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Research note

Effect of pasteurization process and storage on color and shelf-life of pomegranate juices

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ABSTRACT

The effects of two heat processes LTP (low-temperature pasteurization: 65 °C, 30 s) and HTP (high-temperature pasteurization: 90 °C, 5 s) on color quality of pasteurized cloudy and clarified or centrifuged pomegranate juices were evaluated during prolonged storage at room temperature (25 °C) and under refrigeration (5 °C). Both heat treatments combined with refrigeration prevented microbial growth for 120 days. Although processing and storage of pomegranate juice had a decisive impact on the degradation of anthocyanin compounds and the consequent formation of brown pigments, storage temperature was the main factor affecting both browning index (BI) and red color loss in pasteurized pomegranate juices. Samples stored at 5 °C had a lower and slower loss of red color than those stored at 25 °C. Results showed that BIs increased rapidly with time in juices stored at 25 °C, being not acceptable (>1.00) after 7 days. The juices stored at 5 °C showed less browning regardless of pasteurization treatment they were subjected. In particular LTP-treated cloudy and clarified juices stored at 5 °C for 90 days exhibited BI values of 0.93 and 0.85, respectively.

1. Introduction

Interest in pomegranate (*Punica granatum* L.) juice and its products has increased markedly in recent years with a growing number of reports on their potential health benefits, which has placed them in the first line of functional juice market. Pomegranate juice consumption has been associated with inhibition of prostrate cancer in men (Pantuck et al., 2006), reduction in serum oxidative stress in plasma of type-2 diabetes mellitus patients (Rosenblat, Hayek, & Aviram, 2006), a reduced atherosclerosis in diabetic patients (Rock et al., 2008), reduction in blood pressure and low density lipoprotein (LDL) oxidation (Aviram et al., 2004), anti-HIV-1 activity (Neurath, Strick, Li, & Debnath, 2005), and potential protection against colon cancer (Kasimsetty et al., 2010). These beneficial effects of the pomegranate juices were attributed to the antioxidative properties of pomegranate polyphenols anthocyanins and hydrolyzable tannins (such as punicalagins, punicalin, pedunculagin, gallagic and ellagic acid esters of glucose) (Mena, Gironés-Vilaplana, Moreno, & García-Viguera, 2011). Processing and pasteurization conditions play an important role in the color, flavor, texture and antioxidant capacity of juice (Hernández, Melgarejo, Tomás-Barberán, & Artés, 1999).

Color is one of the most important attributes of food and beverages. It is the first thing that consumers notice on the shelves and it may invite or dissuade them in their choice of products (Nachay, 2009). In recent years there has been an increase in the consumption of red beverages, such as red orange, grape, berry and pomegranate juices, due to the healthy properties of the natural pigments present in these fruits, such as anthocyanins (Konczak & Zhang, 2004). Unfortunately, anthocyanins are unstable and susceptible to degradation, leading to a brownish color during processing and storage. The primary color deterioration in fruit juices containing anthocyanins occurs as a result of the degradation of monomeric anthocyanins, polymerization and the subsequent formation of brown pigments (Somers & Evans, 1986). These color changes strongly affect consumer behavior and result in a loss of marketability of processed pomegranate products.

Thermal processing is the most common method to extend shelf life of fruit juices by inactivating microorganisms and enzymes. Heating right after pressing inhibits native polyphenol oxidase (PPO) enzymes that cause brown color formation by oxidizing polyphenols (Skrede, Wrolstad, & Durst, 2000). However,

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conventional heat treatments often lead to detrimental changes in sensory and nutritive properties of juices. Although researchers have optimized time/temperature profiles to minimize the effects derived from the exposure of some foods to heat, the optimization of time/temperature pasteurization treatment is still a great challenge for the incipient processing industry of pomegranate juice.

The aim of the present work was to evaluate and compare the effects of two heat pasteurization processes on inactivation of naturally occurring microorganisms and color deterioration in pomegranate juices during prolonged storage at different temperatures.

2. Materials and methods

2.1. Juice extraction

Second quality pomegranate 'Mollar' fruits, harvested in autumn of 2010 when fully ripened, were provided by Cambayas Coop. V. (Elche, Alicante, Spain). Pomegranates were cut in halves and arils were hand-separated from the pith. Juice was immediately 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 at 2700*g* for 10 min using an Allegra™ 25R Centrifuge (Beckman Coulter Inc., Brea, California, USA).

