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Effect of pectolytic enzyme preparation on antioxidant capacity and color characteristic of rose petals extract (Rosa Damascena Mill.)

K. D. Kalcheva – Karadzhova[1*], K. M. Mihalev[2], D. P. Ludneva[1], V. T. Shikov[2], R. H. Dinkova[2], I. Y. Bakalov[1]

[1]*Food Research and Development Institute – Plovdiv, bul. Vasil Aprilov 154, Bulgaria*
[2]*University of Food Technology – Plovdiv, bul. Marica 26, Bulgaria*

The influence of three commercial pectolytic enzyme preparations (Pectinex Ultra Color, Panzym Pro Color and Panzym BE XXL) on the yield of total polyphenols, anthocyanins, antioxidant activities and color characteristic of extracts from rose petals (*Rosa Damascena* Mill) was investigated. As a result of the enzymatic treatment, the content of total polyphenols and anthocyanins general increase, by 18% and 15%, respectively. There is an overall increase up to 2.2 times in the antioxidant activity and an enhancement on the red color component intensity of the extracts.

Keywords: Rosa Damascena Mill., enzyme-assisted extraction, antioxidants, polyphenols, color

INTRODUCTION

Damask rose (*Rosa damascena* Mill.) is one of the most important medicinal and ornamental plants grown mainly for it's essential oil and medicinal aspects in many areas of the world, such as Bulgaria, Turkey, India and Iran [1].

Antioxidant compounds like phenolic acids, polyphenols such as peroxide, hydro- peroxide or lipid peroxyl inhibit the oxidative mechanisms that lead to degenerative diseases [2].

Rosa damascena is a plant rich in terpenes, glycosides, flavonoids, and anthocyanins but also contains carboxylic acid, myrcene, vitamin C, kaempferol and quarcetin [3, 4, 5, 6]. Its ability to be strengthening the heart, treatment of menstrual bleeding, digestive problems [7, 8]. and reduction of inflammation [3]. It also healing depression, grief, nervous stress and tension [3, 8].

The application of cell-wall degrading enzymes represents another novel approach in spice production. Enzyme-assisted extraction is an effective method for improving and recovery of bioactive compounds from plants and algae [9, 10]. Enzymatic hydrolysis of plant materials is currently being used for a variety of reasons, e.g. improving yields in juice processing [11], enhanced release of secondary plant metabolites [12], recovery of functional food ingredients [13].

To facilitate the extraction of polyphenols by destroying the cell wall matrix food industry is commonly used widely used pectolytic enzyme preparations. The use of pectolytic enzyme preparations cause destruction of the cell wall matrix, resulting in with maximizing juice yield and increased extraction of phenolic compounds, especially anthocyanins [14].

The purpose of this paper was to investigate the possibilities for achieving enhanced extraction using of three commercial pectolytic enzyme preparations (Pectinex Ultra Color, Panzym Pro Color and Panzym BE XXL) on the juice yield, yield of total polyphenols, anthocyanins, antioxidant activities and color characteristic of extracts from rose petals (*Rosa Damascena* Mill). The obteined extract will be a good base to used in different areas of the food industry.

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 three commercial pectinolytic enzyme preparations were used: Pectinex Ultra Color (Novozymes A/S, Bagsvaerd, Denmark),

* To whom all correspondence should be sent:
krasimiradecheva@abv.bg

Panzym BE XXL and Panzym Pro Color were provided from Begerow GmbH& Co, Langenlonsheim, Germany.

Plant material

Rose (*Rosa damascena* Mill.) petals, harvest year 2012, 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

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 (1.0%, v/v) of enzyme preparation 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.

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. The resulting extracts where analysing.

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 as described by Singleton et al. [15] and the pH-differential method [16], respectively, modified as described by Dinkova et al. [17].

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. [18] and Benzie and Strain [19] respectively, with some modifications [Dinkova et al.].

The content of total dry matter (%) in the extract from *Rosa Damascena* petals was determined by weight moisture analyzer (Kern "MLB-50-3, Kern & Sohn GmBH, Germany).

The yield of the extract wre determined by a measuring cylinder after centrifugation and filtration of the sample.

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

The results show (table 1) that the use of other pectinolytic enzyme preparations does not affect the extraction of phenolic compounds, anthocyanins, yield of extract and dry matter.

As a result of the enzymatic treatment, the content of total polyphenols and anthocyanins general increase, by 18% and 15% respectively.

