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# Optimizing Enzymatic Maceration of Blackcurrant Mash by Response Surface Methodology

Krasimira Petrova[1], Todorka Petrova[1], Petya Ivanova[1], Milena Ruskova[1], Ivan Bakalov[1], Kiril Mihalev[2], and Nikolay Penov[2]

*1. Food Research and Development Institute, Bulgaria*

*2. University of Food Technologies, Bulgaria*

**Abstract:** Pre-press maceration with commercial enzyme preparation (Pectinex Ultra Color) was investigated in experimental blackcurrant (Ometa variety) juice production using response surface methodology (RSM). Enzyme dosage and maceration time were varied at a constant temperature of 50°C and the total polyphenols (TPP), total monomeric anthocyanins (TMA), antioxidant capacity and juice yields (Y) were compared. The contents of total polyphenols and anthocyanins ranged from 828.57 to 1274.96 mg GAE/100 g and 326.76 to 383.49 mg CGE/100 g blackcurrant juice, respectively. The total antioxidant capacity determined by the free radical scavenging activity (DPPH) of the juice ranged from 3977.82 to 4580.18 µmol TE/100 g and ferric reducing antioxidant power (FRAP) from 4749.19 to 6579.74 µmol TE/100 g. Juice yields ranged from 44.57 to 50.31% by wet weight of the mash. The criterion established to determine the optimal conditions of enzymatic maceration of blackcurrant mash was to find the conditions leading to high values of total monomeric anthocyanins, total polyphenols, and juice yield.

**Key words:** response surface methodology (RSM), enzymatic maceration, juice, blackcurrant

## 1. Introduction

Blackcurrants (*Ribesnigrum* L.) are one of the most popular and widely cultivated species in Europe. It is traditionally used for producing of juices, nectars, jams, and nutraceutical ingredients. Blackcurrants are rich in phenolic compounds, notably anthocyanins and hydroxycinnamic acids. The water-soluble anthocyanins in black currants are mainly found in the skin [1].

Pectolytic enzymes are commonly used in industrial blackcurrant juice production to facilitate juice extraction [2]. These enzymes are used during conventional processing cause degradation of the cell wall matrix and increased the juice yield [3]. Enzyme-assisted blackcurrant juice processing significantly increases the contents of anthocyanins and other phenolic compounds in the juice [4].

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

Response surface methodology (RSM) is an effective tool which uses quantitative data in an experimental design to optimize a process [6].RSM has been used for optimizing processes in fruit and vegetable juice production [5, 7, 8].

The aim of this study was to optimize the enzymatic mash maceration using RSM, thus allowing extraction of polyphenol-rich blackcurrant juice.

## 2. Material and Methods

### *2.1 Chemicals*

For the analytical purposes DPPH [2,2-diphenyl-1-picrylhydrazyl]; TPTZ [2,4,6-Tris(2-pyridyl)-s- triazine ]; Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchroman

**Corresponding author:** Krasimira Petrova, Ph.D., research area: food technologies. E-mail: krasi_petrova80@abv.bg.

-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); Folin-Ciocalteau's (FC) reagent (Merck, Darmstadt, Germany); and Gallic acid monohydrate (Fluka, Buchs, Switzerland) were used. All the other reagents and solvents used were of analytical grade.

### 2.2 Enzyme Preparation

Pectinex Ultra Color was obtained from Novozymes A/S (Bagsvaerd, Denmark). It is produced from *Aspergillusaculeatus* and *Aspergillusniger*, with declared activity of 10 000 PECTU/mL, and is brown liquid. The suitable maceration temperature is 50°C and is active in the pH range of 3.0-5.0.

### 2.3 Plant Material and Processing

Blackcurrant (*Ribesnigrum* L.) fruits, at a commercial maturity stage (harvest 2015) were from Research institute of mountain stockbreeding and agriculture, Troyan, Bulgaria.

After sorting to eliminate unriped, overriped or damaged fruits, blackcurrant fruits were frozen stored until used.

Frozen fruits were processed according to the flow diagram shown in Fig. 1.

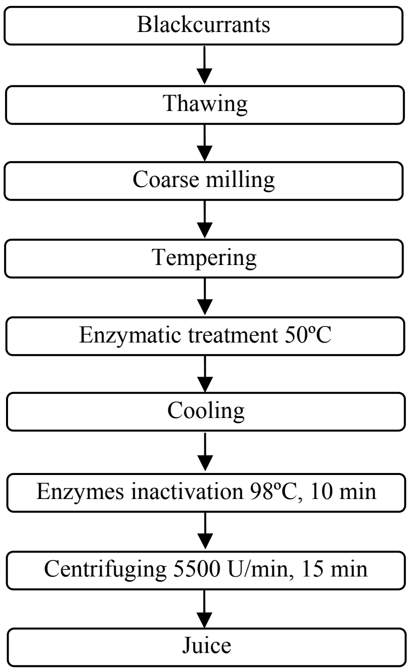


**Fig. 1 Process flowchart for extraction of the blackcurrant juice.**

### 2.4 Analytical Methods

All measurements were performed with a Helios Omega UV-Vis spectrophotometer equipped with VISIONlite software (all from Thermo Fisher Scientific, Madison, WI, 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 Dinkova et al. [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].

### 2.5 Experimental Design and Data Analysis

The results reported in the present study are the mean values of at least three determinations, and the coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were found to be < 5% in all cases.