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). Replicate samples of raw and heat-treated juice contained in screw cap 20 mL polypropylene containers were stored in cooled incubators MIR-153 (Sanyo Electric Co., Ltd., Gunma, Japan) at 25 and 5 °C for 45 and 120 days, respectively.

2.3. Microbiological analysis

Initial microbial levels of cloudy and clarified pomegranate juices before heat treatment were determined by total aerobic plate counts (APCs), using the spread-plate technique. Samples (1 mL) were serially diluted in buffered peptone water (PW; Scharlau Chemie, S.A., Barcelona, Spain) and then 0.1 mL volumes of appropriate dilutions were spread-plated onto duplicate plates of plate count agar (PCA; Scharlau Chemie, S.A.), using a sterile bent glass rod. Plates were incubated a 30 °C for 24–96 h.

2.4. Shelf-life study

Replicate samples of raw and heat-treated pomegranate juice were analyzed for number of microorganisms after 0, 7, 14, 21, 28, 45, 60, 75, 90 and 120 days of storage. From each agitated processed sample, 0.1 mL volumes were taken at each time period and total APCs were performed as previously described. For thermally untreated samples, serial PW dilutions were necessarily made.

2.5. Color measurement

Color intensity of pomegranate juices can be determined by taking into consideration its absorbance values at 520 nm (A_{520}), the wavelength of maximum absorbance of the present monomeric anthocyanins, which give the juice the characteristic red color. Hence, the color of pomegranate juices was determined by reading the A_{520} parameter, using a microplate reader Spectrostar Omega (BMG LABTECH GmbH, Offenburg, Germany). The pomegranate juices were previously diluted (1:2) with distilled water and then filtered through a 0.45 μm nylon filter.

Total color density (TCD) was expressed as total absorbance values of the brown compounds, which show maximum absorbance at 420 nm and absorbance of juice that gives its maximum at 533 nm (Alper, Bahçeci, & Acar, 2005). The pomegranate juices were diluted with distilled water for each test in order to obtain an absorbance below 1.0 measured at 533 nm. TCD was estimated by Eq. (1), where DF was the dilution factor.

$$\mathrm{TCD} = [(A_{420} + A_{533}) - 2(A_{700})] \cdot \mathrm{DF} \quad (1)$$

2.6. Browning index

Browning index (BI) [expressed as the absorbance ratio at 430 nm by that at 520 nm, according to Malien-Aubert, Dangles, and Amiot (2001)] of water diluted (1:2) pomegranate juices was determined using a microplate reader Spectrostar Omega.

2.7. Determination of pH

The pH of the pomegranate juices samples was measured with a pH-meter GLP 21 (Crison Instruments S.A., Barcelona, Spain).

2.8. Statistical analysis

Treatments were performed in triplicate, and all the parameters studied were also determined in triplicate for each storage temperature and time period. Statgraphics® Plus for Windows 3.0 (Statistical Graphic Corp. and Graphic Software Systems Inc., Rockville, Maryland, USA) was used for Statistical Analysis.

3. Results

3.1. Microbial inactivation

APCs of cloudy and clarified pomegranate juices before and after pasteurization treatments were 5.59 ± 0.13 and 5.23 ± 0.04 log colony forming units (CFU)/mL, respectively. The reduction in number of endogenous microorganisms in the clarified pomegranate juice obtained by centrifugation was not statistically significant. Microbiological analysis of the heat-treated clarified juices indicated that both pasteurization treatments were sufficiently effective to decrease the APCs to a level below the detection limit, i.e. no CFU was observed just after treatment. For the heat-treated cloudy juices, microbial counts were almost nil or negligible (≤1.00 ± 0.00 log CFU/mL) after pasteurization processes.

3.2. Shelf-life study and pH analysis

After 7 days storage of untreated pomegranate juice samples at 25 °C, very high levels (≥7.65 log CFU/mL) of microbial growth were observed (Table 1). Microbial count in LTP-treated cloudy pomegranate juice increased up to 3.0 log CFU/mL after 45 days storage at 25 °C. In contrast, microbial growth was negligible in HTP-treated cloudy juice under the same storage conditions. Also, negligible or nil counts were obtained in both LTP- and HTP-treated clarified pomegranate juices.

The increase of microbial populations in pomegranate juices under refrigeration conditions is shown in Table 2. Microbial counts in untreated cloudy and clarified pomegranate juices increased up to 6.65 log CFU/mL after 21 days storage at 5 °C. On the contrary, microbial growth in heat-treated juice was almost nil