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Extraction process optimization for bioactive compounds in pomegranate peel

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ABSTRACT

Pomegranate peel, a waste generated from fruit processing industry, is a potential source of active ingredients such as polyphenols that are known for their antioxidative properties. In this study, optimization of extraction conditions for bioactive compounds from pomegranate (*Punica granatum* L.) peels was done to investigate the effect of solid/solvent ratio (1:10–1:30), incubation time (15–45 min) and temperature (50–70 °C) on polyphenol extraction using response surface methodology (RSM). The solvent concentration of 60% ethanol was used to extract the phenolic compounds in each experimental run. The experiment was designed to study the effect of extraction conditions on response variables such as total phenolic content (TPC), total flavonoids content (TFC), color index, percent reducing sugars and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in each experiment run. The results of RSM revealed that regression model fitted significantly at $p \leq 0.01$. Using optimization technique, solid to solvent ration of 1:30, temperature of 50 °C and time of extraction of 45 min gives highest yield of 68%, total polyphenolic content of 510 mg gallic acid equivalent/gm, total flavonoids content of 16.40 mg quercetin/gm, color index (ΔE) of 4.07, percent reducing sugars of 0.18 mg of invert sugar and DPPH radical scavenging activity of 24.54%.

1. Introduction

Pomegranate (*Punicagranatum L.; Punicaceae*) due to its multifunctionality and nutritional benefit in the human diet has gained popularity in recent years. The fruit is rich in tannins and other biochemicals, particularly phenolics, which have been reported to reduce disease risk (Martınez, MelgarejoHernandezSalazar &Martınez, 2006; Jaiswal,Der Marderosian &Porter, 2010).The peel of pomegranate fruit constituting about 50% of the total weight is often discarded as waste (Al-Said, Opara& Al-Yahyai, 2009). However, it has been reported that fruit peel contains maximum amounts of bioactive compounds than the juice that possesses stronger biological activities (Li et al., 2006; Hajimahmoodi et al., 2008; Gözlekçi et al., 2011). Due to an increasing health consciousness among the consumers, there has been a dynamic increase in the demand for natural antioxidants, which has contributed to nutritional quality of products and this demand for antioxidants can be met by the extraction from natural sources. Plant phenolics are aromatic compounds responsible for the protection against various degenerative diseases and play a major antioxidative role in the diet (Rice-Evans et al., 1997). Food and agricultural waste generated during processing has emerged as an ideal substrate for extraction of bioactive compounds. Several food and agro residues such as onion peels, potato, apple and olive tree leaves (Kaur and Kapoor, 2001), raspberry waste (Laroze et al., 2010) and other food processing waste have been assessed for extraction of polyphenolic compounds. Among these food processing residues, pomegranate peels, can be a potential feedstock for efficient recovery of bioactive and phytochemicals. Studies reveal that pomegranate peel extract had markedly higher antioxidant capacity than pomegranate juice against scavenging of hydroxyl radicals, superoxide anion and $CuSO_4$ inhabitation (induced LDL oxidation) assays (Li et al., 2006). It has also been reported that pomegranate peel extracts possess a wide range of biological actions including antimicrobial activity (Mc Carrell et al., 2008; Endo,CortézUeda-NakamuraNakamura &Filho, 2010), anticancer activity (Ackland,Van De Waarsenburg &Jones, 2005; Kowalski, SamojednyPaulPietsz &Wilczok, 2005; Brusselmans,VrolixVerhoeven &Swinnen, 2005), anti diarrheal activity (Olapour, Mousavi,Sheikhzade,Hoseininezhad& Najafzadeh, 2009), anti-inflammatory (Yoshimura, Watanabe,Kasai,Yamakoshi&Koga, 2005) and anti-diabetic activities (Lansky & Newman, 2007; Althunibat et al., 2010), apoptotic and anti-genotoxic properties (Lin et al., 1999; Seeram et al., 2005). However, the bioavailability of antioxidant compounds may vary, depending upon different pomegranate cultivars, region and uses (Holland, Hatib &Bar-Yaakov, 2009). Therefore, the peel extracts have recently generated interest because of their potential use as a nutraceutical and

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natural food ingredient (Qu,Pan&Ma, 2010). For the utilization of phytochemical in the preparation of dietary supplements, nutraceuticals, food ingredients, pharmaceutical or cosmetic products the important step is the extraction of bioactive compounds from plant materials. Generally, plant samples are treated by milling, grinding and homogenization, followed by air-drying or freeze-drying before carrying out the extraction process (Abascal, Ganora& Yarnell, 2005). Studies found that polyphenols compounds such as gallic acid, flavonols, ellagic tannins, anthocyanin, procyanidins and ellagic acid present in the fruit peel, exhibit various pharmacological activities (Lansky & Newman, 2007; Althunibat et al., 2010; Viuda-Martos,Fernandez-Lopez&Perez-Alvarez, 2010). However, solvent extraction technique due to its efficiency, ease of use and wide applicability is most commonly used procedure for the preparation of extracts from plant materials. However, yield of solvent extraction depends on various factors including the type of solvents with varying polarities, sample-to-solvent ratio, extraction time and temperature, as well as chemical composition and physical characteristics of the pomegranate part (Dai Jin & Mumper Russell, 2010). With this background, the present investigation was undertaken to optimize the extraction of polyphenols from by-products of local pomegranate cultivars.

