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Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage

Feryal Varasteh [a], Kazem Arzani [a,*], Mohsen Barzegar [b], Zabihollah Zamani [c]

[a] Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University (TMU), P.O. Box 14115-336, Tehran, Iran
[b] Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran
[c] Department of Horticultural Science, Faculty of Agriculture Science & Engineering, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

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ABSTRACT

Edible coatings as chitosan treatments (0%, 1% and 2%) were applied to 'Rabbab-e-Neyriz' pomegranate (*Punica granatum* L.). The effect of chitosan coating on individual anthocyanins and colour parameters of the juice during storage at 2 °C or 5 °C was examined. Six predominant anthocyanins were identified in the juice, with up to 935 mg/L total anthocyanins at the time of harvest. Cyanidin 3,5-diglucoside (402 mg/L) was the major pigment. The total anthocyanin content and chroma decreased with storage time in all applied treatments, although lightness and hue angle increased. These changes were reduced with chitosan treatments and at lower storage temperature (2 °C as compared to 5 °C). Based on the obtained results, the diglucoside anthocyanins were more stable than the monoglucosides. Chitosan coating followed by cold storage delayed anthocyanin degradation and prevented colour deterioration in the pomegranate arils.

1. Introduction

Pomegranate (*Punica granatum* L.) from the Punicaceae family is a popular fruit native to Iran and the surrounding area (Varasteh, Arzani, Zamani, & Mohseni, 2009). The edible parts of pomegranates (called arils) make up approximately 50% of the fruit weight and are made up of 76–85% juice and 15–24% seeds. The composition of pomegranate juice and the content of bioactive compounds depends on genetics, fruit maturity, environmental, agronomic, and postharvest conditions, storage, and processing factors (Miguel, Fontes, Antunes, Neves, & Martins, 2004; Poyrazoglu, Gokmen, & Artik, 2002). Fresh pomegranate juice contains 85% moisture, and considerable amounts of sugars, pectin, organic acids, polyphenolic compounds, anthocyanins, amino acids, and minerals (D'Aquino et al., 2010).

Anthocyanins are water-soluble flavonoid compounds that produce colours ranging from orange and red to various shades of blue and purple, and have a critical role in the colour quality of many fresh and processed fruit, vegetables, and plants. In addition to its colourant properties, anthocyanins have been found to exhibit a wide range of biological, pharmacological, anti-inflammatory, antioxidative, and chemoprotective properties (Da Costa, Horton, & Margolis, 2000). Analysing pigment composition of fruit is important in order to optimise postharvest treatments and storage conditions. It has been reported that anthocyanin synthesis continues in harvested fruit even at low storage temperatures, and postharvest treatments may affect anthocyanin biosynthesis, degradation, or both (Goncalves et al., 2007; Holcroft, Gil, & Kader, 1998; Holcroft & Kader, 1999). Anthocyanin pigments, such as pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside, and delphinidin 3,5-diglucoside, are responsible for the red colour of pomegranate peel and aril, which is one of the important quality features (Alighourchi, Barzegar, & Abbasi, 2008a; Miguel et al., 2004). Pomegranate anthocyanins are labile compounds that will undergo numerous degradative reactions during storage and processing (Alighourchi, Barzegar, & Abbasi, 2008b; Wrolstad, Durst, & Lee, 2005).

Recently, more attention has been paid to the potential of biopolymers in fruit packaging applications due to their ecofriendly and biodegradable properties. Most of them can be processed into films or used as fruit surface coatings to enhance storability, retard discolouration, improve quality, and reduce respiration and transpiration rates because of their high gas selectively (CO_2/O_2) and partial moisture barrier (Shahidi, Arachchi, & Jeon, 1999). Chitosan, a high molecular weight cationic polysaccharide, produced by the deacetylation of chitin, is widely used in postharvest trials because of its excellent film forming and anti-fungal, bio-safe and biochemical properties (Lin et al., 2008). The effect of chitosan coating on colour preservation of some fruit, such as litchi (De Reuck, Sivakumar, & Korsten, 2009), strawberry (El Ghaouth, Ponnampalam, & Boulet,

* Corresponding author. Tel.: +98 21 48290; fax: +98 21 48292200.
E-mail address: arzani_k@modares.ac.ir (K. Arzani).

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1991; Zhang & Quantick, 1998), and raspberries Zhang & Quantick (1998) has been investigated.

The assessment of quality parameters must be done carefully on edible-film-coated fruit throughout the storage period (Park, 1999). It has been determined that edible coating acts as a barrier to gas transport which could extend commercial shelf life of coated products by modifying their internal atmosphere and slowing down the respiration rate (Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2006). Holcroft et al. (1998) suggested that CO_2 and O_2 concentration affects anthocyanin synthesis and/or degradation. In addition, cold storage is necessary to slow fruit ripening, discolouration and deterioration. It has also been found that anthocyanins content of pomegranate can be preserved by lowering storage temperature (Artes, Tudela, & Villaescusa, 2000).'Rabbab-e-Neyriz' is one of the most important cultivars in Iran and we can find no information on the effect of chitosan coating on the anthocyanins content of pomegranate fruit. Therefore, the main purpose of this study was: (i) to identify the main anthocyanins present in the fruit juice of Rabbab-e-Neyriz cultivar; (ii) to evaluate the effect of chitosan treatment and storage temperature on the total and individual anthocyanin contents throughout storage and (iii) to monitor changes in juice colour parameters of pomegranate fruit coated with different chitosan concentrations during storage at two temperatures.

