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Approaches to understanding the contribution of anthocyanins to the antioxidant capacity of pasteurized pomegranate juices

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ABSTRACT

Influence of processing and storage conditions on anthocyanin stability and antioxidant activity of clarified and cloudy juices from arils of the 'Mollar' pomegranate variety was studied. Clarification process reduced the content of total monomeric and individual anthocyanins, and increased the antioxidant activity of pomegranate juice. Thermal treatments (65 and 90 °C for 30 or 5 s) decreased the percentage of polymeric anthocyanin form, increasing on the contrary the monomeric one. In any case, storage temperature was the main factor affecting all the parameters tested. Cyanidin 3-*O*-glucoside (Cy3G) was more instable than delphinidin 3,5-di (Dp3,5dG) and cyaniding 3,5-diglucosides (Cy3,5dG). A linear relationship was observed between oxygen radical absorbance capacity (ORAC) and total monomeric anthocyanins, suggesting that they contributed strongly to the antioxidant capacity. Results presented in this study show that hurdle technology (heating plus refrigeration) may help to reduce anthocyanin degradation in pasteurized pomegranate juice, avoiding a dramatic impact on its colour and preserving the beneficial effects of this specific bioactive compounds on human health.

1. Introduction

Juices besides fruits are suitable food products in term of ingestion of health protective phytochemicals (Netzel et al., 2002). The distribution and composition of the phenolic compounds in juice products are dependent on the juice processing application which affects the rupture of the vacuoles and cell wall differently (Bengoechea et al., 1997). The pomegranate is known to contain considerable amount of phenolic compounds, including anthocyanins, ellagic acid, punicalagin isomers, punicalins, granatins, and different flavanols as catechins and gallocatechins (González-Molina, Moreno, & García-Viguera, 2009), and these constituents give the characteristic flavour and also play a large role in the acquisition of sensory properties (colour, bitterness, astringency, etc.) of the juice.

Pomegranate phenolic compounds exhibit a wide range of biological properties, such as antimicrobial, anti-inflammatory, anticancer, antiatherosclerotic, hepato- and neuro-protective (Faria, Monteiro, Mateus, Azevedo, & Calhau, 2007; Negi & Jayaprakasha, 2003; Reddy, Gupta, Jacob, Khan, & Ferreira, 2007; Sartippour, Seeram, Heber, & Pantuck, 2008; Seeram, Lee, & Heber, 2004), and above are now gaining importance because of its potent antioxidant activity (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000; Noda, Kaneyuki, Mori, & Packer, 2002). These health benefits are related to the high antioxidant activity of the pomegranate juice and can be correlated to its phenolic composition (Ferrari, Maresca, & Ciccarone, 2010), being the hydrolysable tannins which have higher antioxidant activity, although anthocyanins also contribute to the total antioxidant capacity of the juice (Gil et al., 2000).

Anthocyanins are the main pigments responsible for the red colour in pomegranates. The predominant anthocyanins present in pomegranates are delphinidin 3,5-di (Dp3,5dG) and 3-*O*-glucoside (Dp3G), cyanidin 3,5-di (Cy3,5dG) and 3-*O*-glucoside (Cy3G), and pelargonidin 3,5-di (Pg3,5dG) and 3-*O*-glucoside (Pg3G) (Miguel, Dandlen, Antunes, Neves, & Martins, 2004; Mousavinejad, Emam-Djomeh, Rezaei, & Khodaparast, 2009). Cy3G is the most abundant anthocyanin identified in the 'Mollar' pomegranate variety (Mena et al., 2011). Anthocyanins readily degrade during thermal processing which can have a dramatic impact on colour and nutritional properties of anthocyanin rich food products. Recent studies reveal that anthocyanin stability is not merely a function of the pasteurization temperature, but is in turn influenced by the intrinsic properties of the product (pH, chemical structure and concentration of anthocyanins, the presence of endogenous

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enzymes and other natural compounds called co-pigments, metallic ions and sugars) and the processing (magnitude and duration of heating, storage temperature and time, the presence of oxygen and/or light) (Patras, Brunton, O'Donnell, & Tiwari, 2010). In addition, co-pigmentation reactions, especially inter- and intra-molecular ones, play a role in anthocyanin stability towards thermal treatment (Rein, 2005). There is hence a real need to minimize the degradation of anthocyanins present in the pomegranate juice during the pasteurization process and the further storage in order to secure its optimal sensorial and nutritional quality.

The Spanish southeast is one of the largest production areas of pomegranate variety 'Mollar'. It is a late-ripening cultivar with delicious and soft arils, being the harvest of fruits from the end of September to mid-November (Artés, Marín, & Martínez, 1996). Pomegranate transformation into juice is crucial for a profitable farming and solves common problems caused by large amounts of discarded fruits, encouraging the full use of the harvest and its introduction in the market of beneficial foods for health. The overall aim of this work was to examine effects of thermal processing and storage on anthocyanin stability and antioxidant activity of clarified and cloudy juices from arils of the 'Mollar' pomegranate variety. The aim was to gain knowledge of anthocyanin chemical behaviour before and after pasteurization and storage to better understand their contribution to the antioxidant capacity of pomegranate juices.

