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Optimization of pectinase and protease clarification treatment of pomegranate juice

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ABSTRACT

Clarification is a fundamental step in the processing of pomegranate juice to improve its appearance and marketability. The objective of this work was to develop a tailored pectinolytic and proteolytic enzymatic clarification process for pomegranate juice. Response surface methodology was used to analyse the effects of incubation time (30–120 min), temperature (25–50 °C) and complex enzyme amount (0.1–0.4 g/100 g of juice) on physical characteristics of pomegranate juice. R^2 and *adjusted* R^2 for chill haze, turbidity, potential turbidity and clarity regression models were greater than 0.9 and 0.8, respectively. The complex enzyme amount was the most important factor influencing the clarification of pomegranate juice. Moreover, it was found that an increase of the complex enzyme amount caused a substantial loss of protein and phenol haze forming activity. The optimum enzymatic treatment conditions were: temperature 25–30 °C, time 100–110 min, protease-pectinase complex enzyme amount (the ratio of protease:pectinase was 1:2) 0.22–0.25 g/100 g of pomegranate juice.

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1. Introduction

Pomegranate fruit juice has come to be regarded as an important functional and healthy drink all over the world (Viuda-Martos, Fernandez-Lopez, & Perez-Alvarez, 2010; Fadavi, Barzegar, & Azizi, 2006). The current interest and consequently the increasing market demand by consumers are supported by its antioxidant properties (Kalaycıoğlu & Erim, 2016). Moreover, it has also been revealed that pomegranate fruit contains anti-carcinogenic (Bell & Hawthorne, 2008), antimicrobial (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007) and anti-artherosclerotic compounds (Aviram et al., 2004). Tannins and anthocyanins are responsible for the higher antioxidant activity of the juice (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000). However, the same molecules contribute to the formation of the undesirable haze and sediment in the bottle during storage, which are often wrongly perceived as deterioration of quality (Mirzaaghaei, Goli, & Fathi, 2016; Onsekizoglu, 2013; Vardin & Fenercioglu, 2003). Thus, clarification could be considered as a necessary step in the processing of fruit juice to improve its appearance and marketability. This is even more so due to the fact that pomegranate juice shows excessive turbidity (Erkan-Koç, Türkyılmaz, Yemiş, & Özkan, 2015; Mirzaaghaei et al., 2016). Conventional clarification methods involve simple filtration and centrifugation (to achieve a clarified but not stabilized juice), fining agents such as bentonite, gelatine and silica sol (Yousefnezhad, Mirsaeedghazi, & Arabhosseini, 2016; Mirzaaghaei et al., 2016; Erkan-Koç et al., 2015). These latter compounds could produce an acceptable clarified and stabilized juice, however these also lead to a substantial loss of phenolic compounds and antioxidant activity (Erkan-Koç et al., 2015; Bagci, 2014). An alternative approach to the fining treatment would be a more targeted enzymatic treatment that can be considered as an advanced step in the industrial production of pomegranate juice. Several studies reported on the depectinization of fruit juices with the aim to increase its clarity (Ghosh, Pradhan, & Mishra, 2016; Pinelo, Zeuner, & Meyer, 2010). As demonstrated by Cerreti, Liburdi, Benucci, and Esti (2016), turbidity in pomegranate juice can be removed by pectinase-catalyzed electrostatic destabilization of suspended cloud-causing pectin particles, and by modification of haze active protein-polyphenol complexes via enzyme catalysis using proteases without altering the anthocyanin composition and juice colour. A significant and synergistic effect of the combined use of pectinase and protease enzymes in terms of turbidity and potential haze formation of the pomegranate juice was revealed (Cerreti et al.,

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2016). This result motivated us to investigate and find a new optimized enzymatic procedure to clarify pomegranate juice. In fruit juice processing, the rate of enzymatic hydrolysis depends on several physical-chemical factors such as incubation time, temperature and enzyme concentration (Chen, Xu, Qin, Ma, & Zheng, 2012; NuŕAliaa, Siti Mazlina, Taip, & Liew Abdullah, 2010). Any optimization procedure implies improving the performance of a process in order to obtain the maximum benefit from it. Traditionally, in order to optimize the operating conditions in a process, the influence of one factor at a time on an experimental response was investigated, while all other factors were kept constant. This optimization technique (one-variable-at-a-time) showed various disadvantages: it did not include interactive effects among the variables, it did not depict the complete effects of the parameter on the response, and it required a large number of experiments and consequently led to an increase of time and expense as well as an increase in the consumption of reagents and materials.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques which are advantageous towards overcoming the above-mentioned problems (Khuri & Mukhopadhyay, 2010; Myers & Montgomery, 2002). RSM has been widely applied and used for developing, improving and optimizing food industry processing, in which a response is influenced by several variables and the objective is to optimize this response (Ahdno & Jafarizadeh-Malmiri, 2016; Ghosh et al., 2016; Tastan & Baysal, 2015).

