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Clarification of pomegranate juice with chitosan: Changes on quality characteristics during storage

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ABSTRACT

In this study, for the first time, the use of chitosan as a clarifying agent in the production of clear pomegranate juice was evaluated and its effects on quality characteristics of juice were investigated. A central composite face centered design was used to establish the optimum conditions for clarification of pomegranate juice (PJ) using response surface methodology. The three factors were concentration of chitosan (10–120 mg/100 ml), process temperature (10–20 °C), and process time (30–90 min) and their effects on turbidity and a^* values were investigated. Using a desirability function method, the optimum process conditions were found to be 68.93 mg/100 ml chitosan at a process temperature and time of 10 °C and 30 min, respectively. PJ was produced using the optimum conditions and the quality characteristics such as turbidity, colour characteristics (L^*, a^*, b^*, C^*), titratable acidity, total phenolic, monomeric anthocyanin, and protein contents were evaluated during storage at 4 and 20 °C for 6 months.

1. Introduction

Pomegranate (*Punica granatum* L.) is one of the important fruits grown in Turkey, Iran, USA, Middle East, Mediterranean, and North Africa. The edible part of the fruit contains a considerable amount of organic acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (Al-Maiman & Ahmad, 2002; Vardin & Fenercioglu, 2003).

There is currently much interest in the pomegranate fruit and juice because of the high content of phenolic compounds, such as catechins, ellagic tannins, and anthocyanins. Anthocyanins are responsible for the bright red colour of pomegranate juice (PJ) and this red colour is one of the major quality parameters of PJ that affects consumer sensory acceptance (Alighourchi & Barzegar, 2009; Borochov-Neori et al., 2009). Additionally, anthocyanins are also responsible for the appearance, astringency, and bitterness of fruit juices (Adhami & Mukhtar, 2006; Alper, Bahceci, & Acar, 2005).

PJ have become the topic of intense research due to their health benefits. In the past decade, numerous studies showed that the antioxidant activity of PJ was higher than the other fruit juices and beverages. Furthermore, it was proposed that PJ consumption is helpful against different pathologies such as cancer, Alzheimer's disease and heart disease. In this sense, PJ decreases cholesterol and low density lipoprotein (LDL) levels while increasing high density lipoprotein (HDL) levels, and the PSA (prostate specific antigen) (Cassano, Conidi, & Drioli, 2011; Shema-Didi, Kristal, Sela, Geron, & Ore, 2014; Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009; Wang & Martins-Green, 2014).

Clarification is an important step in the processing of fruit juice mainly in order to remove pectin and other carbonhydrates which are present in the juice. Generally, conventional clarifying procedures can be achieved by centrifugation, enzymatic treatment,

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applying clarifying agents such as gelatin, bentonite, silica sol, polyvinly pyrrolidone or a combination of these compounds. Clarity and homogeneity are the two important characteristics of clarified products, and are ensured by the complete removal of all suspended solids (Sin, Yusof, Hamid, & Rahman, 2006).

Chitosan (deacetylated chitin) is a nontoxic and biodegradable compound and due to its polycationic in nature, has been found to be an effective coagulating agent. Chitosan coagulates the suspended particles and thus helps their separation from beverages (Chatterjee, Chatterjee, Chatterjee, & Guha, 2004). Successful application of chitosan as clarification agent for apple, grape, lemon, and passion fruit juices have been reported in literature (Chatterjee et al., 2004; Domingues, Junior, Silva, Cardoso, & Reis, 2011).

Response Surface Methodology (RSM) is an effective statistical technique to optimize complex processes. The major advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. For this reason, it is less laborious and time-consuming than other approaches used to optimize a process (Zhong & Wang, 2010). RSM has been frequently used in the optimization of clarification processes in the food industry (Abdullah, Sulaiman, Aroua, & Noor, 2007; Domingues et al., 2011; Lee, Yusof, Hamid, & Baharin, 2006).

Although chitosan applications have been evaluated for other purposes in the food industry, at the best of our knowledge, there are no studies reported in literature about optimizing conditions for clarification of PJs with chitosan using RSM. Furthermore, a study for determining the optimization parameters for clarification using chitosan can provide new opportunities to enhance this technique in the juice processing industry. In the current study, the main objectives were (1) to determine the effects of the concentration of chitosan, process temperature, and process time on the turbidity and a^* values of juice, and optimize the clarification process using chitosan of PJ by RSM, (2) to compare the quality characteristics of PJ produced with traditional clarification methods during storage at 4 and 20 °C for 6 months.

