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The effect of pectinase and protease treatment on turbidity and on haze active molecules in pomegranate juice

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ABSTRACT

Juice clarification strategies have been developed in order to optimize the visual appearance of juice which strongly affects consumer behaviour. Clarity and color during post-bottling storage are important quality factors. This study examined the effects of pectinolytic and/or proteolytic clarification treatment on turbidity and on haze active molecules in pomegranate juice.

A significant and synergic effect of the combined use of pectinase and protease enzymes was demonstrated and the best results in terms of turbidity levels of juice and potential haze formation were obtained. The data indicated that although pectinolytic and proteolytic treatments did not modify the total amount of pectins, proteins and phenols, they affected the haze forming activity of turbidity-causing molecules. It is important to note that enzymatic treatments of this kind did not modify anthocyanin composition and juice color. Therefore, this work contributes to improve the pomegranate juice processing in order to enhance quality attributes.

1. Introduction

In the last decade much attention has been paid to pomegranate fruit and juice due to the biological properties and health benefits of the large amount of polyphenols it contains, such as punicalin, punicalagin, hydrolyzable tannins and anthocyanins (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000; Mousavinejad, Emam-Djomeh, Rezaei, Hossein, & Khodaparast, 2009; Seeram et al., 2005). Nowadays, there is an increasing demand for pomegranate juice worldwide which has consequently led to an increase in the production of pomegranate fruits (Tehranifara, Zarei, Nemati, Esfandiyaria, & Vazifeshenas, 2010; Turfan, Türkyılmaz, Yemiş, & Özkan, 2011). Conventional processing of pomegranate fruit into juice is laborious and involves several steps such as fruit washing, cleaning (removal of peel and separation of arils), pressing, clarification, pasteurization and filtration. As for other types of clear juice production, clarification is a fundamental step of the pomegranate juice process in order to eliminate substances responsible for turbidity in the fresh juice and to avoid the development of turbidity during storage, usually known as haze formation (Cassano, Conidi, & Drioli, 2011; Mirsaeedghazi, Emam-Djomeh, Mousavi, Aroujalian, & Navidbakhsh, 2010; Vardin & Fenercioglu, 2003).

Removing these particles is a critical industrial problem that improves clarity as well as quality and stability of color. The fruit juice industry has investigated various methods to solve this problem since consumer choice is mainly driven by the visual appearance of juice (Costell, Tárrega, & Bayarri, 2010).

Nowadays, conventional clarification procedures rely on the hydrolysis of pectin and starch with pectinases and amylases, respectively; clarifying agents, such as bentonite, gelatin or silica-sol to induce the physico-chemical precipitation of sediments and/or haze-active components and filtration or centrifugation processes (Mirsaeedghazi et al., 2010; Pinelo, Zeuner, & Meyer, 2010; Rinaldi et al., 2013).

In particular, pectinases remove immediate turbidity. Any depectinizing action has two effects: to degrade the viscous soluble pectins and to induce the aggregation of cloud particles. Pectin forms a protective coat around suspended proteins and carries a negative charge in acidic environments which causes them to repel each other. Pectinases degrade the chain of pectins, thus exposing positively-charged proteins. The electrostatic repulsion between the cloud particles is thereby reduced so that they aggregate together (Kashyap, Vohra, Chopra, & Tewari, 2001; Sorrivas, Genovese, & Lozano, 2006).

Moreover, in beverages that are initially free of turbidity, it is

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assumed that the haze formation is caused by interactions between proteins and polyphenols (Siebert, 2006). Proteases break down haze-active proteins, thus preventing protein-phenol interaction and reducing turbidity level without removing phenolics (Pinelo et al., 2010).

There is an increase in interest towards pectolytic enzymes as clarifying agents (Sandri, Lorenzoni, Fontana, & de Silverira 2013), also in the immobilized form (Esawy, Gamala, Kamel, Ismail, & Abdel-Fattah, 2013), yet few studies have so far relied on proteases to clarify fruit juices (Landbo, Pinelo, Vikbjerg, Let, & Meyer, 2006; Meyer, Köser, & Adler-Nissen, 2001). Recently, Pinelo et al. (2010) and Landbo et al. (2006) found that treatment with acid protease improved clarity and had a haze-retarding effect on cherry and black currant juice, respectively.

The aim of this study was to enhance the colloidal stability of pomegranate juice with tailored enzymatic (protease and pectinase) treatments, by examining pectins, proteins and phenol amount and their haze forming activity.

To date, few studies have reported changes in anthocyanins and color of pomegranate juice during the clarification process (Turfan et al., 2011; Turfan, Türkyılmaz, Yemiş, & Özkan, 2012; Vardin & Fenercioglu, 2003), and no data have been published regarding the influence of the enzymatic clarification treatment on the anthocyanin level and chromatic parameters of the juice.

