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ENZYME-ASSISTED EXTRACTION OF POLYPHENOLS FROM ROSE (*ROSA DAMASCENA* MILL.) PETALS

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Abstract: The efficiency of enzyme-assisted extraction for the recovery of polyphenols from rose (*Rosa damascena* Mill.) petals was evaluated performing a simplex centroid experimental design for mixture with three components (pectinolytic, cellulolytic and hemicellulolytic preparation). The ternary enzyme combinations leaded to the highest contents of total polyphenols, reaching 43% higher average value as compared to the control (without enzymatic treatment) sample. Enzymatic treatments also enhanced (9–25%) the extractability of total anthocyanins. The results obtained demonstrate that enzyme-assisted extraction improves the recovery of polyphenolic antioxidants from rose petals, especially using ternary enzyme combinations, comprising pectinolytic, cellulolytic and hemicellulolytic preparation.

Keywords: polyphenols, enzyme-assisted extraction, *Rosa damascena*.

INTRODUCTION

Rosa damascena Mill., which belongs to the family Rosaceae and genus *Rosa*, is an important essential oil crop, with Bulgaria and Turkey being the main rose petal processing countries in the world (Ginova et al., 2013). Plants from genus *Rosa* have been used in phytopharmaceutical, essential oil and food industries for many years (Fenglin et al., 2004). Many studies (Cho et al., 2003; Ng et al., 2004; VanderJagt et al., 2002; Vinokur et al., 2006)

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associated the antioxidant activity of aqueous extracts from rose petals, primarily, with the presence of phenolic compounds. In addition to the antioxidant capacity, rose petal extracts have been reported to possess anti-inflammatory and analgesic (Choi and Hwang, 2003), antibacterial (Anesini and Perez, 1993; Perez and Anesini, 1994), antiviral (Mahmood et al., 1996) and antifungal (Dixit et al., 1976; Anesini and Perez, 1993) effects.

Enzyme-assisted extraction is gaining increasing attention as an advanced process to enhance the recovery of bioactive compounds from plant matrices. Cellulases, hemicelullases, and pectinases, as well as other enzymes, can be used to catalyze the hydrolysis of the cell wall polysaccharides, thus, enabling a better release and a more efficient extraction of phenolic compounds (Pinelo et al., 2008).

Therefore, the present study evaluated the efficiency of enzyme-assisted extraction for the recovery of polyphenols from Rosa damascena petals. In order to determine the optimal enzyme composition, a simplex centroid experimental design for mixture with three components (pectinolytic, cellulolytic and hemicellulolytic preparation) was performed.

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: Finely ground (particle size < 0.63 mm) rose petals were mixed with water (12:1, v/w), acidified (pH 3.0) with 1 M HCl, and left overnight for rehydration at 10 C. After pH adjustment (pH 3.0), the suspension (100.0 g) was placed in a 50 °C water bath for 20 min before 10 mL of an acidified water solution (1.2%, v/v) of enzyme preparation (single or mix) were added. After incubation for 2 h at 50 °C, the sample was placed

in a boiling water bath for 10 min to inactivate enzymes, then immediately cooled in an ice bath and centrifuged (4200 g × 15 min, 25 °C). The supernatant obtained was filtered through a paper filter.

Enzyme preparations were applied according to the following simplex centroid experimental design (Figure 1): 1 - 100% Pectinex Ultra Color (X1); 2 - 100% Rohament CL (X2); 3 - 100% Xylanase AN (X3); mix 1 - X1:X2 = 1:1; mix 2 - X1:X3 = 1:1; mix 3 - X2:X3 = 1:1; mix 4, 5, 6 - X1:X2:X3 = 1:1:1.

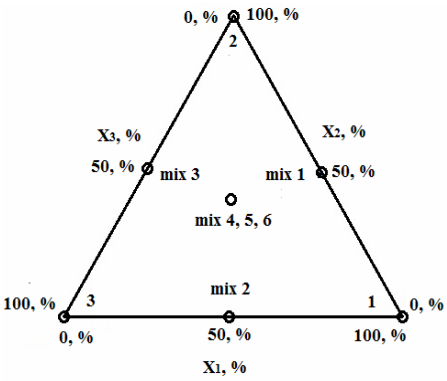

Figure 1. Ternary diagram for the simplex centroid experimental design: X1 - Pectinex Ultra Color; X2 – Rohament CL; X3 - Xylanase AN.

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.

