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Phenolic compounds extraction from Iranian pomegranate *(Punica granatum)* industrial waste applicable to pilot plant scale

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ABSTRACT

Industrial pomegranate waste (IPW) derived from the commercial processing of about 0.35 MMT fresh pomegranate is commonly disposed of at the Iranian landfills at a high cost and risk to environment. This paper describes a successful utilization of process condition optimization and modeling of the solvent extraction of phenolic compounds from IPW with preserved high antiradical and/or antioxidant activity. They are value added products with potential commercial application in food industries and preventive medicine due to their well-known beneficial health effects.

Process conditions to maximize the total phenolic content (TPC), hydrolysable tannins (HT), total flavonoids (TF), radical scavenging activity (assessed as DPPH%) and ferric reducing antioxidant power (FRAP), were considered as optimal extraction conditions. HT as major constituents of TPC (about 50%) were also characterized and quantified by LC/MASS analysis.

The optimum operating conditions were an ethanol concentration of 60%, a solvent to dry waste ratio of 30 mL/g, the temperature of 46°C and the time of 6 h, which were also successfully implemented in a pilot plant scale. The FRAP and the DPPH results correlated to the TPC and the HT, and were influenced mostly by the solvent to dry waste ratio, followed by the ethanol composition.

Other objectives considered in the present study included reductions in the process steps and operating costs, the use of an environmental friendly extraction solvent and its recovery, and the minimization of waste.

1. Introduction

An increasing demand for environmentally compatible production processes, coupled with rising operational and waste treatment costs, has led the food industries to focus on the waste reduction to reduce costs and provide new sources of income. Therefore, the implementation of new and advanced integrated waste preventive or recovery approaches for valuable natural by-products has become one of the most important objectives of the Iranian food and agricultural industries.

Recently the principles and five important ultimate goals of any applied technology for the recovery of valuable products from food and agricultural industrial wastes, has been reviewed. Maximizing the yield, avoiding any alteration and maintaining the food grade nature of the target compounds during and after the recovery processing, are amongst these goals (Galanakis, 2012).

Amongst these natural components, the significance of antioxidants (also known as radical scavengers or antiradical compounds) in preventive medicine as well as their beneficial health effects is well known. The antioxidant properties of phenolic compounds can preserve flavor and color while preventing vitamin destruction in foods (Ahmed et al., 2015; Devatkal and Naveena, 2010). Furthermore, they protect living systems from oxidative damage (Althunibat et al., 2010), protect cells from free radicals (Costantini et al., 2014), and prevent serious diseases (Al-Jarallah et al., 2013; Ammar et al., 2015; Anibal et al., 2013; Askari et al., 2014; Chrubasik-Hausmann et al., 2014). In this regard, numerous research teams have conducted investigations towards the development of methods for the extraction of antioxidants (Bertran et al., 2004; Costa et al., 2014; Diñeiro García et al., 2009; Kallel et al., 2014; Karacabey and Mazza, 2010; Santos et al., 2013).

According to International Trade Centre *UNCTAD*/WTO (*ITC*) statistics (Brinckmann, 2011), the total worldwide production of pomegranates is approximately 1.5-2 million metric tons (MMT)/annually, and the Iranian contribution is about 40% (above 0.75 MMT) of this production. In Iran, pomegranates are mostly consumed fresh and roughly 0.35 MMT are processed into juice, jams, syrup and sauce (Anonymous, 2009). According to research conducted on twenty Iranian pomegranate cultivars (Tehranifar et al., 2010), the edible

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portion (aril) of the fruit is about 38–65% of the total fruit weight and consists of about 27–47% juice and 53–73% seed. Therefore, a considerable amount of industrial pomegranate waste (including husk (75–80%) and seed (25–20%) on wet basis (w.b.)) are produced annually in Iran. This waste is mostly used as animal feed.

Predominant class of polyphenols in pomegranate fruit is hydrolysable tannins. These include gallotannins, derivatives of gallic acid, and ellagitannins such as punicalagin A and B isomers (Adams et al., 2006) including gallagic acid (ellagic aid with 2 gallic acid moieties) and hexahydroxydiphenoyl (HHDP)–linked glucose that yields gallagic acid and/or punicalin upon hydrolysis. These are mainly located in the fruit peel, mesocarp and aril, and are attributed to their strong radical scavenging and antioxidant ability (Fischer et al., 2011; Li et al., 2006). Although they are extracted into the juice upon commercial processing of the whole fruits, but considerable amounts still remain in the waste, which after commercial scale recycling may provide antioxidant rich health supplements (Ahmed et al., 2015; Çam et al., 2014; Devatkal and Naveena, 2010). Hydrolyzable tannins are considered as the most active antioxidants amongst the other groups of polyphenols in pomegranates. It is believed that these compounds act synergistically together, so their extraction, purification, and bioavailability have been the subject of intensive research over the last ten years (Fischer et al., 2013; Saad et al., 2012; Safavi et al., 2014; Seeram et al., 2007).

Due to the high reactivity of natural polyphenols, the potential for polyphenolic profile alteration during the extraction process exists. Therefore, an important challenge in any efficient extraction process is to concentrate the desired components with the preserved properties, which are the function of the process parameters such as sample particle size, solvent concentration and composition, solvent to dry waste ratio, temperature and time of extraction.

