several whole fruit such as sweet cherry, table grape and nectarine (Valverde et al., 2005; Martínez-Romero et al., 2006; Ahmed et al., 2009; Castillo et al., 2010). However, few studies have been devoted to the use of *A. vera* gel on minimally processed fruit, apart from recent reports on kiwifruit or apple slices, for which *Aloe* gel-coated slices showed improved quality during storage (Chauhan et al., 2011; Benitez et al., 2013). Interestingly, the antifungal activity of *Aloe* gel from several species has been correlated with the content of aloin, one of the major phenolic compounds of *Aloe* leaves (Zapata et al., 2013). When edible coatings are applied to minimally processed fruit, the incorporation of citric acid and ascorbic acid have positive effects on reducing browning and microbial spoilage (Pérez-Gago et al., 2010). Thus, the objective of this work was to investigate for the first time the

* Corresponding author. Tel.: +34 96 6749720; fax: +34 96 6749678.
E-mail address: dmromero@umh.es (D. Martínez-Romero).

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effect of *A. vera* gel (alone or in combination with ascorbic and citric acids) applied as a coating on the overall quality of minimally processed pomegranate arils during storage under MAP conditions.

2. Materials and methods

2.1. Plant material and experimental design

Pomegranate fruit (*P. granatum* L., cv. Mollar de Elche) were harvested at commercial ripening (fully mature according to commercial practice) from a plot located in Elche (Alicante) and immediately transported to the laboratory. Pomegranates with defects (sunburn, cracks, bruises and cuts in the husk) were discarded and only fruit with healthy outer skins and uniform in size and appearance were used. Husks (about 300) were carefully cut with sharpened knives and arils manually extracted. The arils were collected in a tray, washed in a solution containing 100 $\mu L\,L^{-1}$ chlorine (NaOCl) for 5 min and further rinsed in tap water, drained and excess water removed from arils with paper towels, as reported by López-Rubira et al. (2005). The arils were divided into 7 lots for the following treatments: (a) water (control washed arils); (b) acids 0.5% (citric acid 0.5% + ascorbic acid 0.5%); (c) acids 1.0% (citric acid 1.0% + ascorbic acid 1.0%); (d) *A. vera* 50% (*A. vera* gel diluted with distilled water 50–50 v/v); (e) *A. vera* 100% (*A. vera* gel); (f) *A. vera* 50% + acids 0.5% (treatments b + d); (g) *A. vera* 100% + acids 1.0% (treatments c + e).

Acid treatments (0.5% or 1%, treatments b and c, respectively) were prepared by dissolving citric or ascorbic acid powder (Sigma-Aldrich, Madrid) at individual concentrations of 0.5 or 1.0%, the pH of the solutions being 2.2 and 2.6, respectively. Freshly *A. vera* gel was prepared according to a previous report (Navarro et al., 2011). Briefly, for each leaf the spikes along their margins were removed before longitudinally slicing to separate the rind from the inner leaf gel. The gel fillets were crushed to yield a mucilaginous gel which was filtered to discard the fibrous fraction, this gel being used for treatment *A. vera* 100%. The gel diluted with distilled water (50:50 v/v) was used for the *A. vera* 50% treatment. In both cases, acids (citric acid and ascorbic acid powder) were added at 0.5% or 1.0%, and pH of the gels adjusted to 3.7.

Treatments were performed by dipping the arils in the corresponding solution for 5 min, then drained using a colander, collected on a tray and left to dry. After coating, arils (130 g) were placed directly in rigid polypropylene boxes (280 mL) and covered with airtight lids (Lealplast S.L., Murcia, Spain). A silicone septum was deposited on the lid for gas extraction and O_2 and CO_2 quantification. For each treatment 30 boxes were used, from which 10 were sampled after 4, 8 and 12 days of storage at 3 °C and 90% RH for analytical determinations, respectively. For day 0 measurements the arils (10 boxes) after chlorine washing were used. In addition, microbial analyses were also performed on 10 samples before chlorination.