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 (1965) and the pH-differential method (Giusti and Wrolstad 2001), respectively, modified as described by Dinkova et al. (2012).

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. (1995) and Benzie and Strain (1996), respectively, with some modifications (Dinkova et al., 2012).

Determination of enzyme activities: Endoglucanase, xylanase, β-xylosidase, β-glucosidase, polygalacturonase and protease activities of the enzyme preparations were determined using carboxymethyl cellulose (Dobrev et al., 2012), oat xylan (Dobrev et al., 2007), p-nitrophenyl-β-D-xylopyranoside

(Ponpium et al., 2000), p-nitrophenyl-β-D-glucopyranoside (Ponpium et al., 2000), pectin (Miller, 1959) and casein (Kaverzneva, 1971) as substrate, respectively.

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. The means were compared using one-way ANOVA, performed with Microsoft Excel, and Tukey's test at a 95% confidence level.

RESULTS AND DISCUSSIONS

Significant increases in the extract yields, contents of total polyphenols and anthocyanins and antioxidant capacity values were observed in response to the enzymatic treatments (Table 1).

As seen in Figure 2b, the ternary enzyme combinations leaded to the highest contents of total polyphenols, reaching 43% higher average value for the mix 4–6 extracts (Table 1) as compared to the control (without enzymatic treatment) sample. Consistent with Miron et al. (2013), these results suggest extensive cell wall degradation due to the combined action of the pectinolytic, cellulolytic and hemicellulolytic preparation.

Table 1.Treatment variants and results[a] for the simplex centroid design

Treatment variant	Yield (%)	TPP[b] (mg GAE/ 100 g)	DPPH[c] (µmol TE/ 100 g)	FRAP[c] (µmol TE/ 100 g)	TMA[d] (mg CGE/ 100 g)
Control (no enzyme)	34 ± 1a	308 ± 4a	2163 ± 45a	1114 ± 15a	12.6 ± 0.1a
1	46 ± 1b	389 ± 5b	2733 ± 56b	1864 ± 25b	15.1 ± 0.1b
2	41 ± 1c	325 ± 4c	1714 ± 35c	1445 ± 19c	13.8 ± 0.1c
3	50 ± 1d	359 ± 4d	2786 ± 57bd	1745 ± 23bd	14.4 ± 0.1d
Mix 1	50 ± 1d	358 ± 4d	2692 ± 55bd	1835 ± 24bd	15.6 ± 0.1e
Mix 2	50 ± 1d	352 ± 4d	2537 ± 52e	1855 ± 25e	14.6 ± 0.1df
Mix 3	50 ± 1d	368 ± 4de	2405 ± 50e	1774 ± 24f	13.7 ± 0.1cg
Mix 4,5,6	51 ± 1d	441 ± 5f	2767 ± 57bdf	1784 ± 24bdg	15.7 ± 0.1eh

[a] Means ± standard deviations (n = 3). Different letters within a column indicate significant differences (Tukey's test, $P < 0.05$).
[b] Results are expressed as mg gallic acid equivalents (GAE) per 100 g.
[c] Results are expressed as µmol Trolox equivalents (TE) per 100 g.
[d] Results are expressed as mg cyanidin 3-glucoside equivalents (CGE) per 100 g.

Enzymatic treatments enhanced (9–25%) the extractability of total anthocyanins (Table 1, Figure 2e). However, increasing the proportions of the

cellulolytic (X2) and/or hemicellulolytic (X3) preparation, the anthocyanin recovery decreased, which may be attributed to the secondary activity profiles (Table 2) of the enzyme preparations.

