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Optimizing enzymatic extraction from rose petals (Rosa Damascena Mill.)

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Response surface methodology (RSM) has been used to optimize the extraction conditions of antioxidants and anthocyanins from rose petals (*Rosa Damascena* Mill.) by using enzyme - assisted extraction. A ternary enzyme combinations included pectinolytic, cellulolytic and hemicellulolytic preparation was used. The variation in enzyme dosage (0.052 - 0.132%) and maceration time (125 - 205 min) define the optimal maceration conditions to prepare extracts with high antioxidant and anthocyanins content

Keywords: Response surface methodology (RSM), polyphenols, anthocyanins, enzyme-assisted extraction, Rosa Damascena Mill

INTRODUCTION

The interest in drug–and product derived from plants has made it important to search for effective extraction method to maximize the yield of bioactive component such as vitamin C, anthocyanin and phenolic compounds [1]. Response surface methodology (RSM) is an effective statistic technique which uses quantitative data in an experimental design to optimize a process [2].

RSM was demonstrated to be a potent tool in optimizing experimental parameters maximizing numerous responses [3]. Central composite design, which is one of the most common designs, has been widely used to optimize phenolic compounds extraction [4].

Optimal central composite design (OCCD) is an experimental approach to define empirical models or equations for describing the effect of test variables and th3ir interactions on the respective responses [5, 6, 7].

In previous investigations the efficiency of enzyme-assisted extraction with three components enzyme mixture (pectinolytic, cellulolytic and hemicellulolytic preparation) on the recovery of polyphenols from *Rosa damascena* petals using a simplex centroid experimental design was evaluated [8].

So the purpose of this study is to optimize enzymatic treatment conditions from rose petals (*Rosa Damascena* Mill.) using variation in dosage obtained extract can be used in food and parapharmaceutical industry.

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MATERIALS AND METHODS

Chemicals

For analytical purposes the following reagents were used: DPPH [2,2-diphenyl-1-picrylhydrazyl] and Trolox [(+/−)-6-hydroxy-2,5,7,8- tetramethyl-chroman-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); TPTZ [2,4,6-tripyridyl-s-triazine] and gallic acid monohydrate (Fluka, Buchs, Switzerland); Folin-Ciocalteau's reagent (Merck, Darmstadt, Germany). All the other reagents and solvents used were of analytical grade.

Enzyme preparations

The following commercial enzyme preparations were used: pectinolytic preparation Pectinex Ultra Color (Novozymes A/S, Bagsvaerd, Denmark); cellulolytic preparation Rohament CL (AB Enzymes GmbH, Darmstadt, Germany); hemicellulolytic preparation Xylanase AN (Biovet JSC, Peshtera, Bulgaria). Plant material:

Rose (*Rosa damascena* Mill.) petals, harvest year 2013, were supplied by Ecomaat Ltd. (Mirkovo, Bulgaria). The petals were dried in a thin layer at room temperature (25-27°C) for one week before final hot air drying (50°C, 1 h). Dried rose petals were stored in a desiccator in dark until used.

Enzyme - assisted extraction

Extracts from *Rosa damascena* petals were processed according to the flow diagram shown in Fig.1.

Sample preparation

An aliquot (5 g) of filtered extract was transferred into 50 mL volumetric flask using 40 ml of acidified (0.1% HCl) methanol. After extraction for 24 h at 10°C, the flask was filled up to the mark with acidified methanol and filtered through a paper filter. Extraction was performed in triplicate.

