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Implementation of response surface methodology to optimise extraction of onion (*Allium cepa*) solid waste phenolics

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ABSTRACT

An experimental setup based on a 2^3-full factorial, central composite design was implemented with the aim to optimising recovery of polyphenols from onion solid wastes (OSW). In order to allow for the establishment of a sustainable process, reusable and non-toxic solutions composed of water/ethanol/citric acid were employed as extracting media. The factors considered were (i) the pH of the medium, (ii) the extraction time and (iii) the ethanol concentration. The model obtained produced a satisfactory fitting of the experimental data with regard to total polyphenol extraction (R^2=0.97, p=0.0025) and the reducing power of the extracts (R^2=0.97, p=0.0033), but not with the antiradical activity (R^2=0.89, p=0.0592). The 2nd order polynomial equations obtained after elaboration of the experimental data indicated that all parameters considered were significant in respect with the efficiency of total polyphenol recovery. The highest total polyphenol yield was theoretically predicted to be 9342±1435 mg gallic acid equivalents per 100 g dry weight, under optimal conditions (60% EtOH, pH 2 and 4.2 h). Liquid chromatography-electrospray ionisation mass spectrometry of the optimally obtained extract revealed that the principal phytochemicals recovered were quercetin 3,4′-diglucoside, quercetin 4′-glucoside and quercetin. Simple linear regression analysis between the total polyphenol and the antiradical activity of the OSW extracts showed that there was no correlation in a statistically significant manner, as opposed to reducing power.

Industrial relevance: The recovery of value-added substances from agri-food industrial wastes is an issue with importance pertaining to both the reduction of the waste load released to the environment, and the development of novel, natural food additives with functional properties. Up to date, the examinations pertaining to the clarification of factors that can affect extractability were based on rather unilateral assessment, while it is generally accepted that the retrieval depends on several parameters, which render the phenomenon a particular complexity. In this view, the implementation of factorial design with respect to investigating in parallel several factors pertaining to efficient polyphenol recovery becomes imminent. Thus the establishment of models on such a sound experimental basis is expected to provide a reliable background for more costand resource-effective processes, with a potential direct industrial applicability.

1. Introduction

The recycling of residues has become an important task to every food processing activity, in recognition that a systematic reduction in waste disposal is profitable under both economical and ecological aspects. Concepts like the differentiation and separate treatment of waste materials support this trend, and in this connection special attention has been drawn to the recovery of valuable substances (Laufenberg, Kunz, & Nystroem, 2003).

By-products and wastes of plant food processing, which represent a major disposal problem for the industry concerned, are very promising sources of value-added substances, with particular emphasis being given to the retrieval of bioactive and technologically important secondary metabolites (Schieber, Stintzing, & Carle, 2001). In this regard, numerous studies have been dealing with the development of efficient processes of recovering such phytochemical substances (Moure et al., 2001; Shi, Nawaz, Pohorly, Mittal, Kakuda, & Jiang, 2005).

More than 450,000 tonnes of onion solid waste (OSW) is produced each year in Europe (Moure et al., 2001). The outer dry layers and the apical parts of onion bulbs, which are not edible and removed before processing, have been shown to contain a wide spectrum of polyphenolic components (Ly, Hazama, Shimoyamada, Ando, Kato, & Yamauchi, 2005; Ramos et al., 2006). Most of these substances have not been reported to occur in the scales of the bulb, but they represent oxidation products of quercetin and its glucosides. Some of these

Abbreviations: AAE, Ascorbic acid equivalents; A_{AR}, antiradical activity; Dw, dry weight; OSW, onion solid waste; P_R, reducing power; RSM, response surface methodology; S.D., standard deviation; TP, total polyphenols; TRE, trolox equivalents.

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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
Ethanol content (%)	X_1	40	50	60
pH	X_2	2	4	6
Time (h)	X_3	1	3	5

phenolics have been shown to possess antiplatelet properties (Furusawa, Tsuchiya, Nagayama, Tanaka, Nakaya, & Iinuma, 2003) and strong antioxidant activity in vitro (Ly et al., 2005; Ramos et al., 2006).

Furthermore, the application of onion extracts to a range of food matrices including chicken (Karastogiannidou, 1999), minced sardines (Serdaroğlu & Felekoğlu, 2005), corn oil (Navas, Carrasquero-Durán, & Flores, 2006) and turkey (Tang & Cronin, 2007), was shown to provide effective protection against lipid peroxidation and formation of thiobarbituric acid-reactive substances (TBARS).

