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Anthocyanin and colour changes during processing of pomegranate (*Punica granatum* L., cv. Hicaznar) juice from sacs and whole fruit

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ABSTRACT

The effects of clarification and pasteurisation on anthocyanins (ACNs) and the colour of pomegranate juice (PJ) produced from sacs and whole fruits were investigated. Clarification caused a loss of 4% of ACNs in juice from sacs (JFS) and a loss of 19% in juice from whole fruit (JFWF). After pasteurisation, there was an 8–14% and 13–9% loss of ACNs from unclarified and clarified JFS and JFWF samples, respectively. Polymeric colour was very high even in unclarified samples (25–29%). Compared to JFS, higher polymeric colour was formed in JFWF. HPLC analyses of PJ revealed that cyanidin-3,5-diglucoside was the major ACN, followed by cyanidin-3-glucoside and delphinidin-3-glucoside. Cyanidin-3,5-diglucoside showed higher stability to clarification and pasteurisation than cyanidin-3-glucoside in both PJ samples. Cold clarification with only gelatin is recommended for PJ. To prevent excessive ACN loss and the formation of brown colouring, PJ should be subjected to minimal heating.

1. Introduction

Turkey is the fourth major producer of pomegranates in the world, following India, China and Syria. Production in Turkey accounts for approximately 5% of the total world production or approximately 2,000,000 metric tons per year (www.tagem.gov.tr). In recent years, there has been a steady increase in pomegranate production in Turkey due to a high demand for Turkish pomegranate juice (PJ) concentrates. In fact, production in metric tons was 80,000 in 2005, 91,000 in 2006 and 102,000 in 2007 (www.tarimmerkezi.com.tr). With new plantations, pomegranate production is expected to increase dramatically in the coming years. Turkey has a very rich genetic source of pomegranate cultivars. Over 30 registered varieties have been grown in addition to many local varieties. The pomegranate cultivars in highest demand by the Turkish fruit juice industry are Hicaznar and Silifke Asisi, which have a deep violet-red colour and a sweet and sour taste. These properties make the cultivars perfect for juice production.

The high demand for PJ is the result of studies that have shown the health benefits of biological compounds, specifically, phytochemicals, in pomegranates. The primary phytochemicals in pomegranates are the polyphenols, including anthocyanin (ACN) pigments, flavonol glycosides, procyanidins, phenolic acids and ellagic acid derivatives (Negi & Jayaprakasha, 2003). Polyphenols are found in all fruits and vegetables and play a major role in their colour, flavour, texture as well as antioxidant (Hernandez, Melgarejo, Tomas-Barberan, & Artes, 1999) and antibacterial activities (Negi & Jayaprakasha, 2003). Due to their antioxidant properties, phenolic compounds including ACNs are thought to have preventive roles in a number of chronic diseases such as cardiovascular disease and cancers (Heinonen, Meyer, & Frankel, 1998). The primary antioxidative phenolics in PJ are punicalagins, followed by hydrolysable tannins, ACNs and ellagic acids (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000).

An attractive red colour is the most important quality criteria for fruit juices containing anthocyanin, including PJ. ACNs are also responsible for the orange, red and blue colours of many fruits and vegetables. Unfortunately, ACNs are unstable and susceptible to degradation, leading to a brownish colour during processing and storage. The primary colour deterioration in fruit juices containing ACNs occurs as a result of the degradation of monomeric ACNs, polymerisation of ACNs and the subsequent formation of brown colour (Somers & Evans, 1986). These colour changes strongly affect consumer behaviour and result in a loss of marketability of processed pomegranate products.

Various factors affect the stability of ACNs, including the temperature of processing and storage, the chemical nature of ACNs

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(acylation or glucosylation), pH, ascorbic acid, hydrogen peroxide, sugars, light and metals. Clarification and pasteurisation during the production of fruit juices also affect the stability of ACNs. Clarification is the most important step in the production of clear fruit juice. Clarification agents are used to achieve clarity and improve colour, flavour and physical stability. After pressing, clarification agents such as bentonite, gelatin and kieselsol are added to cloudy juice to remove colloidally suspended particles that may be present in the form of proteins, polyphenols, pectins and gums. Removal of these particles is critical for improving the clarity and colour of fruit juices. Bentonite is added to fruit juices to remove proteins, while gelatin is used for the removal of high molecular weight (>500 Dalton) polyphenols that otherwise cause turbidity during storage. Kieselsol is added to fruit juices to remove excessive gelatin, which also causes turbidity during storage.

The other important step in processing pomegranates is the heating of fruit juices at various stages. Heating right after pressing inhibits native polyphenol oxidase (PPO) enzymes that cause brown colour formation by oxidising polyphenols (Skrede, Wrolstad, & Durst, 2000). PPO also degrades monomeric ACNs indirectly by forming *O*-quinones from polyphenols during enzymatic browning that react with and degrade monomeric ACNs. Pasteurisation is the process of heating before packaging to inactivate pathogenic or spoilage microorganisms. Heat applied during juice processing causes the degradation of monomeric ACNs, polymerisation of ACNs and the formation of brown colour in red fruit products (Somers & Evans, 1986).