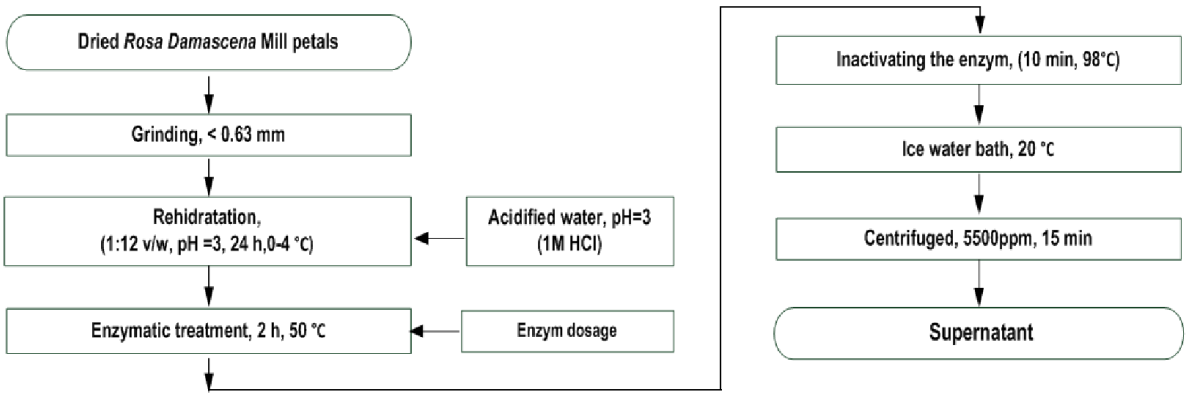

Fig.1. Process flowchart for extraction of rose petals (*Rosa Damascena* Mill.)

Analytical methods

All measurements were performed with a Helios Omega UV-Vis spectrophotometer equipped with VISIONlite software (all from Thermo Fisher Scientific Inc., Waltham, MA, USA) using 1 cm path length cuvettes.

The contents of total polyphenols (TPP) and total monomeric anthocyanins (TMA) were determined by the method of Singleton and Rossi [9] and the pH-differential method [10], respectively, modified as described by [11].

The total antioxidant capacity was determined by the DPPH (free radical scavenging activity) and FRAP (ferric reducing antioxidant power) assay, following the methods of Brand-Williams et al. [12] and Benzie and Strain [13], respectively, with some modifications [11].

Statistical analysis

The results reported in the present study are the mean values of at least three analytical determinations and the coefficients of variation, expressed as the percentage ratios between the standard deviations and the mean values, were found to be < 5% in all cases. SYSTAT statistical software (SPSS Inc., Chicago USA, version 7.1) and Excel were used to analyze the data.

Experiment design

An OCCD of the type 2^n+2n+n_0 was applied. The influence of the independent variables was determined by means of the RSM [2, 14].

Table 1 shows the levels of the two independent variables – enzyme dose (0.02–0.18%E/S) and reaction time (30–210 min).

Considering two parameters and a response, experimental data were fitted to obtain a second-degree regression equation of the form:

$$y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} b_{ij} x_i x_j \quad (1)$$

where y is the predicted responses for TMA, TPP, DPPH, FRAP, yield of extract, respectively; X_1 is the enzyme concentration and X_2 is the incubation time; b_0 is the value of the fitted response at the centre point of the design and b_i, b_{ii} and b_{ij} are the coefficients of the regression equation

Table 1. Independent variable values and corresponding levels

Factor	Minima	Center point	Maxima	Axial piont, α
Enzyme dose (%E/S[a]) – X_1	0.02	0.1	0.18	-α=-1 +α=+1
Time (min) – X_2	30	120	210	-α=-1 +α=+1

[a] ml enzyme preparation per 100 g substrate

RESULTS

The statistical combinations of variables in coded and actual values along with the predicted and experimental responses are presented in Table 2. There is an increase in the values of TMA and TPP (Table 2 and Fig.2) by prolong the time of processing, leading to reduction of enzyme dosage mixture.

The positive effect of prolonged treatment means that the enzyme mixture possess the pectolytic, cellulitic and hemicellulitic activities that are able to catalyze the degradation of the polysaccharides in the cell wall, resulting in increased extraction of polyphenols and anthocyanins.

Higher content of enzyme mixture dosage leads to increased values of bioactivities compounds and yield in a short time of processing. The effect of enzyme dosage decrease with decreasing of time treatment.

The regression equation characterizing the influence of different variables on extraction process is obtained (2-6). The high values of the

coefficient of determination (R^2) indicate a reasonable agreement of the corresponding model with the experimental results [16].

Pareto chart Fig.3, was used to visualize the interaction effects of all factors to the response variable.