The values of antioxidant capacity of enzymaticaly treated samples were 1.1 - 1.2 times, and 1.6 - 2.2 times higher, for the DPPH- and FRAP -test, compared the control. This is with positive correlation with the increased values of polyphenols.

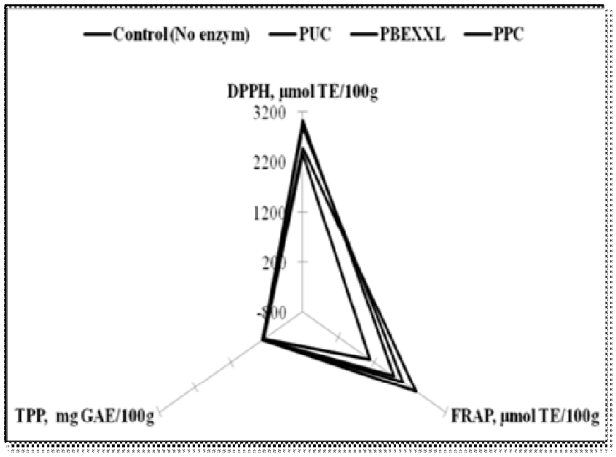

Fig.1. Radar chart of the antioxidant capacity of the extract of rose petals depending on processing different pectinolytic preparations

The radar diagram (Fig.1) of Terashima et al. [20] was used for characterization of total antioxidant capacity. The polyphenols antioxidants, acting as both a donor of electrons (FRAP-test) and hydrogen atoms (DPPH-test) are essential for the activities of extracts from rose petals (*Rosa Damascena* Mill.).

The color indicators in CIE*Lab* and CIE*Lch* system were given in Fig.2.

Enhance of the red color component intensity of the extracts, evaluated in the CIE*Lab* and CIE*Lch* system, was reported by the increase in the value of the red component (a^*) and decrease the hue angle ($h°$). Results are consistent with an increase in anthocyanin content as a result of enzymatic treatment (Table 1).

Table 1. Treatment variants and results for the extracts (supernatant) from Rosa Damascena petals (*Rosa Damascena* Mill.)

Treatment variant	TMA[1], mgCGE/100g	TPP[2], mgGAE/100g	DPPH[3], µmolTE/100g	FRAP[3], µmolTE/100g	Yield, %	Dry matter,%
Control (no enzyme)	6.7 ± 0.3a	271.0 ± 12a	2463.0 ± 111a	1078.0 ± 47a	30.5 ± 1a	3.2 ± 0.1a
PUC	7.7 ± 0.3b	320.0 ± 14b	2720.0 ± 122b	1750.0 ± 79b	48.5 ± 2b	4.0 ± 0.2b
PBEXXL	7.7 ± 0.4b	320.0 ± 14b	2882.0 ± 130b	2002.0 ± 90c	47.0 ± 2b	4.1 ± 0.2c
PPC	7.7 ± 0.3b	318.0 ± 14b	2865.0 ± 129b	2367.0 ± 107d	50.0 ± 2b	4.3 ± 0.2d

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

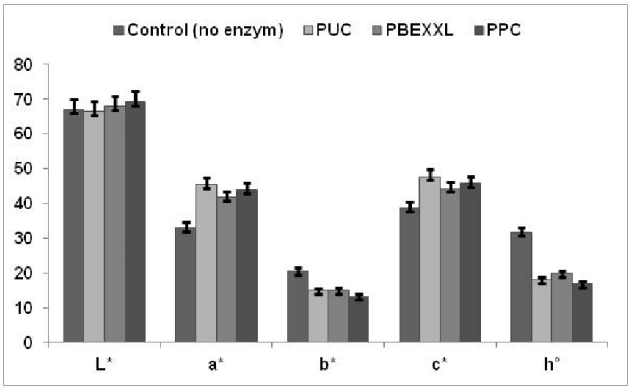

Fig.2. Color indicators in CIE*Lab* and CIE*Lch* system on extracts from petals of *Rosa damascena* depending on the various pectinolytic enzyme preparations (mean ± SD; n= 3)

CONCLUSIONS

The results demonstrate that enzyme-assisted extraction enhances the recovery of polyphenolic antioxidants from rose petals (*Rosa Damascena* Mill.), with all types of pectolytic enzyme preparations. This new process may offer an environmentally-friendly alternative to the conventional organic solvent extraction. Due to the high extraction yields, and high total polyphenols content of extracts from rose petals it could be used in food, cosmetic and pharmaceutical industries.

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