To our knowledge, this is the first study on pomegranate juice, which highlights the individual and interactive effects of pectinase, protease, pectinase-protease treatments on turbidity and haze formation during cold storage, as well as on haze-active molecule content and color stability.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteau reagent, tannic acid, gelatin, bovine serum albumin (BSA) and the other chemicals used for analytical purposes were purchased from Sigma Aldrich (Milan, Italy).

Klerzyme 150 pectinase preparation (A) from *Aspergillus niger* was purchased from DSM company (Barcelona, Spain), while the two native plant cysteine proteases, bromelain (B_1) and papain (B_2) were purchased from Sigma Aldrich (Milan, Italy).

2.2. Pomegranate juice preparation

Pomegranate juice was obtained from fresh pomegranates using a laboratory-type press, avoiding the seeds crushing. Before juice extraction, the pomegranates were washed and drained then cut into two pieces and processed into juice. The pomegranate juice was then divided into equal parts (325 mL in triplicate) in test tubes and was subjected to various experimental enzymatic clarification treatments as follows: (Control) no added enzyme preparation, (A) pectinase, (B_1) bromelain, (B_2) papain, (AB_1) pectinase and bromelain, (AB_2) pectinase and papain, (AB_1B_2) pectinase with both proteases, according to Table 1. The tubes with samples were then shaken and placed in a water bath at 50 °C for exactly 2 h. Following the enzymatic clarification treatment, the samples were immediately heated to 85 °C for 1 min in order to inactivate the enzyme. All juice samples were centrifuged at 15,000 rpm for 10 min before being placed in darkness in cold storage at 4 °C for up to 14 days. During processing, pH, tritable acidity and soluble solid content (°Brix) of pomegranate juice were monitored. pH was measured potentiometrically with a Mettler Toledo pH meter (Steroglass, Perugia, Italy). Tritable acidity was determined as g anhydrous citric acid/L of juice by tritating 10 mL of pomegranate juice with NaOH 0.1 M reaching pH 8.1 and °Brix measurements were carried out at 20 °C with a digital refractometer HI 96801 (Hanna Instruments, Milan, Italy). The pH, tritable acidity and soluble solid content of juice samples ranged from 2.97 to 3.05, 15.60–15.68 g/L (as anhydrous citric acid) and 14.73 to 14.85 °Brix, respectively, thus indicating that enzymatic treatment did not affect the amounts of organic acid and sugar contained in the pomegranate juice. Moreover, several samples were withdrawn from any tube at various storage times (0, 1, 7, 14 days) and assayed to quantify the amount of pectins, proteins and phenols.

Table 1
Enzyme preparation dosage ($g_{protein}$/L of juice).

Enzyme	Clarification treatment						
	Control	A	B_1	B_2	AB_1	AB_2	AB_1B_2
Klerzyme 150	–	0.02	–	–	0.02	0.02	0.02
Bromelain	–	–	0.02	–	0.02	–	0.02
Papain	–	–	–	0.02	–	0.02	0.02

'–' No added enzyme preparation.

2.3. Turbidity measurement and heat stability test

The turbidity of the juice was measured with a HD 25.2 turbidimeter (Delta Hom, Padua, Italy) and expressed in nephelometric turbidity units (NTU). The immediate turbidity was measured immediately after the clarification treatment (day 0) and the same measurement was performed after 1, 7, and 14 days of cold storage at 4 °C.

The potential turbidity of pomegranate juice was determined by heat test: juice samples were incubated at 80 °C for 6 h and then kept at 4 °C for 16 h (Vincenzi, Marangon, Tolin, & Curioni, 2011). Haze formation was measured after equilibration at room temperature (approximately 25 °C).

2.4. Induction of haze in pomegranate juice

Haze forming activity was assessed by adding a known amount of gelatin (3 g/L) or tannic acid (2.5 g/L) to the treated juice following 14 days of storage. All the samples were incubated for 30 min in a water bath at 25 °C and haze determinations were carried out as reported above. The corresponding turbidity indicates the relative amounts of haze-active (HA) phenols and HA proteins in the pomegranate juice, after adding the enzyme (Siebert, Carrasco, & Lynn, 1996).

2.5. Determination of pectin content

The total pectin content was determined with the K-PECID 11/11 kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The pectate is incubated with pectate lyase, which cleaves the polygalacturonic acid, releasing unsaturated oligosaccharides, which absorb strongly at 235 nm. From the increase in absorbance (ΔA) the amount of unsaturated product produced can be calculated as:

$$\text{Unsaturated product} = \Delta A/L \times \varepsilon$$

where:

ΔA = Reaction Absorbance (after 30 min) − Blank Absorbance.
L = path length of the reaction cuvette (=1 cm).
ε = the molar extinction coefficient of the reaction product (4600 M^{-1} cm^{-1}).