Table 2. Experimental design matrix and results for the optimal central composite design

№	Coded values		Enzyme dose (%E/S[a])	Time (min)	TMA[b] (mgCGE/100g)	TPP[c] (mgGAE/100g)	DPPH[d] (mgTE/100g)	FRAP[e] (mgTE/100g)	Yield[f], %
			X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5
1	-	-	0.02	30	14.4	302.5	1880.0	1493.0	34.0
2	+	-	0.18	30	15.7	424.5	2637.0	1808.0	45.5
3	-	+	0.02	210	18.3	494.2	2796.0	2180.0	34.5
4	+	+	0.18	210	18.5	481.3	2950.0	2237.0	37.0
5	-	0	0.02	120	17.6	561.1	3242.0	1830.0	42.6
6	+	0	0.18	120	18.4	615.6	3697.0	2012.0	49.1
7	0	-	0.1	30	14.0	245.3	1965.0	1538.0	32.2
8	0	+	0.1	210	17.3	369.5	2579.0	2085.0	29.5
9	0	0	0.1	120	17.6	473.6	3187.0	1740.0	42.0
10	0	0	0.1	120	17.2	458.2	3157.0	1798.0	42.0
11	0	0	0.1	120	15.9	478.6	3185.0	1868.0	42.0

[a] ml enzyme preparation per 100 g substrate.
[b] Results are presented as mg cyanidin 3-glucoside equivalents (CGE) per 100 g
[c] Results are presented as mg gallic acid equivalents (GAE) per 100 g
[d] Results are presented as mg trolox equivalents (TE) per 100 g
[e] Results are presented as mg trolox equivalents (TE) per 100 g
[f] Results are presented as % per 100 g

