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Optimisation of Anthocyanin Recovery from Onion (*Allium cepa*) Solid Wastes Using Response Surface Methodology

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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 anthocyanin recovery from onion solid wastes. 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 the pH of the medium, the extraction time and the ethanol concentration. The 2nd order polynomial equation obtained after elaboration of the experimental data indicated that extraction time mostly affected the extraction yield.

Key words: Anthocyanins, extraction, onions, response surface, wastes, concentration

INTRODUCTION

Anthocyanin pigments are of prominent importance in foods because of their dual role; first they constitute an integral part of the sensory attributes, since their levels, various forms and derivatives pertain directly to the coloration of the final product; second they have been claimed to possess diverse biological properties and therefore they are considered as secondary metabolites with potential nutritional value (Wrolstad, 2004).

Despite their advantages with respect to heat, light, pH stability and purity compared with natural colorants such as anthocyanins, synthetic pigments are increasingly rejected by consumers owing to health concerns. Thus there is worldwide interest in additional use of anthocyanins as a consequence of perceived consumer preferences as well as legislative action which has continued the delisting of approved artificial dyes (Socaciu, 2009).

Anthocyanin-rich extracts might thus have potential as food additives and in this view a number of residual sources have been proposed for anthocyanin recovery, including grape pomace (Makris *et al*., 2008), tropical fruits (Vera de Rossoa and Mercadante, 2007), carrot (Ersus and Yurdagel, 2007) and roselle (Mourtzinos *et al*., 2008). However, the exploitation of onion anthocyanins as food colorants has never been examined, although their dyeing properties are promising (Guinot *et al*., 2007). This study was undertaken with the aim of identifying the effect of some key factors such as the amount of ethanol, the pH and extraction time, on the recovery of anthocyanin pigments from onion solid wastes. The investigations were focused on the implementation of response surface methodology for optimising recovery, through combinations of the above mentioned variables.

MATERIALS AND METHODS

Chemicals and plant material: Absolute ethanol and HCl were of analytical grade. 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 until analysed.

Extraction procedure: The onion solid waste was frozen with liquid nitrogen and ground with a pestle and a mortar. An amount of approximately 500 mg of ground tissue was placed in a 30 mL glass vial and 10 mL of solvent was added. 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.

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Table 1: Experimental values and coded levels of the independent variables used for the 2^3 full factorial, central composite design

		Coded variable level		
Independent variables	Code units	-1	0	1
Ethanol content (%)	X_1	40	50	60
pH	X_2	2	4	6
Time (h)	X_3	1	3	5

Table 2: Measured and predicted value of Total Anthocyanin (TA) content determined for individual design points

	Independent variables			Response (TA, mg CyE/100 g dw)	
Design point	X_1	X_2	X_3	Measured value	Predicted value
1	-1	-1	-1	78.50	77.85
2	-1	-1	1	164.41	174.45
3	-1	1	-1	78.32	86.04
4	-1	1	1	168.00	162.84
5	1	-1	-1	83.42	87.46
6	1	-1	1	198.90	190.06
7	1	1	-1	76.10	64.95
8	1	1	1	148.24	147.75
9	-1	0	0	164.00	152.03
10	1	0	0	132.94	149.29
11	0	-1	0	174.41	169.79
12	0	1	0	143.72	152.73
13	0	0	-1	35.61	34.72
14	0	0	1	120.00	124.42
15	0	0	0	137.47	133.78
16	0	0	0	139.02	133.78

Experimental design: A 2^3 full factorial experiment design was used to identify the relationship existing between the response function (anthocyanin yield) 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). Each variable to be optimised was coded at three levels-1, 0 and 1 (Table 1).

For each factor an experimental range based on the results of literature data and on the performance of preliminary experiments was chosen. The three independent variables were coded according to the following equation:

$$x_i = \frac{X_i - X_o}{\Delta X_i}$$

Where:

X = 1, 2, 3

xi and X_i = 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

Δx_i = The step change of X_i corresponding to a unit variation of the dimensionless value

Response 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 significance of the model. Response surface plots were obtained using the fitted model by keeping the independent variables simultaneous.

Moisture content: The moisture content of the onion solid wastes used was determined by drying in an oven at 80°C for 4 days.

Total anthocyanins: A modification of a previously published protocol was used (Dourtoglou *et al*., 2006). Briefly an aliquot of extract was combined with ethanolic HCl solution (0.25 M) to give a dilution 1:10. The solution was mixed thoroughly and the absorbance at 520 nm (A_{520}) was read after 5 min using the ethanolic HCl solution as blank. Total anthocyanin content was determined as cyanin (Cyaniding 3-O-glucoside) Equivalents (CyE) per 100 g fresh tissue using as ε = 26900 and MW = 449.2.

Statistical analyses: All determinations were carried out at least in triplicate (n =3). For all statistics, JMP ™ 5.1 and Microsoft Excel™ 2000 were used.

RESULTS AND DISCUSSION

The experimental screening performed was designed to assess the influence of three factors that is the ethanol concentration, the pH and the extraction time. In Table 1 can be seen the experimental values and coded levels of the three independent process variables used for the 2^3 full factorial, central composite, experimental design implemented. Values of the independent process variables (X_1, X_2 and X_3) considered as well as measured and predicted values the response (TA) are analytically shown in Table 2.