The effect of the polarity efficiencies of the solvents on the polyphenol extraction from the pomegranate peel has been studied using organic solvents such as acetone, ethyl acetate, methanol, and ethanol alone or in combination with water at defined extraction conditions (Wang et al., 2011). Hence, a combination of alcohol and water is more effective in extracting the phenolic compounds than alcohol or water alone (Markom et al., 2007). Due to the economic and safety merits, hydroethanol is considered as an environmentally friendly solvent for producing food grade antioxidants from PIW. Contact time and temperature are amongst the other process conditions to be optimized. It is well accepted that increasing temperatures promote extraction by, enhancing solubility of polyphenols and increasing the extraction coefficient. In contrast, the possibility of polyphenols degradation increases at high temperatures (Pinelo et al., 2005b; Spigno et al., 2007).

Decreasing of the processing time and improvements in heat and mass transfer as well as improvement in the final product quality are top of the list of the main challenges, which should be well considered in any technology to be applicable in a commercial scale. In this regards, inspiring researchers are urged to optimize the critical parameters in any appropriate technology chosen for the recovering of the value added compounds from industrial wastes (Galanakis, 2013; Galanakis and Schieber, 2014).

With reference to these criteria, the main objective of the present study was to identify the most suitable operating conditions for green antioxidants extraction for commercial purposes from one of the main pomegranate processing factories in Iran (i.e. Sanich Co.). Sanich uses the cultivars grown under the same geographical conditions (i.e. Markazi province area). The conventional optimization techniques, such as the one-factor-at-a-time (OFAT) procedure, is not only time consuming but also ignores the interaction of the variables (Liyana-Pathirana and Shahidi, 2005). Therefore, more reliable optimization techniques such as RSM have been adopted. In RSM, a collection of statistical and mathematical techniques are used for the evaluation and quantization of the effects of several process parameters and their interactions on response variables. It has been successfully used for developing, improving, and optimizing the extraction of phenolic compounds from a variety of natural products (Heydari Majd et al., 2014; Karacabey and Mazza, 2010; Liu et al., 2013; Morelli and Prado, 2012). To our best knowledge, there are a limited number of reports on the green, feasible, and economic extraction of these compounds from the waste of fruit processing industries for commercial purposes. Also, the optimization of effective variables has been ignored in the literature (Amyrgialaki et al., 2014; Pan et al., 2012). Moreover the structural composition alterations and quantification of the main group of pomegranate polyphenol (i.e., hydrolysable tannins) after large scale extraction process has not been reported so far. The operating conditions are expressed in terms of green solvent composition, time and temperature of extraction, and solvent to dry waste ratio (as independent variables). To the best of our knowledge, there are no reports on the optimization of the four independent variables used in this study to maximize the total polyphenolic production while preserving the radical scavenging/antioxidant properties (as response variables). A 3^4-full factorial design and RSM were employed, which led to the development of a feasible and flexible process that would be suitable for use on a commercial scale.

In present study in addition to the Folin-Ciocalteu method for quantifying the TPC, the hydrolysable tannins and the total flavonoides as two major classes of pomegranate polyphenolic compounds were also quantified spectrometrically. Amongst the latter compounds, the hydrolysable tannins in addition to hydroxy cinnamic and hydroxyl benzoic acids derivatives were also fractionated, characterized and quantified by SPE- HPLC-DAD/MASS analysis. Moreover, the Ferric Reducing Antioxidant Potential (FRAP) assay and the radical scavenging capacities using DPPH were also determined for the IPW extracts. The main ground for this approach was the potential interferences in various quantifying methods, stemmed from the heterogeneity (different reactivity) and the complex nature of natural polyphenolic extracts e.g pomegranate polyphenolic extracts (Singleton et al., 1999).

Antioxidant capacity assays were classified in two main groups of the "hydrogen atom transfer (HAT)" and the "electron transfer (ET)" reaction based analysis (Huang et al., 2005). According to this classification DPPH and FRAP methods are Hat- and ET- based assays, respectively. In the HAT-based assays the capacity of a compound (or mixed polyphenols in crude natural extracts) to scavenge free radicals by hydrogen atom donation and in the ET-based assays, its (or their) ability to reduce an oxidant compound are measured (Huang et al., 2005). The oxidant compound in FRAP assays is colorless Fe^{3+}(TPDZ) complex which turns to a blue-colored (Fe^{2+}(TPDZ)) complex after the reaction with electron-donating antioxidants. The degree of color change is attributed quantitatively to the concentration of antioxidants.

2. Materials and methods

2.1. Materials

Pomegranate waste including the peel, mesocarp, aril, and seeds was provided immediately after processing on November 2015 by the Sanich Co. (Iran). The waste was transferred within a few hours to a laboratory, dried in an air flow cabinet oven at 30°C for 48 h, and powdered in a hammer mill crusher. The ground material was consecutively passed through the sieves of 35, 60 and 120 mesh sizes and the fraction between 35 and 60 mesh sizes (a mean particle size of 0.25–0.50 mm) was collected for further processing. The sized material was placed in an opaque plastic bag and stored at room temperature (20-25°C) in a dry ventilated area until used. The water content of the waste powder was determined with an oven method by drying to a constant weight at 105°C; it was 11 g/kg.

The solvent absorption of the pulverized pomegranate waste was 3 mL/g, which was determined by adding 20 and 40 mL of solvent to 5 and 10 g of waste, respectively. After two hours, the samples were filtered and the filtrate was measured. The maximum amount of solvent absorbed by the powdered waste was approximately one-fifth of its