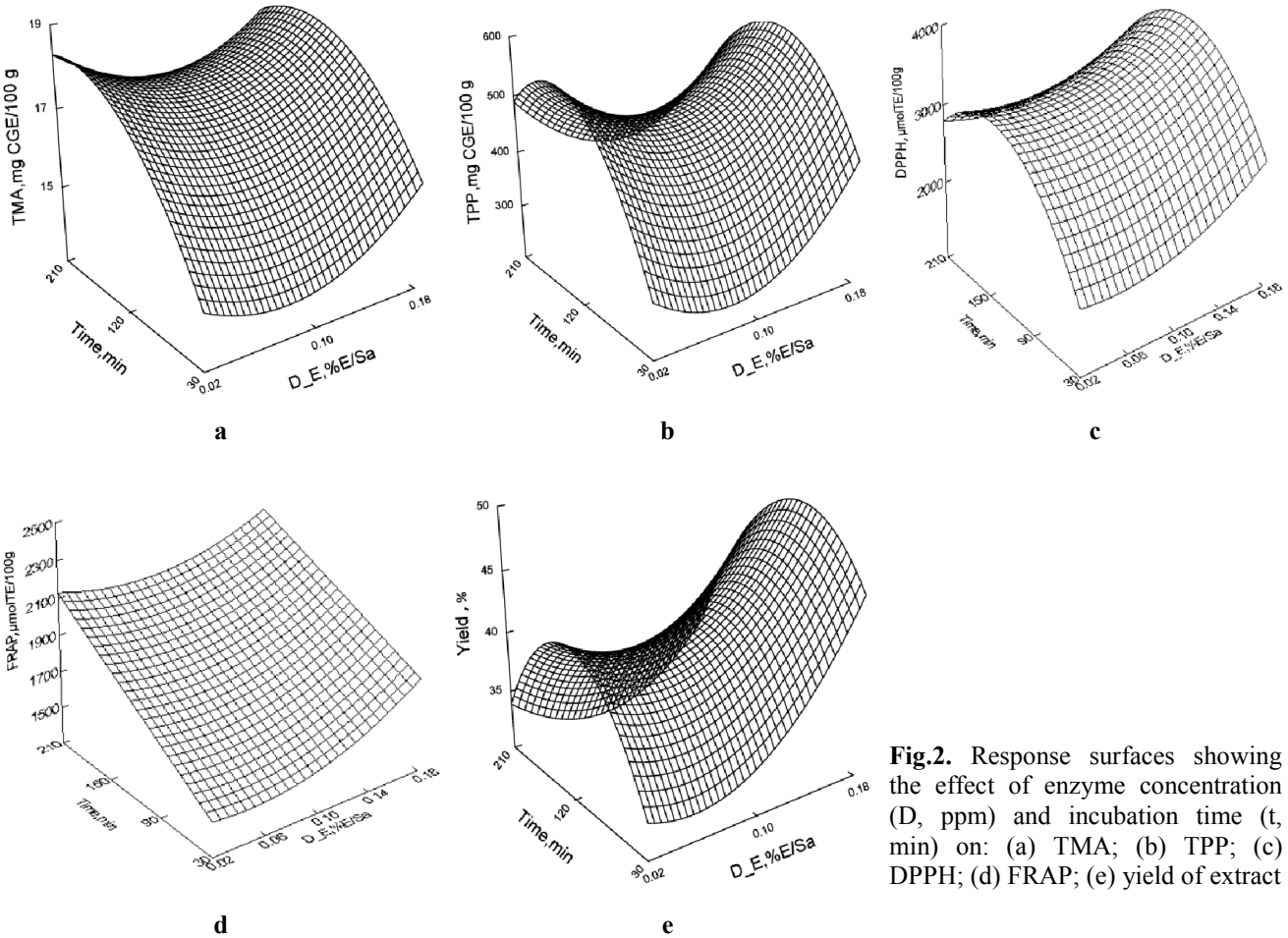

Fig.2. Response surfaces showing the effect of enzyme concentration (D, ppm) and incubation time (t, min) on: (a) TMA; (b) TPP; (c) DPPH; (d) FRAP; (e) yield of extract

$$Y_1 = 13.191 - 25.030.X_1 + 0.06.X_2 + 171.505.X_1^2 - 0.038.X_1.X_2 - 0.0002.X_2^2,\ mg\ CGE/100\ g;\ R^2 = 0.937 \quad (2)$$

$$Y_2 = 190.88 - 2390.52.X_1 + 5.57.X_2 + 16525.5.X_1^2 - 4.68.X_1.X_2 - 0.01.\ X_2^2,\ mg\ GAE/100\ g;R^2 = 0.993 \quad (3)$$

$$Y_3 = 1082.22 - 3812.72.X_1 + 32.29.X_2 + 45855.3.X_1^2 - 20.94.X_1.X_2 - 0.11.X_2^2,\ \mu molTE/100g;\ R^2 = 0.999 \quad (4)$$

$$Y_4 = 1410.81 - 1473.14.X_1 + 3.71.X_2 + 18511.5.X_1^2 - 8.96.X_1.X_2 + 0.001\ X_2^2,\ \mu molTE/100g;\ R^2 = 0.986 \quad (5)$$

$$Y_5 = 26.978 - 66.10.X_1 + 0.27.X_2 + 745.07X_1^2 - 0.31.\ X_1.X_2 - 0.001.X_2^2\ ,\%;\ R^2 = 0.96 \quad (6)$$

