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Optimisation of the extraction of pomegranate (*Punica granatum*) husk phenolics using water/ethanol solvent systems and response surface methodology

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ABSTRACT

Pomegranate (*Punica granatum* (Linn.)) husk is an agri-food residual material, particularly rich in polyphenolic phytochemicals. An experimental setup based on a 2^3-full factorial central composite design was implemented with the aim to optimising recovery of polyphenol extraction from pomegranate husks, using ethanol/water/citric acid as the extracting medium. The factors considered were (i) the pH of the medium, (ii) the ethanol concentration and (iii) the extraction time. Optimal extraction afforded extracts exceptionally enriched in total polyphenols, with the yield being 324.9 mg gallic acid equivalents per g of dry weight. Liquid chromatography–electrospray ionisation mass spectrometry of the optimally obtained extract revealed that the principal phytochemicals recovered were punicalins A and B and ellagic acid.

1. Introduction

Food industry generates a high volume of processing by-products and wastes, which in several instances might pose severe environmental problems. Waste valorisation is widely recognised as a solution of preference for waste management, reducing the polluting load of dumped material and providing opportunities for the development of novel, natural products. These residual sources represent a major pool of substances with very high potential to the pharmaceutical, food and cosmetics manufacturing, while the development of processes for their (bio)-production and recovery would provide an irrefutable economic benefit for the agri-food sector and a direct, positive environmental impact (Schieber et al., 2001; Laufenberg et al., 2003; Montgomery, 2004).

One of the higher value options is the efficient recovery of bioactive polyphenolic phytochemicals, which occur widely in a number of agri-food residues. Polyphenols may serve both as food additives, for effective protection of various foods, and bioactive substances in cosmetics and pharmaceuticals, hence the efforts for commercialisation of novel products focus mainly on applications of polyphenols or polyphenol-containing extracts. The utilisation of other substances with proven antioxidant ability, with the exception of tocopherol mixtures, is rather limited, due to difficulties related with their recovery from natural sources, their chemical stability and cost (Schieber et al., 2001; Moure et al., 2001; Balasundram et al., 2006).

For this reason efforts have been focused on the development of cost-effective methodologies, to meet an increasing demand for shorter time and reduced manufacturing cost (Galanakis, 2012). Although some waste material can be used following minimal processing, extracts containing a specific class of phytochemicals are preferred, as they may possess peculiar properties and provide distinct and targeted final product attributes. Thus most commercial processes include recovery and purification steps. The objective is to separate manly a class of compounds at high yield, discarding other inert constituents and undesired substances. In such a process, hazardous separating agents (*e.g.* solvent residues) should not remain in the product above an acceptable level. Taking into consideration these demands, as well as the increasing necessity to reduce time-to-market and development cost, it is highly advantageous to develop straight-forward recovery processes, which will

Abbreviations: AAE, ascorbic acid equivalents; A_{AR}, antiradical activity; CCD, central composite design; C_{EtOH}, ethanol concentration (%, v/v); dw, dry weight; S.D., standard deviation; *t*, time; Y_{TP}, total polyphenol yield; TRE, trolox equivalents.

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be characterised by high extraction yield, low cost, selectivity and if possible, zero waste.

The search for plant food residues as cost-effective sources of multifunctional phytochemicals encompasses efforts in the direction of (i) exploiting materials with high burden and versatile composition, (ii) implementing efficient recovery tools and methodologies and (iii) ascertaining production of novel commodities without further generation of waste. In this line, the use of benign solvent systems for the recovery of target compounds becomes imminent. Ethanol is a bio-solvent, produced *via* fermentation of various carbohydrate-containing raw materials. Unlike other solvents such as methanol or acetone, ethanol is non-toxic and can be reused following its recovery after removal from the extract through distillation, thus generating practically zero wastes. Therefore, ethanol can be considered as an environmentally benign solvent. Water/ethanol mixtures have been employed for the extraction of several classes of polyphenolic compounds, including anthocyanins from black currants (Cacace and Mazza, 2003) and phenolics from grapevine shoots (Luque-Rodríguez et al., 2006), olive leaves (Japón-Luján et al., 2006), grape seed meal (Shi et al., 2003), grape seeds (Yilmaz and Toledo, 2006), red grape pomace (Makris et al., 2008), barley, and aromatic plants (Tsakona et al., 2012).

