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Effects of various protein- and polysaccharide-based clarification agents on antioxidative compounds and colour of pomegranate juice

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ABSTRACT

The effects of natural sedimentation and clarification agents [protein-based (albumin, casein and gelatin) and polysaccharide-based (chitosan and xanthan gum)] on total phenolics, hydrolysable tannins, anthocyanins and antioxidant activity of pomegranate juice were investigated. While protein-based agents led to higher reductions in the contents of total phenolics (7.2–17.2%), hydrolysable tannins (16.7–59.5%) and anthocyanins (11.7–23.7%) ($p < 0.05$) than natural sedimentation, polysaccharide-based agents had similar effects on these compounds, like natural sedimentation ($p > 0.05$). The stabilities of β-punicalagin and α-punicalagin (the major hydrolysable tannins) against clarification were similar. Moreover, antioxidant activities of the samples clarified with polysaccharide-based agents were higher (2.4–26.6%) than those of the protein-based agents. For both high total phenolic, hydrolysable tannin and anthocyanins contents, as well as high antioxidant activity, we suggest that polysaccharide-based agents be used for the clarification of pomegranate juice. In fact, chitosan is especially recommended for this purpose since chitosan (10.3 NTU) led to lower turbidity as compared to xanthan gum (20.0 NTU).

1. Introduction

Pomegranates are generally processed into juice with their rinds, constituting 48% of the whole fruit (Kulkarni & Aradhya, 2005). During pressing of pomegranates, hydrolysable tannins (e.g. punicalagins and punicalin), which are the main phenolic group in the rinds, pass from the rinds into juice (Muhacir-Güzel, Türkyılmaz, Yemiş, Tağı, & Özkan, 2014). And, they cause the formation of cloudy appearance as well as astringency and bitterness in juice (Spanos & Wrolstad, 1992). The cloudy appearance negatively affects consumer attitude toward pomegranate juice since consumers question the safety and quality of turbid products.

The removal of hydrolysable tannins should be targeted to produce a clear pomegranate juice (Muhacir-Güzel et al., 2014; Turfan, Türkyılmaz, Yemiş, & Özkan, 2011) because pomegranates contain insignificant amounts of the other compounds (pectin and protein) causing the turbidity in juices (Benk, 1971). For the removal of tannins, cold-clarification of pomegranate juice with only gelatin is suggested (Özkan, Yemenicioğlu, Asefi, & Cemeroğlu, 2002). However, gelatin removes the significant amounts of not only hydrolysable tannins (74% reduction, Muhacir-Güzel et al., 2014) but also anthocyanins (19% reduction, Turfan et al., 2011), which are responsible for the attractive red colour of pomegranate juice, from the juice. Therefore, the effects of different clarification agents [polyvinylpolypyrolidone (PVPP) (Vardin & Fenercioğlu, 2003) and albumin (Valero, Vegara, Martí, & Saura, 2014)] and clarification methods [natural sedimentation (Vardin & Fenercioğlu, 2003), laccase treatment (Neifar et al., 2011) and microfiltration (Mirsaeedghazi, Emam-Djomeh, Mousavi, Aroujalian, & Navidbakhsh, 2010)] on tannin and anthocyanin contents of pomegranate juice have been extensively investigated in various studies. However, an efficient clarification agent or a method removing tannins, but at the same time preventing anthocyanin loss from pomegranate juice, has not still been found.

New clarification methods and different clarification agents should be investigated to solve the clarification problems in pomegranate juice. Although the removal effects of chitosan (Fang, Zhang, Du, & Sun, 2007; Imeri & Knorr, 1988), casein (Cosme, Ricardo-da-Silva, & Laureano, 2008) and xanthan gum (Fang et al., 2007) on haze precursors in various fruit juices (apple and bayberry juice) and white wine were determined in previous studies, the effects of these clarification agents on the turbidity of pomegranate juice have not been investigated.

In literature, there are conflicting statements about clarification mechanism of chitosan. While Bassi, Prasher, and Simpson (2000)

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reported that chitosan served as an effective coagulating agent in removing proteins, Imeri and Knorr (1988) showed that chitosan has a good affinity for negatively charged phenolic compounds such as flavan-3-ols (especially catechin), tannins, cinnamic acids and their derivatives since chitosan carries strong positive charge. On the contrary to the findings of Imeri and Knorr (1988) and Oszmiański and Wojdyło (2007) reported that chitosan had no impact on the phenolic contents of apple juice. Similar to chitosan, the clarification mechanism of casein has not been full elucidated, yet. Although its mechanism on clarification is not known, the significant reducing effect of casein on hydrolysable tannins was reported (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Compared to chitosan and casein, xanthan gum is a newer substance used in the clarification of fruit juices. The removing effect of xanthan gum on juice colloids has also not clearly shown yet.

The primary purpose of this study was to determine the effects of various clarification agents (e.g. gelatin, albumin, casein, chitosan and xanthan gum) and natural sedimentation on turbidity, total phenolics, hydrolysable tannins, colour (total monomeric anthocyanin, individual anthocyanins and polymeric colour) and antioxidant activity of pomegranate juice. Additionally, the correlations between antioxidant activity and the contents of anthocyanins, hydrolysable tannins and total phenolics of the samples were also investigated.