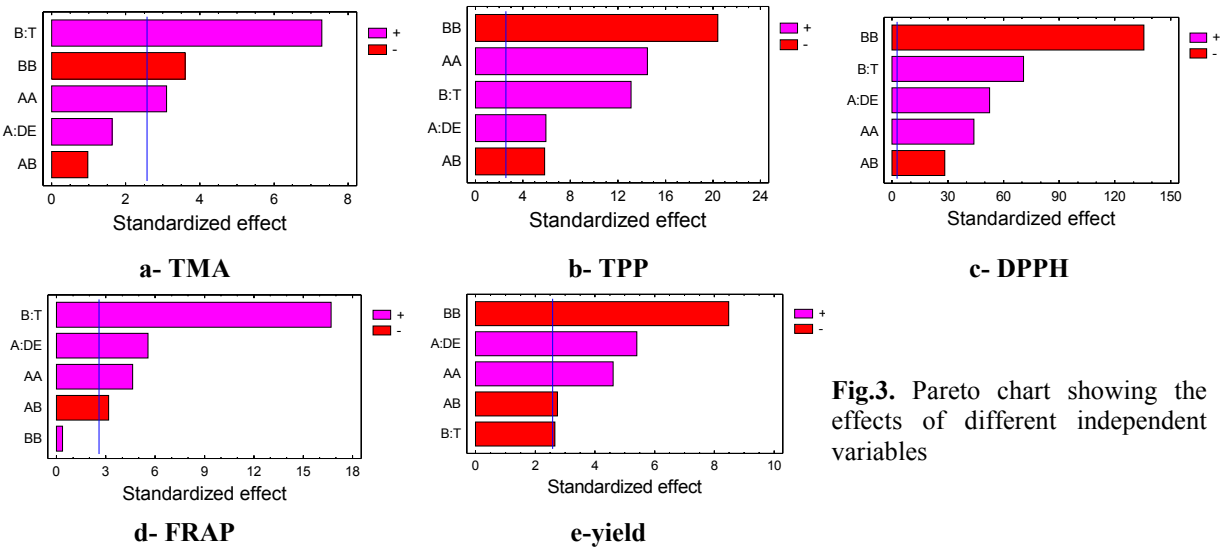

Fig.3. Pareto chart showing the effects of different independent variables

The model shows that time of enzymatic treatment influence the greatest selected dependent variables, such as the yield of extracts, TPP and radical scavenging activities (DPPH - test) the effect is quadratic. The effect is linear to the TMA and ferric reducing antioxidant power (FRAP).

In order to prepare the extract with high polyphenol and anthocyanin content graphical optimization was made (Fig.4).

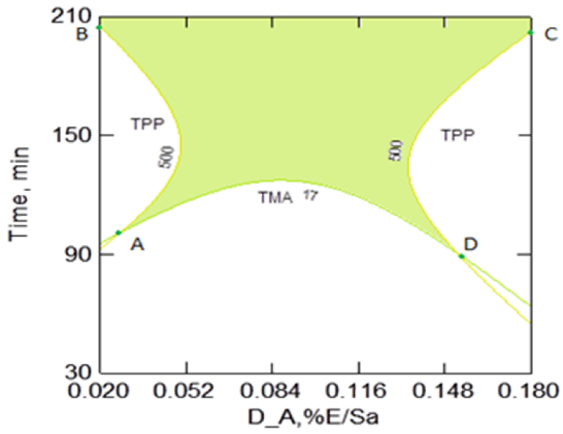

Fig.4. Graphics optimization of enzymatic extraction of rose petals as a function of extraction time and enzyme dosage

Optimization was carried out by the superposition of several contour surfaces of competing responses. The response surface plots were generated for interaction of two independent variables. The limits of target functions for optimization of enzymatic extraction of rose petals (*Rosa damascena* Mill.) were presented in Table 3.

Table 3. Limits of target functions for optimization of enzymatic extraction of rose petals

Equation №	Target function	Limits of target function
2	TMA,mg CGE/100 g	TMA > 17.0
3	TPP,mg GAE/100 g	TPP > 500.0

Intervals of variations of the enzyme mixture dosage (0.052 to 0.132%) and enzymatic treatment time (125-205 min) determine the field for obtaining the extracts with high content of total polyphenols and anthocyanins content.

CONCLUSIONS

The effect of ternary enzyme combinations included pectolytic, cellulytic and hemicellulytic preparation were investigated to optimize the extraction conditions of antioxidants and anthocyanins from rose petals (*Rosa Damascena* Mill.). The OCCD results show that the increment

of incubation time significantly affects the contents of total polyphenols and anthocyanins. A decrease in the time of enzyme treatment could be achieved with increasing of dosage of enzyme mixture.

The optimization process was focused on the total anthocyanins and total polyphenols content. Intervals of variations in the enzyme mixture dosage (0.052 to 0.132%) and the time of enzymatic treatment (125-205 min) determine the best conditions for preparing rose petals extracts with high content of total polyphenols and anthocyanins content.

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