The experimental values of the response were analysed by multiple regression to fit the following second-order polynomial equation:

$$\begin{aligned} TA\ (mg\ CyE/100\ g\ dw) = {} & 133.78 - 1.37X_1 - 8.53X_2 + 44.85X_3 - \\ & 7.68X_1X_2 + 1.50X_2X_3 - 4.95X_1X_3 + \\ & 16.88X_1^2 + 27.48X_2^2 - 54.21X_3^2 \end{aligned}$$

The quality of fit was ascertained using the coefficients of determination (R^2). The experimental data obtained showed a good fit with the equation (R^2 = 0.95)

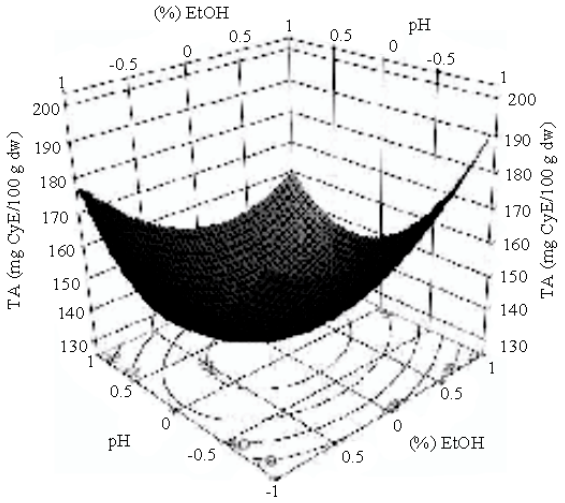

Fig. 1: Response surface plot showing the effect of the simultaneous variation of EtOH and pH on the TA recovery

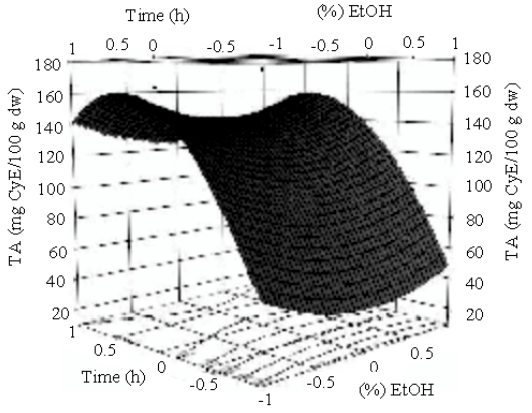

Fig. 2: Response surface plot showing the effect of the simultaneous variation of EtOH and time on the TA recovery

which was statistically acceptable at 99.9% significance level ($p<0.001$). This fact indicated a satisfactory agreement between observed and predicted responses and that the equation found can adequately predict the experimental results. The utilisation of the predictive model enabled the theoretical calculation of the optimal sets of conditions under which maximal value (TA = 183.85 mg CyE/100 g dw) could be attained:

$$[EtOH] = 60\%, pH = 2, t = 3.7 \text{ h}$$

The trends revealed in each case were recorded in the form of three-dimensional plots (Fig. 1-3) where the effect of simultaneous variation of EtOH, pH and time is shown. The trend seen for TA recovery upon

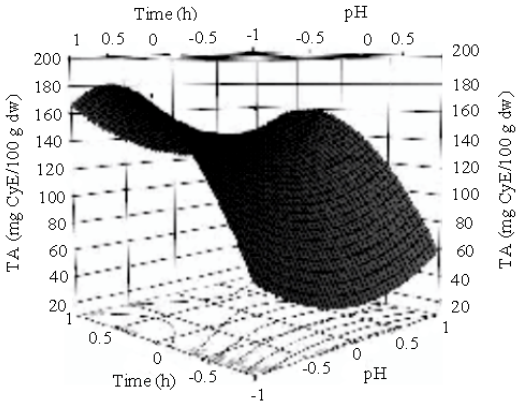

Fig. 3: Response surface plot showing the effect of the simultaneous variation of pH and time on the TA recovery

simultaneous variation of EtOH and pH indicated that maximal yield can be achieved at low pH and high EtOH values. At intermediate values for both variables, recovery showed a declining tendency. With regard to the extraction time, maximal yield was found for intermediate duration while shorter and longer durations were not proven favourable.

Water/ethanol mixtures have been employed for the extraction of anthocyanins from purple sunflower hulls (Gao and Mazza, 1996), black currants (Cacace and Mazza, 2003) and grape pomace (Makris *et al*., 2008) but such an examination has never been performed on onion wastes.

In an effort to examine some basic factors that are likely to govern the extractability of anthocyanin pigments from onion solid wastes 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.

The regulation of pH using an organic acid such as citric acid was judged necessary, since in general acidic solvents are more efficient for anthocyanin extraction compared with neutral ones (Revilla *et al*., 1998). On the other hand, solvent composition affects profoundly its physical properties such as density and dynamic viscosity which affect diffusion and rate of extraction. Also composition influences the dielectric constant. It has been proposed that reduction of dielectric constant of a protic solvent such as water ($\varepsilon H_2O = 78.5$) into the range of intermediate-behaviour solvents such as

methanol (ε MeOH = 32.6) or ethanol (ε EtOH = 24.3) by modifying pressure or temperature can improve extraction of anthocyanins (Cacace and Mazza, 2002).

Under the optimal conditions established by deploying response surface methodology it was found that an amount of 183.85 mg CyE/100 g dw can be recovered. Comparing this yield with the one reported for grape pomace, amounting 266.20 mg/100 g dw (Makris *et al*., 2008) it can be claimed that onion solid wastes might be a residual source of water-soluble pigments that should not be overlooked. On the other hand, the utilisation of wastes from more pigmented onions as well as the consideration of factors such as temperature and particle size could reveal onion wastes as a very good source of anthocyanins.

CONCLUSION

The most important outcomes of this study can be summarised as follows: The implementation of response surface methodology for the recovery of anthocyanins from onion solid wastes showed that extraction time was statistically significant. Optimal conditions required the highest value of ethanol amount tested (60%), a pH as low as 2 and an extraction time of 3.7 h. The amount of anthocyanins recovered was comparable to that reported for grape pomace which is considered one of the richest food residues and thus onion wastes appear as a promising source of water-soluble pigments.

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