Previous investigations on waste material such as vinification by-products (Karvela et al., 2009a,b), olive leaves (Mylonaki et al., 2008) and onion peels (Kiassos et al., 2009) demonstrated that solvent systems composed of ethanol, water and citric acid (pH regulator) can afford very satisfactory yields of polyphenolic phytochemicals with significant antioxidant properties. In this concept, this study was undertaken to investigate the potential of retrieving polyphenolic antioxidants from *Punica granatum* husks, using similar solvent systems and response surface methodology.

This specific food processing residual appears to be exceptionally rich in polyphenols (Elfalleh et al., 2009; Pan et al., 2012; Qu et al., 2010; Salgado et al., 2012; Wang et al., 2011), especially hydrolysable tannins (Aqil et al., 2012; Fischer et al., 2011; Glazer et al., 2012). This peculiar composition makes pomegranate husks unique compared with other plant food wastes, which contain primarily flavonoids and other simpler phenolics (Makris et al., 2007a). Thus, the examination of this material with regard to its potential as a residual source of valuable, bioactive substances would merit higher attention, as it may possess significant functional properties, such as antioxidant and cytotoxic activities (Okonogi et al., 2007), antimutagenic activity (Negi et al., 2003), and antimicrobial activity (Negi and Jayaprakasha, 2003; Osorio et al., 2010; Tehranifar et al., 2011).

Table 1
Experimental values and coded levels of the independent variables used for the 2^3 full-factorial design.

Independent variables	Code units	Coded variable level		
		−1	0	1
C_{EtOH} (%, v/v)	X_1	40	50	60
pH	X_2	2	4	6
t (h)	X_3	1	3	5

2. Materials and methods

2.1. Chemicals

All solvents used for chromatographic purposes were HPLC grade. Folin–Ciocalteu phenol reagent was from Fluka (Steinheim, Germany). Gallic acid, ellagic acid, trolox™ and 2,2-diphenylpicrylhydrazyl (DPPH•) stable radical were from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

2.2. Waste material

P. granatum husks were obtained from a local pomegranate-processing plant (Chania, Greece), immediately after processing. The husks were transferred within a few hours to laboratory, dried in an oven at 60 °C for 48 h and pulverised in a domestic blender. Pulverised material was placed in plastic, screw-cup jars and stored at −40 °C until used.

2.3. Extraction procedure

An amount of approximately 0.5 g of pulverised tissue was placed in a 30-mL glass vial with 20 mL of solvent, composed of varying amounts of aqueous ethanol. All solvent systems used contained citric acid (1 g L^{-1}) and were adjusted to the desired pH using 1 N NaOH. Extractions were carried out under magnetic stirring at 400 rpm, at room temperature (22 ± 2 °C) for predetermined time periods. Upon completion of extraction, the extracts were filtered through paper filter, and stored at −20 °C until analysed. All extracts were also filtered through 0.45-μm syringe filters prior to determinations.

Table 2
Measured and predicted Y_{TP} and A_{AR} values determined for individual design points.

Design point	Independent variables			Responses			
	X_1	X_2	X_3	Y_{TP} (mg GAE g^{-1} dw)		A_{AR} (mM TRE g^{-1} dw)	
				Measured	Predicted	Measured	Predicted
1	−1	−1	−1	314.8	324.9	2.82	2.87
2	−1	−1	1	329.6	309.4	2.91	2.87
3	−1	1	−1	310.2	301.8	2.91	2.81
4	−1	1	1	297.4	305.8	2.65	2.74
5	1	−1	−1	370.8	354.7	2.83	2.69
6	1	−1	1	335.8	336.5	2.74	2.79
7	1	1	−1	267.8	280.4	2.64	2.63
8	1	1	1	299.4	281.7	2.76	2.66
9	−1	0	0	286.4	296.5	2.88	2.88
10	1	0	0	279.0	299.4	2.56	2.76
11	0	−1	0	148.7	155.9	1.28	1.26
12	0	1	0	130.4	116.9	1.14	1.17
13	0	0	−1	152.1	153.9	0.96	1.15
14	0	0	1	118.2	146.8	1.15	1.16
15	0	0	0	146.4	136.4	1.27	1.22
16	0	0	0	150.6	136.4	1.37	1.22