2. Materials and methods

2.1. Materials

Pomegranates (*P. granatum* L., var. Hicaznar) and traditional clarification agents (bentonite and gelatin) were provided by ASYA Fruit Juice and Food Ind. Inc. (Isparta, Turkey). Commercial water soluble chitosan (Carboxymethyl chitosan, CAS No. 83512-85-0) (deacetylation degree of 90–95%, medium molecular weight and food grade) was purchased from Qingdao Reach International Inc. Company (China). Pomegranates were stored in a refrigerator at +4 °C and 85–90% humidity for a maximum of 24 h before processing.

2.2. Methods

2.2.1. Processing methods

2.2.1.1. Preliminary tests. Initial chitosan concentration range used in preliminary trials were chosen according to literature (Chatterjee et al., 2004; Domingues et al., 2011; Rungsardthong, Wongvuttanakul, Kongpien, & Chotiwaranon, 2006) and were between 10 and 250 mg/100 ml. The chitosan added PJ was stirred at 20 °C and the turbidity were determined. After obtaining the minimum turbidity value of samples at the chitosan concentration of 100 mg/100 ml, effect of temperature on clarification was investigated especially that of cold clarification (below 20 °C) (Cemeroglu, 2009) and process temperatures of 10, 15, and 20 °C were tested. Finally process time was optimized under constant temperature and chitosan concentration and the turbidity values were measured with 30 minute intervals. After determining ranges of process variables by preliminary experiments, a trial design was formed for the production of clear PJ.

2.2.1.2. Pomegranate juice extraction. A process flow sheet is shown in Fig. 1. Pomegranates were washed with water and separated from husk and fruit pericarp manually. Juice sacs were placed in a muslin cloth and pressed using a laboratory press (Karl Kolb, D-6072, Germany). Raw PJ (16°Bx, pH 3.35) was centrifuged (Hettich, 320 r, Germany) at 4000 rpm for 5 min. Since pomegranates contain an insignificant amount of pectin (Cemeroglu, 1977), PJ samples were not depectinized. In addition, this information was confirmed by analysis of pectin content in PJ (Section 2.2.2.3). After centrifugation, cloudy juices were aliquoted into two groups and were clarified using chitosan (CH) or with traditional method (T). Industrial clarification methods were chosen as the traditional method and the traditional clarification agents bentonite and gelatin (A type, 80–100 Bloom strength) were used at a concentration of 90 mg/100 ml and 17 mg/100 ml fruit juice, respectively. The dosage of bentonite and gelatin were selected according to clarification process of pomegranate juice applied in the fruit juice industry. Traditional clarification was carried out at room temperature, first bentonite and then gelatin were added to raw PJ, followed by continuous mixing in a magnetic stirrer (Wise Stir, MSH-20A, Germany) for 5 min. At the end of a process time of 30 min, after clarification, the fruit juices were filtered with a plate filter (Seitz enzinger noll, 6136 D-6800, Mannheim). The juice samples were then transferred into 200 ml hermetically capped glass bottles, and pasteurization was carried out in a pasteuriser at 95 °C for 1 min. The juice samples were then immediately cooled to room temperature.

For clarification of PJ using chitosan, the optimum concentration obtained from RSM was used (68.93 mg/100 ml). Chitosan was added to the PJ supernatant after centrifugation and mixed as described above. Finally PJ were filtered, pasteurized in glass bottles and cooled as described above and in Fig. 1.

Quality characteristics such as turbidity, colour, pH, titratable acidity, total soluble solids, total phenolic, monomeric anthocyanin,

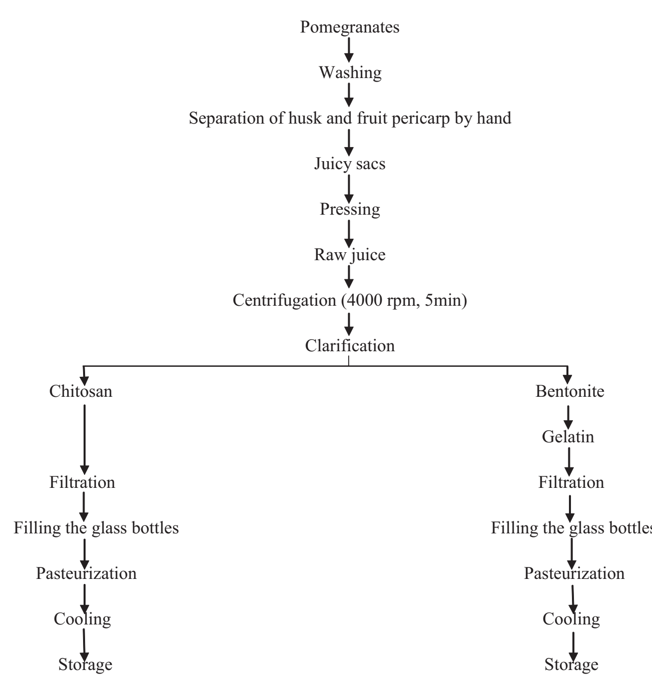

Fig. 1. Clear pomegranate juice production.