Hence it could be hypothesised that OSW phenolics can have a significant prospect in applications to foods, neutraceuticals, cosmetics and pharmaceuticals, but in spite of their obvious usefulness studies pertaining to their efficient recovery are limited (Kefalas & Makris, 2006a). In an effort to examine some basic factors that are likely to govern the extractability of polyphenols from OSW, this investigation was undertaken with the view to identifying optimal operational conditions. To develop a sustainable process, extracts were obtained utilising cheap and non-toxic solvents, composed of water/ethanol/citric acid mixtures and the process was optimised on the basis of a 2^3-full factorial experimental design and response surface methodology (RSM).

2. Materials and methods

2.1. Chemicals

All solvents used for chromatographic purposes were HPLC grade. Absolute ethanol was of analytical grade. Folin-Ciocalteu phenol reagent and ascorbic acid were from Fluka (Steinheim, Germany). Gallic acid, trolox^R, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2-diphenyl-picrylhydrazyl (DPPH$^\bullet$) stable radical, and quercetin were from Sigma Chemical Co (St. Louis, MO, U.S.A.).

2.2. Onion solid wastes (OSW) collection and pre-treatment

The outer dry and semi-dry layers and the apical trimmings of brown-skin onion bulbs (*Allium cepa*) were collected immediately after processing from a local catering facility (Chania, Crete). The tissues were frozen in liquid nitrogen and ground with a pestle and a mortar. The ground tissue was placed at −20 °C for no longer than a week before the analysis.

2.3. Extraction procedure

An amount of approximately 500 mg of ground waste 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.

2.4. Experimental design

A 2^3 full-factorial experiment design was used to identify the relationship existing between the response functions and process variables as well as to determine those conditions that optimised the extraction process. The three independent variables or factors studied were ethanol concentration [X_1, varying between 40 and 60% (v/v)], pH (X_2, varying between 2 and 4) and extraction time (X_3, varying between 1 and 5 h). The selection of ranges within which each factor varied was based on preliminary experimentation and literature data (Cacace & Mazza, 2003; Yilmaz & Toledo, 2006). Each variable to be optimised was coded at three levels −1, 0 and 1 (Table 1).

The three independent variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i},\ x_i = 1,\ 2,\ 3$$

where x_i and X_i are the dimensionless and the actual value of the independent variable i, X_0 the actual value of the independent variable i at the central point, and ΔX_i the step change of X_i corresponding to a unit variation of the dimensionless value. Response (total polyphenols, antiradical activity and reducing power) at each design point was recorded (Table 2). Data from the central composite experimental design were subjected to regression analysis using least square regression methodology to obtain the parameters of the mathematical models. The Student's *t*-test permitted to check the statistical significance of the regression coefficients deriving from the model. Analysis of variance (ANOVA) was applied to evaluate the statistical

Table 2
Responses of the dependent variables to extraction conditions

Design point	Independent variables			Responses					
	X_1	X_2	X_3	TP (mg GAE/100 g dw)		A_{AR} (mM TRE/g dw)		P_R (mM AAE/g dw)	
				Measured	Predicted	Measured	Predicted	Measured	Predicted
1	−1	−1	−1	2518	2330	8.3	8.6	5.8	5.9
2	−1	−1	1	7108	7491	13.2	14.3	14.8	15.3
3	−1	1	−1	3338	3663	6.1	7.4	5.7	6.3
4	−1	1	1	8224	7905	21.7	22.0	16.0	15.8
5	1	−1	−1	2788	3071	10.4	10.0	7.6	7.5
6	1	−1	1	9300	8939	16.2	14.6	17.8	17.0
7	1	1	−1	3743	3324	7.8	6.5	8.1	7.4
8	1	1	1	8121	8273	20.5	20.0	17.3	17.0
9	−1	0	0	7714	7514	16.1	13.1	16.4	15.4
10	1	0	0	7724	8069	9.2	12.9	15.0	16.8
11	0	−1	0	7577	7461	14.9	15.4	15.7	15.9
12	0	1	0	7533	7794	17.4	17.5	15.5	16.1
13	0	0	−1	2125	2339	3.6	4.0	4.3	5.0
14	0	0	1	7250	7395	12.8	13.5	13.7	14.5
15	0	0	0	6800	7331	14.0	12.6	16.0	15.2
16	0	0	0	8151	7331	12.8	12.6	15.9	15.2