2. Material and methods

Fresh pomegranate was purchased from local market of Palampur (Himachal Pradesh, India) and processed to separate the peels followed by drying using hot air drier (MAC Instruments, New Delhi) and finally ground to a fine powder and stored in a cool and dry place. The processed pomegranate powder was further used for extraction and optimization in experimental design.

3. Experimental design (central composite rotatable design)

The Response Surface Methodology (RSM) is a widely adopted tool for the quality of optimizations processes (Nazni & Karunathara, 2011). The RSM, originally described by Box and Wilson (Box & Wilson, 1951), is effective for responses that affect many factors and their interactions. The central composite rotatable design (CCRD), was adopted to predict responses based on few sets of experimental data in which all factors were varied within a chosen range (Box & Hunter, 1957). The experiment consisted of 8 factorial runs, 6 axial runs and 6 center runs. The 3 independent variables were solid/solvent ratio (X_1), temperature (X_2) and time of extraction (X_3). Each variable was set at 5 levels and a total of 20 experiments were designed whereby formulation15, namely the center-point formulation, was repeated 6 times. The independent variables and their variation levels are shown in Table 1. The levels of each variable were established according to literature information and preliminary trials. The outline of the experimental layout with the coded and natural values is presented in Table 2. Homogeneous variance is a necessary pre-requisite for (linear) regression models. Therefore, a reduction invariability within the objective response (dependent variables) due to transformation of data to standardized scores $Z = X - X/S$ where X = dependent variable of interest; X = mean of dependent variable of interest and S = standard deviation. For each standardized score, analysis of Variance (ANOVA) was conducted to determine significant differences among the treatment combinations. Also, data were analyzed using multiple regression procedures. This will study the effect of process variables such as solid/solvent ratio, temperature and time of extraction on response viz Total phenolic content (TPC), total flavonoids content (TFC), color index (ΔE), % reducing sugars and DPPH assay for its significant fitness in regression models. The standardized scores were fitted to a quadratic polynomial regression model by employing a least square technique (Gacula & Singh 1984; Wanasaundara & Shahidi, 1996). The model proposed for each response of Y was:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where Y is the response, X_1 = solid/solvent ratio, X_2 = temperature, X_3 = time of extraction, β_0 = intercepts, β_1, β_2, β_3 are linear, β_{11}, β_{22}, β_{33} are quadratic and β_{12}, β_{13} and β_{23} are interaction regression coefficient terms, respectively. Coefficients of determination (R^2) were computed. The adequacy of the model was examined on the basis of three criterion such as F value, Lack of Fit (LoF) and adequate precision value. The optimization was done by numerically. Constraints were set to get the optimized coded value of the variable between the upper and lower limits of the variable. For every response, response surface plots were produced from the equations, by holding the variable with the least effect on the response equal to a constant value, and changing the other two variables.

Table 1
Independent variables and levels used for central composite rotatable design.

Independent variable		Variables with their coded levels				
		−1.68	−1	0	1	1.68
Solid/solvent ratio	X_1	3.18	10	20	30	36.8
Temperature	X_2	43.2	50	60	70	76.8
Time of extraction	X_3	4.8	15	30	45	55.2

Table 2
Design of experiment with coded and actual values.

Run	Coded values			Actual values		
	X_1	X_2	X_3	X_1	X_2	X_3
1	1.00	−1.00	1.00	30	50	45
2	0.00	−1.68	0.00	20	43.2	30
3	−1.00	1.00	−1.00	10	70	15
4	−1.00	−1.00	−1.00	10	50	15
5	−1.00	−1.00	1.00	10	50	45
6	0.00	0.00	0.00	20	60	30
7	0.00	0.00	0.00	20	60	30
8	0.00	0.00	−1.68	20	60	4.8
9	0.00	0.00	1.68	20	60	55.2
10	0.00	0.00	0.00	20	60	30
11	1.00	−1.00	−1.00	30	50	15
12	1.68	0.00	0.00	36.8	60	30
13	0.00	0.00	0.00	20	60	30
14	0.00	0.00	0.00	20	60	30
15	0.00	0.00	0.00	20	60	30
16	−1.00	1.00	1.00	10	70	45
17	1.00	1.00	−1.00	30	70	15
18	1.00	1.00	1.00	30	70	45
19	−1.68	0.00	0.00	3.18	60	30
20	0.00	1.68	0.00	20	76.8	30

X_1 = Solid/solvent ratio, X_2 = Temperature of extraction, X_3 = Time of extraction.

4. Proximate analysis

The design of experiment was performed and phytochemical extraction were done using 60% ethanol as solvent with different variables i.e. solid/solvent ratio (1:10–3:10), temperature (50–70 °C) and time of extraction (15–45 min). Different extracts obtained from each run of experiment were then used for further proximate analysis. TPC was determined according to the modified