2. Materials and methods

2.1. Juice extraction

'Mollar' pomegranate is a late-ripening cultivar with sweet arils that have showed fewer anthocyanins and superior organoleptical properties than 'Wonderful' variety (Mena et al., 2011). Second quality pomegranate fruits from var. 'Mollar', harvested in autumn of 2010 were provided by Cambayas Coop. V. (Elche, Alicante, Spain). Pomegranates were cut in halves and arils were hand-separated from the pith. Juice was obtained by applying pressure on arils inside a nylon mesh with a laboratory pilot press (Zumonat C-40; Somatic AMD, Valencia, Spain). The extracted cloudy juice contained 2% pulp. For obtaining clarified juice, the cloudy juice was centrifuged (Allegra™ 25R Centrifuge, Beckman Coulter Inc., Brea, California CA, USA) at 2700*g* for 10 min.

2.2. Juice pasteurization and storage

Both cloudy and clarified juices were subjected to heat treatments at 65 °C for 30 s (LTP, low temperature pasteurization) or 90 °C for 5 s (HTP, high temperature pasteurization) in a semi-tubular pasteurizer 25 L/h (Mipaser Prototype, Murcia Spain). The juices were stored in cooled incubators MIR-153 (Sanyo Electric Co., Ltd., Gunma, Japan) at room temperature (25 °C) and refrigeration (5 °C) for 45 and 120 days, respectively.

2.3. Co-pigmented, monomeric and polymeric anthocyanins

Co-pigmented, monomeric and polymeric anthocyanins were determined using a modified method of Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999). Briefly, 10 μL of 1% (v/v) acetaldehyde was added to 100 μL of juice sample filtered through a 0.45 μm syringe filter. The mixture was allowed to sit for 45 min at room temperature before measuring absorbance at 520 nm (A^{acet}). To another 100 μL of juice sample, 13 μL of a 5% (w/v) SO_2 solution were added, and then A_{520} nm was measured (A^{SO_2}). The A_{520} nm was also measured directly from the juice sample. This reading was multiplied by 2 (dilution factor) to give the A^{juice}. From these readings, the anthocyanin forms were expressed as: co-pigmented anthocyanins = $A^{acet} - A^{juice}$, monomeric anthocyanins = $A^{juice} - A^{SO_2}$ and polymeric anthocyanins = A^{SO_2}, being total anthocyanins = A^{acet}. The percent distribution of the different forms of anthocyanins was calculated as:

$$\%\text{co-pigmented} = [(A^{acet} - A^{juice})/A^{acet}]^*100$$

$$\%\text{monomeric} = [(A^{juice} - A^{SO2})/A^{acet}]^*100$$

$$\%\text{polymeric} = [A^{SO2}/A^{acet}]^*100$$

2.4. Determination of total monomeric anthocyanins

Replicate samples of raw and heat-treated pomegranate juice contained in screw cap 20 mL polypropylene containers were analyzed for total monomeric anthocyanin content, total phenols and antioxidant capacity after 0, 14, 28, 45, 60, 90 and 120 days of storage at different temperatures (5 and 25 °C). All samples were centrifuged in a Hettich EBA 21 centrifuge (Andreas Hettich GmbH & Co.KG, Tuttligen, Germany) at 10,480*g* during 10 min at room temperature. The supernatant was filtered through a 0.45 μm nylon membrane (Waters Corporation, Milford, Massachusetts MA, USA).

Qualitative analysis of anthocyanins was performed by High-performance Liquid Chromatography (HPLC) on a Model L6200 liquid chromatograph (Merck-Hitachi, Darmstadt, Germany) equipped with a SPD-M6A UV–VIS photodiode array detector (Shimadzu, Kyoto, Japan) and a Model 234 automatic sample injector (Gilson International Bv, Barcelona, Spain). Chromatograms were recorded and processed on a LC Workstation Class M10A Shimadzu PC-based chromatography data system.

A 20 μL sample was analyzed on a Luna® 5 μm C_{18} column (25 × 0.46 cm) (Phenomenex Ltd., Macclesfield, UK) with a security guard cartridge system C_{18} ODS (4 × 3 mm), using a mobile phase of water/formic acid (95:5 v/v) (solvent A) and HPLC grade methanol (solvent B). Elution was performed at a flow rate of 1 mL/min. The linear gradient started with 1% B, keeping isocratic conditions during 5 min, reaching 20% B at 20 min, 40% B at 30 min, 95% B at 35 min and 1% B after 41 min. UV chromatograms were recorded at 520 nm and compared with those obtained with analytical standards: anthocyanin monoglucosides (Polyphenols Laboratories AS, Sandnes, Norway) and diglucosides (Extrasynthese, Genay Cedex, France). Anthocyanins were quantified by the absorbance of their corresponding peaks as Cy3G at 520 nm.

2.5. Determination of total phenols

For total phenolic compounds determination, 1:10 dilutions of the juices were used. Total soluble phenolic compounds (TPC) were determined with Folin–Ciocalteu reagent (Sigma–Aldrich Corp., Saint Louis, Missouri MO, USA) in a SPECTROstar Omega UV/VIS absorbance microplate reader (BMG LABTECH GmbH, Offenburg, Germany) (Pérez-Vicente, Serrano, Abellán, & García-Viguera, 2004). 10 μL of sample dilution, 50 μL Folin–Ciocalteu reagent, 100 μL of aqueous 20% Na_2CO_3 (Panreac Química S.A., Barcelona, Spain) and 100 μL of distilled water were mixed. The mixture was allowed to stand for 30 min at room temperature before measuring absorbance at 750 nm. Gallic acid (Sigma–Aldrich Corp.) was used as standard. Results were expressed as gallic acid equivalents (mg GAE/L).

2.6. Oxygen radical absorbance capacity (ORAC) antioxidant assay

The ORAC test is currently the standardized method used by the USDA for testing foods and supplements antioxidant properties. To