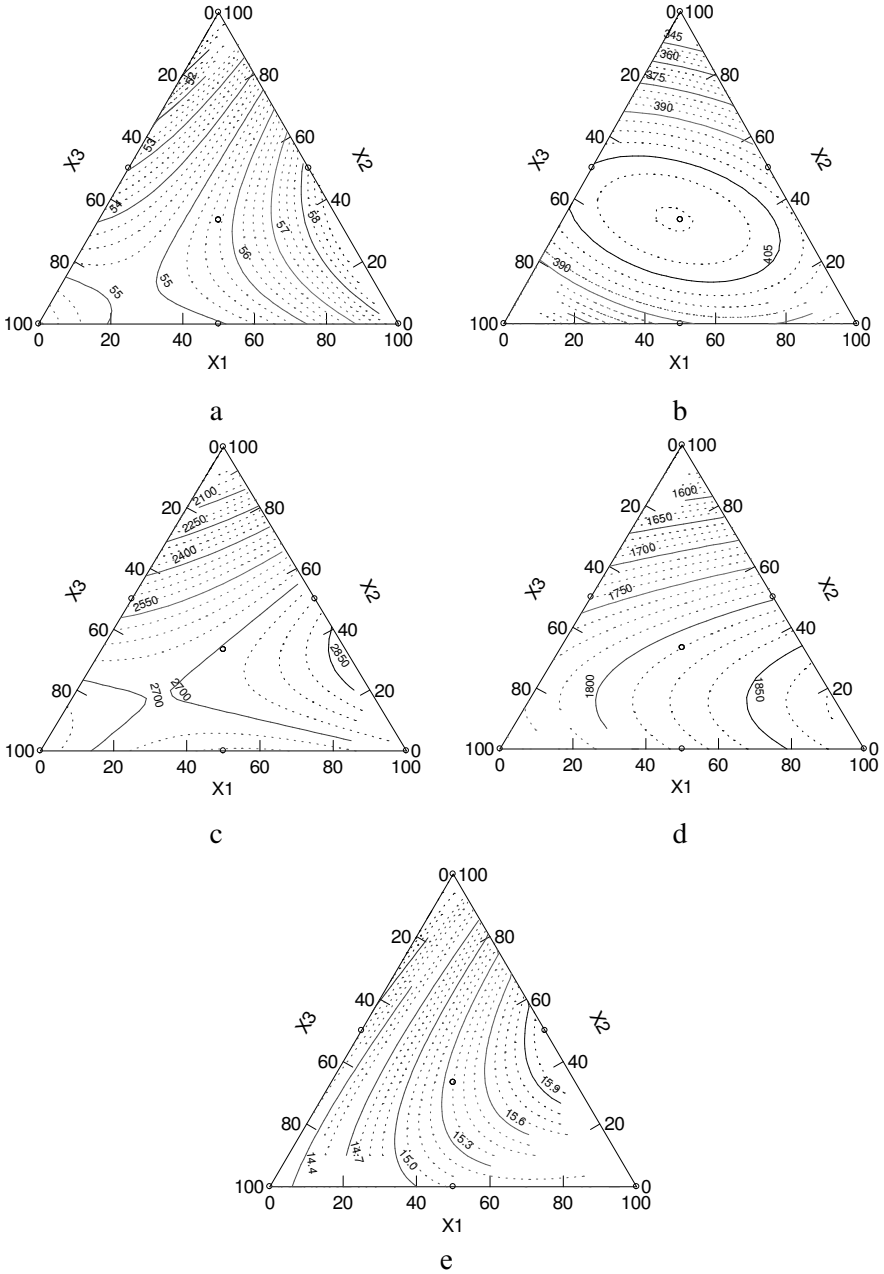

Figure 2. Simplex contour plots for the extract yield (a), TPP (b), DPPH (c), FRAP (d) and TMA (e). Enzymatic treatment variants as in Table 1.

The presence of β-glycosidases causes deglycosylation of the anthocyanidin glycosides, which in turn results in unstable anthocyanin aglycons and a subsequent decrease in anthocyanin levels.

Table 2.Activity profiles of the enzyme preparations

Enzyme activity	Enzyme preparation		
	X1[a]	X2[a]	X3[b]
Xylanase	1790.4	2086.1	6286.8
Polygalacturonase	706.5	290.4	411.7
β-Xylosidase	7.2	49.3	20.0
β-Glucosidase	9.6	80.9	-
Protease	12.7	-	36.4
Endoglucanase	437.5	2333.1	619.7

[a] Results are expressed in U/mL; [b] Results are expressed in U/g.

The two assays used represent different mechanisms of evaluating antioxidant capacity. While the DPPH assay measures the ability of plant extracts to scavenge free radicals, the FRAP assay quantifies the total concentration of redox-active compounds (Magalhães et al., 2008). In general, the changes of the total antioxidant capacity (Table 1, Figure 2c and Figure 2d) correspond to the results obtained for the total polyphenols.

CONCLUSIONS

The results obtained demonstrate that enzyme-assisted extraction enhances the recovery of polyphenolic antioxidants from rose petals, especially using ternary enzyme combinations, comprising pectinolytic, cellulolytic and hemicellulolytic preparation. This new process may offer an environmentally-friendly alternative to the conventional organic solvent extraction. However, further studies optimizing enzymatic treatment conditions, e.g. enzyme combination dosage and time-temperature regime, are required.

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