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From waste to wealth: High recovery of nutraceuticals from pomegranate seed waste using a green extraction process

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ABSTRACT

Waste pomegranate seed (WPS) from the pomegranate juice industry is an interesting substrate for the food processing industry as it contains high quality oil which is rich in conjugated fatty acids, high quality proteins and dietary fibres. However, the majority of the work reported extraction of only oil and employed organic solvents which decrease the nutritional quality of pomegranate seed protein and fibres. Therefore, in this study, a one pot enzymatic green process was investigated for recovery of high quality oil, food-grade proteins and fibres from WPS. The WPS were treated with protease followed by centrifugation to recover the oil, protein and insoluble fibres in different phases. The mechanism of extraction was confirmed by FTIR-imaging and scanning electron microscopy analysis of WPS. The highest oil recovery of 22.9% (out of which 97.4% in free form) and protein recovery of 13.2% (out of which 90.2% in free form) were obtained, when WPS was incubated with protease at a concentration of 50 U/g for 14 h, at 45 °C and pH 7.2. The remaining WPS residue was rich in insoluble fibres (97.6 g per 100 g WPS residue). The protease-derived oil had 2.3% higher content of conjugated fatty acids and 1.4 times higher total phenolic content than the hexane-extracted oil. Moreover, the protease-derived oil displayed 4% higher antioxidant activity than the hexane-extracted oil. The extracted free proteins were in protein hydrolysate form and had high values of the essential amino acid index (91.6%), protein efficiency ratio (5) and biological value (88.5) confirming their high quality. The insoluble fibre rich WPS residue possessed improved water and oil holding capacity, glucose absorption capacity and glucose dialysis retardation index compared to raw WPS.

1. Introduction

India is the world's largest pomegranate producer with 2.3 million tons produced in 2016 (NHB, 2016). In recent years, the production and consumption of processed pomegranate products, especially juice, have greatly increased throughout the world due to the health-promoting effect of different components of pomegranates. Pomegranate (*Punica granatum* L.) seed is a waste produced from the pomegranate juice industry (Kalaycıoğlu and Erim, 2017). After juice extraction, the waste pomegranate seeds (WPS) accounts for 10 wt% of the fruit most of which are not utilized (Abbasi et al., 2008). The WPS can be considered as a source of nutraceuticals such as high quality oil (12–24%) (Liu et al., 2012; Tian et al., 2013), protein (10–20%) (Elfalleh et al., 2012) and insoluble fibres (30–50% is cellulose and hemicellulose) (Uçar et al., 2009; Taher-Maddah et al., 2012). Pomegranate seed oil is a rich source of polyunsaturated fatty acids (PUFAs), especially the conjugated fatty acid punicic acid (18:3 n-5) with IUPAC name 9Z,11E,13Z-octadeca-9,11,13-trienoic acid due to which it possesses many health promoting effects (Aruna et al., 2016). Several studies have demonstrated that pomegranate seed oil displays variety of functional and medicinal effects, such as antioxidant and radical scavenging activity; anticancer activity against prostate cancer, breast cancer, colon cancer, skin cancer; neuroprotective activity against neurodegenerative diseases; antidiabetic and antiobesity property; nephroprotective effects against nephrotoxicity; protective effect against atherosclerosis; anti-inflammatory activity preventing intestinal damage and bone loss; modulating immune function, etc. (Aruna et al., 2016). Pomegranate seed proteins are rich in essential amino acids and meet WHO requirements of essential amino acids for adult humans (Elfalleh et al., 2012). Therefore, WPS proteins could be used as protein resource for human nutrition. As insoluble fibres are dietary fibres responsible for increasing fecal bulk, improving peristalsis, providing laxative effect and anti-hyperglycemic activity in diabetes (Maphosa and Jideani, 2016), WPS being rich in insoluble fibres could also be

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used as a source of dietary fibres. When the agro-industrial processing waste is rich in multiple bioactive compounds and nutraceuticals, it can be utlized as a feedstock for the extraction of these products which reduces the waste generation and pollutant loadings (Arshadi et al., 2016; Banerjee et al., 2017). This also provides an additional revenue to agro-processing industries contributing to their sustainable development and achievement of a circular economy based on zero waste (Clark et al., 2016; Mohan et al., 2016). However, the research carried out on WPS utilization has always focused on extraction of a single component, such as the oil and the remaining major WPS organic fraction, rich in proteins and insoluble fibres, would be still disposed of into the environment. In addition, the methods used for WPS oil extraction either yield less oil, employ toxic organic solvent or are not economical. For instance, cold pressing requires significant energy input and the WPS oil extraction yield is only 40–50% (Khoddami et al., 2014). Therefore, the yield loss and energy requirement in cold pressing can be a major factor in the overall cost consideration (Dominguez et al., 1994). Soxhlet extraction (Abbasi et al., 2008), ultrasound (Goula, 2013; Kalamara et al., 2015; Barizão et al., 2015) and microwave (Çavdar et al., 2017) assisted extraction employ organic solvent (mainly hexane) and obtained higher yields (95–99%) of WPS oil. However, because of the safety, environmental contamination and human health hazards associated with the use of hexane, the cost of construction and operating costs of hexane extraction facilities are high (Rosenthal et al., 1996). Supercritical CO_2 extraction of WPS oil avoids the use of hexane and a 60–70% oil yield could be obtained (Liu et al., 2009; Liu et al., 2012; Đurđević et al., 2017). However, supercritical CO_2 extraction technology requires a high capital investment and, if the protein and insoluble fibre co-products are desired, would still need a separate protein and insoluble fibre extraction facility (Wilken et al., 2016). Drastic mechanical processing, thermal treatment and organic solvents used during the above WPS oil extraction methods not only affect the oil quality but also denature the proteins and decrease their economic and nutritional value (Latif et al., 2011). Therefore, in the scope of industrial application, a mild one pot green process acheiving the extraction of oil, proteins and dietary fibres from WPS in the same facility would be an attractive proposition.