2. Materials and methods

2.1. Plant material and experimental design

Pomegranate fruit 'Rabbab-e-Neyriz' were harvested in October at the commercial harvest stage from a commercial orchard located in Neyriz, east of Shiraz in the Fars Province, Iran. On the same day, harvested fruit were transported by a ventilated car to the laboratory of the Department of Horticultural Science at Tarbiat Modares University in Tehran. Pomegranate fruit were selected for uniformity in size (300–350 g), shape and colour. Fruit with signs of mechanical damage, sunburn, blemishes, disease and pest damage were discarded. Selected fruit samples were randomly distributed into groups of 15 fruit and for each treatment three replicates were used. Aqueous solutions of 1–2% chitosan (medium molecular weight, Fluka, Buchs, Switzerland) were prepared according to the method described previously by Jiang, Li, and Jiang (2005) with some modification. Pomegranate fruit were dipped in 1% or 2% aqueous chitosan solutions (w/v) with 1% acetic acid (v/v). Control fruit were dipped in distilled water with 1% acetic acid without chitosan treatment. Fruit were allowed to dry for 12 h at room temperature. The day after treatment, fruit were placed in baskets (15 fruit per basket) and stored at 2 ± 0.5 °C and 5 ± 0.5 °C at 90 ± 5% RH. At the end of each storage period (every 45 days), three replicates of each treatment were transferred to shelf life conditions for three days at 20 °C. The assessments and analyses of fruit quality parameters during cold storage were performed on Days 45, 90 and 135. The same assessment was performed on fruit at the time of commercial harvest (Day 0).

2.2. Pomegranate juice preparation and storage conditions

To prepare pomegranate juice, six fruit of each replicate were individually cut and the peels covering the arils removed. The arils were then manually separated. The aril juice was extracted using a garlic press. Fifty millilitres of juice were centrifuged (15 min at 12,000*g* at 4 °C), placed in small vials and stored at −80 °C prior to analysis.

2.3. Chemicals

Cyanidin 3,5-diglucoside (Cy 3,5-dG), cyanidin 3-glucoside (Cy 3-G), delphinidin 3,5-diglucoside (Dp 3,5-dG), delphinidin 3-glucoside (Dp 3-G), pelargonidin 3,5-diglucoside (Pg 3,5-dG), and pelargonidin 3-glucoside (Pg 3-G) standards were purchased from Apin Chemicals Ltd. (Abingdon, UK). Methanol (HPLC grade) and formic acid were obtained from Caledon Laboratories (Halton Hills, Ontario, Canada) and Merck (Darmstadt, Germany), respectively. Ultra-pure water was prepared with the Purise System (Seoul, South Korea).

2.4. Anthocyanin analysis

Anthocyanins were determined according to the method described by Alighourchi et al. (2008b). In order to analyse the juice's anthocyanins, juices from different treatments were gently thawed at refrigerator temperature (4 °C) and filtered through a 0.20-μm filter. Twenty microlitres of clarified juice were injected onto a Waters (Milford, MA) HPLC system equipped with Empower software, a pump (Waters 600), a Rheodyne 7125i six-way injector with 20-μL sample loop, and a UV–Vis detector (Waters model 2487). The juice sample was separated using a μBondapack™ C_{18} column (4.6 × 250 mm, dp 10 μm; Waters). The elution was carried out at room temperature using 5% formic acid aqueous solution (**A**) and methanol (**B**) in a linear gradient from 15% to 35% **B** at 15 min, then isocratic to 20 min. Flow rate was 1 mL/min with detection at 510 nm.

Calculation of the concentrations was based on the external standard method and anthocyanins were identified by comparison of their retention times with those of pure standards (Table 1). All data are the average of triplicates.

2.5. Pomegranate juice colour measurement

Pomegranate juice colour was evaluated using a Hunter colorimeter, which provided *CIEL*a*b** measurements. L^* defines the lightness, and a^* and b^* define the red-greenness and blue-yellowness, respectively. Three measured colour parameters were converted into chroma $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$, which expresses the purity or saturation of the colour, and hue angle $h^\circ = \text{arctangent}[b^*/a^*]$, which indicates the colour nuance (Goncalves et al., 2007; Wrolstad et al., 2005). The analyses were performed in triplicates.

2.6. Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) using SPSS software Version 15.0 (SPSS Inc., Chicago, IL). The results are expressed as means ± SE and least significant difference (LSD) values were calculated for mean comparisons.

Table 1
Linear calibration equations of individual anthocyanin standards.

Compound	t_R (min)	Linear range (mg/L)	Linear equation	r^2
Dp 3,5-dG	9.5	1–200	$A = 18260C - 39649$[a]	0.9989
Cy 3,5-dG	10.3	1–500	$A = 18648C - 30888$	0.9995
Pg 3,5-dG	11	0.1–250	$A = 25728C - 50808$	0.9978
Dp 3-G	11.7	0.1–500	$A = 33825C - 108509$	0.9992
Cy 3-G	12.4	1–500	$A = 64276C - 154546$	0.9995
Pg 3-G	13.1	0.01–250	$A = 69830C - 30157$	0.9999

[a] *A*: peak area; *C*: concentration (mg/L).