To our knowledge the effects of incubation time, temperature and enzyme concentration on chill and permanent haze, heat turbidity potential and clarity of pomegranate juice and their further optimization have not been previously reported. In this regard, the objective of this work was to establish the optimal enzymatic treatment conditions to clarify pomegranate juice using RSM. Different clarification parameters such as time, temperature and protease-pectinase complex enzyme amounts were studied, as well as the effect of protease-pectinase treatment on haze active proteins and phenols present in the juice.

2. Materials and methods

2.1. Materials

2.1.1. Fruits

Fresh pomegranate (*Punica granatum* L., var Wonderfull) were provided by Fruttaweb.com (Bologna, Italy). Fruits were stored at 4 °C until use.

2.1.2. Enzymes and other chemicals

Native plant cysteine protease, papain from papaya latex (A) was purchased from Sigma Aldrich (Milan, Italy), while Klerzyme 150 pectinase preparation (B) from *Aspergillus niger* was purchased from DSM (Barcelona, Spain). The powder of papain was solubilized to give the same final concentration of protein as the Klerzyme 150 liquid preparation (8 g/L). All other reagents used in this study were purchased from Sigma Aldrich (Milan, Italy) and were of analytical grade.

2.2. Methods

2.2.1. Pomegranate juice process

Fresh fruits were selected, washed manually to eliminate any particles and drained. Then, pomegranate fruits were cut into two pieces and processed into juice with a laboratory-type press. The pH, titratable acidity and soluble solids content of juice obtained were 3.3, 16.3 g/L (as anhydrous citric acid) and 15.2 °Brix, respectively.

The pomegranate juice obtained was divided into equal parts in test tubes and subjected to different clarification treatments, firstly to investigate the optimal ratio of protease and pectinase in the complex enzyme solution and then to further optimize the clarification conditions of pomegranate juice: time, temperature and concentration of complex enzyme preparation. The temperature of each clarification treatment was adjusted to the desired level using a thermostat bath (Kottermann, Hänigsen, Germany). At the end of each enzymatic treatment, the juice was heated at 85 °C for 2 min to inactivate the enzymes and then centrifuged at 15,000 rpm for 15 min. The supernatant was filtered through PES membrane filter with a pore size of 0.45 μm and placed in dark cold storage at 4 °C until further analysis.

2.2.2. Orthogonal test design to optimize protease:pectinase ratio mixture

An orthogonal L9 $(3)^4$ test design was applied to investigate the optimal papain:pectinase ratio in the complex enzyme preparation. As seen from Table 1, nine experiments were conducted with 2 factors and 3 levels. The samples were shaken and placed in a water bath at 50 °C for exactly 2 h. Following the enzymatic treatment each sample was treated as reported above. The turbidity level results were determined after 1 d of cold storage (4 °C) and for up to 21 d.

2.2.3. Optimization of pomegranate clarification conditions

Optimization of the process conditions for the enzymatic clarification of pomegranate juice was performed with RSM. Experimental design and statistical analysis were performed using Minitab 17 software (Minitab Inc., State College, PA, USA). Central composite design (CCD) was employed to study the combined effect of three independent variables namely incubation time, temperature and complex enzyme amount, coded as x_1, x_2, and x_3,

Table 1
Orthogonal experiment design using protease (A), papain from papaya latex, and pectinase (B) Klerzyme 150.

No.	(A) Papain concentration (g/100 g)	(B) Pectinase concentration (g/100 g)	Turbidity (NTU)
1	0 (0)	0 (0)	18.3
2	0	1 (0.125)	14
3	0	2 (0.25)	11.5
4	1 (0.125)	0	11.3
5	1	1	11
6	1	2	10.3
7	2 (0.25)	0	25
8	2	1	11
9	2	2	10
k_1	−23.13	−24.76	
k_2	−20.72	−21.53	
k_3	−22.93	−20.49	
R	2.41	4.27	

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