2. Materials and methods

2.1. Chemicals and reagents

Punicalagin standard, Sephadex LH-20, albumin (from chicken egg white, grade II), xanthan gum and casein (from bovine milk, technical grade) were purchased from Sigma (St. Louis, MO, USA). Cyanidin 3,5-*O*-diglucoside, pelargonidin 3-*O*-glucoside, pelargonidin 3,5-*O*-diglucoside and delphinidin-3-*O*-glucoside standards were obtained from Fluka (Seelze, Germany). Chitosan (from crab shells, practical grade) was purchased from Aldrich (Milwaukee, WI, USA). Gelatin (A type, 80-100 Bloom strength) was obtained from Döhler (Geisenheim, Germany). 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

2.2.1. Juices

Pomegranates (*Punica granatum* L., cv. Hicaznar) were obtained from Alata Horticultural Research Institute (Erdemli, Mersin, Turkey) and immediately processed into juice as reported previously (Turfan et al., 2011). A flow diagram for the production of pomegranate juice is shown in Fig. 1.

The pomegranates (292 kg) with their rinds were cut into quarters and pressed on a-rack-and-cloth-press (Bucher-Guyer, Niederweningen, Switzerland) in a fruit juice pilot plant at Ankara University by gradually increasing the pressure up to a maximum of 15 MPa within 30 min. The amount of extracted juice was 102 kg and juice yield was 34.9%. The resulting juice was filtered through muslin cloth to remove the coarse particles and was then divided into seven parts. A part of this unprocessed juice was called as "unclarified (control)." The turbidity value of the unclarified juice was 534 NTU.

2.2.2. Clarification

The rest of juice sample was separately clarified with natural sedimentation, gelatin, chitosan, casein, albumin and xanthan gum. To obtain the lowest possible but similar ($p > 0.05$) turbidity values in juice samples, the most suitable concentrations (0.05–1.00 g/L) of clarification agents, incubation temperatures (4 and 20 °C) and times (2–22 h) were determined by preliminary tests. The results were given below:

- *Clarification with natural sedimentation (NS)*: Pomegranate raw juice was holded at 2 °C for 16 h (Vardin & Fenercioğlu, 2003). The turbidity value of this juice was 32 NTU.
- *Clarification with gelatin*: Pomegranate raw juices were clarified using different amounts (0.0625, 0.125, 0.250, 0.375, 0.500 and 0.625 g/L) of gelatin solution (1%, w/v) at 4 °C for 16 h (Muhacir-Güzel et al., 2014; Turfan et al., 2011). The lowest turbidity (6.04 NTU) was obtained in the juice clarified using 0.375 g/L gelatin at 4 °C for 16 h.
- *Clarification with casein*: Pomegranate raw juices were clarified using different amounts (0.0625, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875 and 1.0 g/L) of casein solution (1%, w/v) prepared in sodium carbonate solution (1%, w/v) (Castillo-Sánchez et al., 2008). The clarification was carried out at two different temperatures (4 and 20 °C) for 16 h (Castillo-Sánchez et al., 2008). The lowest turbidity (6.53 NTU) was obtained in the juice clarified using 0.375 g/L casein at 4 °C for 16 h.
- *Clarification with chitosan*: For the preparation of chitosan solution (1%, w/v), 50 mL 2% chitosan solution was added into 50 mL 2% citric acid solution and the mixture was homogenised at 9500 rpm for 3 min (Heidolph SilentCrusher M, Schwabach, Germany). After the solution was heated to 60 °C and filtered using Whatman No 541 filter paper, chitosan solution became ready for clarification trial. Pomegranate raw juices were then clarified with different amounts (0.1, 0.25, 0.50, 0.75 and 1.0 g/L) of chitosan solution. Since there is no information in the literature about incubation temperature and time for the clarification of pomegranate juice with chitosan, the effects of different incubation temperatures (4 and 20 °C) and times (2, 4, 6, 8, 10, 16 and 22 h) on turbidity of the samples clarified with chitosan were also determined. The lowest turbidity (10.3 NTU) was obtained in the juice clarified using 0.50 g/L chitosan at 20 °C for 16 h.
- *Clarification with albumin*: Pomegranate raw juices were clarified using different amounts (0.0625, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875 and 1.0 g/L) of albumin solution (1%, w/v) prepared in pure water (20 °C). The clarification was carried out at two different temperatures (4 and 20 °C) for 16 h (Castillo-Sánchez et al., 2008). The lowest turbidity (10.1 NTU) was obtained in the juice clarified using 0.125 g/L albumin at 4 °C for 16 h.
- *Clarification with xanthan gum*: Pomegranate raw juices were clarified using different amounts (0.05, 0.075, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90 and 1.00 g/L) of xanthan gum solution (1%, w/v) prepared in pure water at 20 °C (Fang et al., 2007). The lowest turbidity (20.0 NTU) was determined in the juice clarified using 0.075 g/L xanthan gum at 4 °C for 16 h. The turbidity value of this juice was significantly different than those of the pomegranate juices clarified with other agents ($p < 0.05$). However, although 12 preliminary tests for the clarification with xanthan gum were carried out, turbidity value lower than 20 NTU could not have been reached.

After incubation, the flocks were removed from all the clarified juice samples by filtration through a coarse filter paper.

2.3. Compositional analysis

The total soluble content (°Brix) of pomegranate juice samples was determined by an automatic digital refractometer (Atago Rx-7000α, Tokyo, Japan). pH was measured potentiometrically with