An emerging technology involving aqueous enzymatic processing has attracted significant attention for one pot green extraction of both oil and proteins from oilseeds (Yusoff et al., 2015). The aqueous enzymatic process involves treatment of oilseeds with single enzyme or mixture of enzymes in an aqueous medium, followed by centrifugation to separate the slurry into free oil, emulsion (oil rich), aqueous (protein rich) and residual solid phases (Liu et al., 2016a). Recently, Yusoff et al. (2015) and Liu et al. (2016a) reviewed application of protease, carbohydrases and their mixtures for the extraction of oil and protein from oilseeds by the aqueous enzymatic process. The challenge in using this process is to improve the recovery of free oil and protein with no or little emulsion formation (Yusoff et al., 2015). When protein is the dominant barrier for removal of oil, proteolytic enzymes seem to be effective as they hydrolyze all proteins, including the lipophilic protein surrounding the oil bodies, thereby decreasing the emulsion formation and enabling removal of free oil and proteins (De Moura et al., 2008; Jiang et al., 2010; Latif and Anwar, 2011; Latif et al., 2011; Zhang et al., 2011). In addition, the recovery of oil and proteins would concentrate insoluble fibres in the seed residue. Due to the functional properties such as water and oil holding capacity, glucose adsorption and dialysis retardation, the insoluble fibres can be used as dietary fibres (Maphosa and Jideani, 2016). Therefore, proteolytic enzymes not only separate oil and proteins but also allow the utilization of the remaining seed residue as a potential source of dietary fibre, leading to a zero waste process. Recently, Goula et al. (2018) applied cell wall degrading enzymes cellulase and pectinase to recover only oil from pomegranate seeds despite the availability of other natural product such as protein and dietary fibres of high commercial value therewith. Due to the use of cell wall degrading enzymes dietary fibres are degraded, hence loosing this product. In addition, oil recovery of 70% of hexane-extracted oil (which is less than that obtained in present work) and use of two enzymes may increase the cost of overall process. Our preliminary histochemical analysis of pomegranate seeds showed that the oil bodies are enmeshed in a cytoplasmic network rich in proteins. We hypothesized that the protein is a major component holding oil and acting as a barrier for its extraction. Therefore, we employed a protease to break the cytoplasmic network of pomegranate seed cells to release the oil and proteins and obtain pomegranate seed residue rich in dietary fibres.

So far as we know, no previous studies have been conducted on the recovery and concentration of nutraceuticals such as oils, proteins, and dietary fibres from WPS in a single pot. Therefore, the objective of this work was to develop a one pot green process using protease for simultaneous recovery of oil, proteins and insoluble fibres. The effects of protease concentration and incubation time were evaluated for oil yields, fatty acid composition and antioxidant activity of the obtained oil, and these product parameters were compared with hexane-extracted oil. The proteins were characterized in terms of nutritional value based on their amino acid composition, antioxidant activity and molecular weight distribution. The seed residue remaining after oil and protein extraction was characterized in terms of insoluble fibre content and dietary fibre properties, such as water and oil holding capacity, glucose adsorption capacity and glucose dailysis retardation index. Furthermore, light microscope and FTIR-images, and SEM images of seed samples before and after protease treatment were taken in order to understand the extraction mechanism.

2. Materials and methods

2.1. Materials

Protease from *Aspergillus oryzae* (500 U/mL), Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, St. Louis (USA). Other reagents used were of analytical grade and obtained either from Sigma-Aldrich or Merck. Fresh pomegranates (5 kg) of Bhagava cultivar were procured from a local market in Mumbai, India. The pomegranate arils were manually separated from the fruit and crushed to separate the seeds from the juice. The seeds were carefully washed with 1 L tap water three times for the removal of pomace residues and dried in hot air oven (model RDHO-50, Remi, India) at 50 °C for 48 h. The dried seeds were ground into a powder (mean particle size 500 mm) in a mixer grinder (model Bajaj GX8 500, India) and kept in a sealed bag at 4 °C until use.

2.2. Conventional soxhlet extraction

The pomegranate seed powder (10 g on dry weight basis) was packed into a cellulose thimble, and this thimble was placed in a Soxhlet extractor fixed with a 250 mL round-bottom flask and a water condenser. The round-bottom flask was filled with 150 mL of hexane. The extraction process was executed at 60 °C for 4 h (Tian et al., 2013). After extraction, hexane was removed by evaporation at 40 °C under reduced pressure using a rotary evaporator. The obtained oil was weighed and the extraction yield was calculated by dividing the mass of oil obtained per 100 g of seed powder on dry weight basis. The recovered oil was considered as total oil content of pomegranate seed powder and stored at 4 °C, until use for further experiments.

2.3. One pot extraction of oil, protein and insoluble fibres from WPS

The extraction process presented in this study comparises protease treatment of WPS followed by centrifugation for separation of the oil, protein and insoluble fibres in separate phases as depicted in Fig. 1. The details of extraction are as follows:

Ten grams (dry basis) of pomegranate seed powder was added to 79 mL of 50 mM sodium phosphate buffer at pH 7.2 and incubated for