To date, there have been no detailed studies that report changes in ACNs and the colour of PJ during processing. The primary purpose of this study was to determine the changes in ACN content and brown colour formation during clarification and pasteurisation of PJ from sacs and whole fruits. The secondary purpose was to identify the ACN profile of the major Turkish pomegranate cultivar Hicaznar, which is highly preferred by the Turkish fruit juice industry.

2. Materials and methods

2.1. Chemicals and reagents

Cyanidin 3-*O*-glucoside and cyanidin 3,5-*O*-diglucoside standards were purchased from Sigma (St. Louis, MO); pelargonidin 3-*O*-glucoside and pelargonidin 3,5-*O*-diglucoside were purchased from Fluka (Seelze, Germany); and delphinidin-3-*O*-glucoside was purchased from Polyphenols Laboratories AS (Sandnes, Norway). All reagents used for liquid chromatography were HPLC grade and purchased from Merck (Darmstad, Germany). All other reagents were analytical grade and obtained from Merck.

2.2. Samples

Pomegranates (*Punica granatum* L., cv. Hicaznar) were obtained from the Alata Horticultural Research Institute (Erdemli, Mersin) and processed immediately. A flow diagram of PJ processing is shown in Fig. 1. Before juice extraction, pomegranates were washed in cold tap water and drained. Damaged pomegranates were discarded. The top and bottom of the pomegranate husks were removed with a sharp stainless steel knife to prevent microbial contamination.

After washing, 10 kg of pomegranates were selected and the outer skins were hand-peeled. The juicy sacs from fruit pericarp were separated by hand, placed in a muslin cloth and pressed with a laboratory hand press. The resulting juice was labelled "juice from sacs" (JFS). The rest of the pomegranates (50 kg) were cut into four pieces and processed into juice at the fruit juice pilot plant at Ankara University. Pomegranate quarters were pressed on a rack-and-cloth press (Bucher-Guyer, Niederweningen, Switzerland). The resulting juice was filtered through muslin cloth to remove particles and was labelled "juice from whole fruits" (JFWF).

The resulting cloudy juices from sacs and whole fruits were divided into two groups. One group was clarified using only gelatin at 5 °C (cold clarification). The other group was not clarified. Gelatin solutions at 0.5% and 1% (w/v) were used for the clarification of JFS and JFWF, respectively.

Since pomegranates do not contain pectin (Cemeroğlu, 1977), PJ samples were not depectinised. However, PJ, especially JFWF, contains a significant amount of polyphenols and needs to be clarified to remove some of the high molecular weight polyphenols that otherwise cause turbidity during storage. In fact, JFS and JFWF were shown to contain 2073 and 2773 mg of total phenolics expressed as gallic acid equivalents/l, respectively (Güzel, 2010). PJ obtained from whole fruits also contains high levels of tannins, which cause astringency (Güzel, 2010). Bentonite was not used during the clarification of PJ samples because pomegranates contain an insignificant amount of protein. Gelatin (A type, 80–100 Bloom strength) was used at a concentration of 0.4 g/l for juice from sacs and 2 g/l for juice from whole fruits and was the only agent used for the clarification of PJ. There was no need to use kieselsol because cold clarification was applied and the PJ contained a high amount of polyphenols. After clarification, the turbidity of the juice from sacs and whole pomegranates was 2.23 and 1.75 NTU (Nephelometric turbidity unit), respectively.

Clarified and unclarified juices from sacs and whole fruits were again divided into two groups. One group was pasteurised, and one was not. The juice samples were transferred into 200 ml hermetically capped glass bottles, and pasteurisation was carried out in a water bath at 95 °C for 10 min. After pasteurisation, the juice samples were immediately cooled to room temperature.

2.3. Compositional analysis

The total soluble content (°Brix) of PJ samples was determined by an automatic digital refractometer (Atago Rx-7000α, Tokyo, Japan). The brix was determined after the cloudy juice samples were filtered through a 0.45 μm PVDF (polyvinylidene fluoride) filter (Millipore, Bedford, MA) to reduce turbidity. °Brix measurements were carried out at 20 °C. pH was measured potentiometrically with a pH metre (WTW Inolab Level 1, Weilheim, Germany). Titratable acidity was determined according to the method outlined by IFU (1968) and expressed as "*g anhydrous citric acid/100 ml or g sample*."

2.4. Monomeric ACN analysis

The total monomeric ACN content was determined using the pH differential method described by Giusti and Worlstad (2005). The pH of juice samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. The dilutions were then allowed to equilibrate for 15 min at room temperature (~22 °C). Prior to absorbance measurements, the solutions were filtered through a 0.45 μm PVDF filter to remove the haze. The absorbance of equilibrated solutions at 512 nm (λ_{max}) for ACN content and 700 nm for haze correction was measured on a UV–VIS double beam spectrophotometer (ThermoSpectronic Helios–α, Cambridge, England) with 1 cm path length disposable cuvettes (Brand Gmbh, Wertheim, Germany). All absorbance measurements were carried out at room temperature against distilled water as a blank. Pigment content was calculated as cyanidin-3-glucoside (Cy-3-glu) equivalents with a molecular weight of 449.2 and an extinction coefficient of 26 900 L cm^{-1} mol^{-1}. The difference in absorbance