2.2. Gas composition and odour

The gas composition was measured inside each box and treatment by measuring the CO_2 and O_2 concentrations. Measurements were performed in duplicate by withdrawal of 1 mL of the headspace atmosphere using an air-tight syringe through the silicone septum and injected into a gas chromatograph GC 14B (Shimadzu, Tokyo, Japan) equipped with a thermal conductivity detector (TCD). CO_2 and O_2 were separated on a molecular sieve 5A column, 80–100 mesh (Carbosieve SII. Supelco Inc., Bellefonte, USA), of 2 m length and 3 mm i.d. Oven and injector temperature were 50 and 110 °C, respectively. Helium was used as carrier gas at a flow rate of 50 mL min^{-1}. Results (mean ± SE) were expressed as kPa O_2 and kPa CO_2 inside the boxes.

Internal odour was evaluated immediately after opening the boxes according to the protocol described below (Section 2.5).

2.3. Microbiological analysis

For each box and treatment, samples (10 g) were obtained under sterilized conditions (laminar fume cupboard, gloves and scalpels), which were homogenized in 90 mL of sterile peptone water using a stomacher (Model Seward, Laboratory Blender Stomacher 400, London, UK). Serial dilutions were carried out and 1 mL was added to plate count agar for mesophilic aerobic and for mould and yeast counts (Petrifilm™ Aerobic Count Plate, Laboratories 3M™ Santé, France), and only counts of 30–300 colony forming units (CFU) were considered. The same procedure was carried out on recently harvested arils (day 0) and after chlorination. All plates were incubated for 3 days at 25 and 30 °C for mesophilic and mould and yeast, respectively, and results (mean ± SE) expressed as CFU g^{-1}.

2.4. Aril quality parameters

Aril firmness was determined in each box using a TX-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) interfaced to a PC, and a forward extrusion cell. The cell was filled at half with arils and compression force (3% of the aril cell height) was applied using a 40 mm flat probe. The results were expressed as force-deformation ratio (N mm^{-1}) and are means ± SE.

Total soluble solids (TSS) were determined in duplicate in the juice obtained from 10 g of each box with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) at 20 °C, and expressed as % (°Brix). Total acidity (TA) was determined in the same juice in duplicate by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 mL of diluted juice in 25 mL distilled H_2O, and results expressed as g malic acid equivalent per 100 g^{-1} fresh weight. Results were expressed as mean ± SE. Colour was determined with a Minolta colorimeter (CR200, Minolta Camera Co., Japan) and expressed by the CIE Lab System. Results were means ± SE of 3 determinations for each sample and expressed as Hue angle (arctg b^*/a^*).

Total phenolics and total anthocyanins were determined according to a previous report (Sayyari et al., 2010). Briefly, phenolic extraction was performed using water:methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation) and quantified using the Folin–Ciocalteu reagent. Results were expressed as mg gallic acid equivalent 100 g^{-1} FW. Total anthocyanins were calculated from the above methanolic extract as cyanidin 3-glucoside equivalent (molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}) and results (mean ± SE) expressed as mg 100 g^{-1} FW.

2.5. Sensory evaluation

Sensory analyses to compare the internal package odour of treated and control arils were carried out by 10 trained adults, aged 25–50 years (5 female and 5 male). The panel was trained in a pretest for evaluating the aroma of pomegranate arils. A laboratory of sensory analyses with an individual booth for each panellist was used. For each treatment and sampling date, the ten boxes were opened and each judge evaluated immediately the box head space odour on a ranked scale of 5 to 1, where 5 = fresh fruit, 4 = ripe, 3 = over-ripe, 2 = slightly fermented, and 1 = off-odour.

The same panel evaluated the following aril quality attributes: colour, aroma, texture, flavour and purchase decision. Panellists were pre-trained in aril visualization, smelling and tasting, and each judge evaluated 1 sample for each treatment. Samples were blind labelled with random three digit codes, and the sample order