An optimal central composite design (OCCD) was used to investigate the effect of the enzyme dosage($X_1$) and maceration time($X_2$) on the response (y) in 11 runs [6]. The outline of the experimental design is outlined in Table 1.

**Table 1 Experimental design for the enzymatic maceration.**

| Run № | Coded levels | | Actual levels | |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | Enzyme dosage (%E/S[a]) | Maceration time (min) |
| 1 | -1 | -1 | 0.02 | 30 |
| 2 | +1 | -1 | 0.18 | 30 |
| 3 | -1 | +1 | 0.02 | 210 |
| 4 | +1 | +1 | 0.18 | 210 |
| 5 | -1 | 0 | 0.02 | 120 |
| 6 | +1 | 0 | 0.18 | 120 |
| 7 | 0 | -1 | 0.10 | 30 |
| 8 | 0 | +1 | 0.10 | 210 |
| 9 | 0 | 0 | 0.10 | 120 |
| 10 | 0 | 0 | 0.10 | 120 |
| 11 | 0 | 0 | 0.10 | 120 |

[a] ml enzyme preparation per 100 g substrate.

A second order polynomial model for the dependent variables TMA, TPP, DPPH, FRAP, and juice yield (response, y) was established to fit the experimental data:

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

where $b_0$ = intercepts, $b_i$ are linear, $b_{ii}$ are quadratic, and $b_{ij}$ are interaction regression coefficient terms.

SYSTAT statistical software (SPSS Inc., Chicago, USA, version 7.1) and Excel were used to analyze the data results.

## 3. Results and Discussion

The mean values of TMA, TPP, DPPH, FRAP, and juice yield are shown in Table 2. The contents of total polyphenols and anthocyanins ranged from 828.57 to 1274.96 mg GAE/100 g and 326.76 to 383.49 mg CGE/100 g blackcurrant juice, respectively. The total antioxidant capacity determined by the free radical scavenging activity (DPPH) of the juice ranged from 3977.82 to 4580.18 µmol TE/100 g and ferric reducing antioxidant power (FRAP) from 4749.19 to 6579.74 µmol TE/100 g. Juice yields ranged from 44.57 to 50.31% by wet weight of the mash.

**Table 2 TMA, TPP, DPPH, FRAP, and blackcurrant juice yield.**

| Run № | TMA[a] | TPP[b] | DPPH[c] | FRAP[c] | Yield (%) |
|---|---|---|---|---|---|
| 1 | 326.76 | 828.57 | 3977.82 | 4749.19 | 44.57 |
| 2 | 366.78 | 893.32 | 3944.34 | 5178.07 | 47.58 |
| 3 | 383.49 | 1138.27 | 4330.31 | 6288.62 | 46.00 |
| 4 | 370.90 | 1274.96 | 4580.18 | 6579.74 | 46.19 |
| 5 | 353.34 | 966.50 | 3979.66 | 5988.87 | 44.87 |
| 6 | 369.94 | 1128.12 | 4231.05 | 6182.20 | 45.20 |
| 7 | 355.76 | 1017.01 | 3971.74 | 5181.64 | 50.31 |
| 8 | 379.33 | 1259.28 | 4715.08 | 6547.19 | 50.09 |
| 9 | 375.87 | 1136.29 | 4270.88 | 6244.37 | 49.60 |
| 10 | 375.32 | 1172.40 | 4298.94 | 6434.10 | 49.41 |
| 11 | 367.64 | 1086.43 | 4358.94 | 6102.71 | 49.12 |

[a] Results are presented as mg cyanidin 3-glucoside equivalents (CGE) per 100 g juice;
[b] Results are presented as mg gallic acid equivalents (GAE) per 100 g juice;
[c] Results are presented as µmoltrolox equivalents (TE) per 100 g juice.

The regression equations describing the effect of enzymatic maceration of blackcurrant mash on the TMA, TPP, DPPH, FRAP, and juice yield are given in Table 3. The resulting equations with high accuracy describe the change of the contents of the dependent variable ($R^2 > 0.9$). The coefficients in the regression equations can be used to examine the significance of each term relative to each other when used with coded values [14].

Each of the estimated effects and interactions are shown in the standardized diagram — the Pareto diagram (Fig. 2). It consists of horizontal blocks with lengths proportional to the absolute values of the estimated effects, divided by their standard errors. The vertical line in the Pareto diagram represents the value of the Student criterion at 95% confidence level and separates factors that are significant to those that are not. The diagrams show the predominance of the maceration time (factor B) for TMA, TPP, DPPH, and FRAP. The quadratic effect due to the enzyme dosage (factor A) has the highest impact on the juice yield.

The effect of changes in enzyme dosage and maceration time on TMA, TPP, DPPH, FRAP, and juice yield is given in Fig. 3. The values of anthocyanins, total polyphenols and antioxidant capacity increased with an increase in the maceration time. The yield increased between 0.02 and 0.12% E/S and then decreased between 0.12 and 0.18% E/S. Similar results were reported by Dinkova et al. [15].

**Table 3 Coefficients of regression models for TMA, TPP, DPPH, FRAP, and blackcurrant juice yield.**

| Term | TMA | TPP | DPPH | FRAP | Yield |
|---|---|---|---|---|---|
| | Coefficients | | | | |
| constant | 304.72 | 727.25 | 3793.15 | 4092.97 | 42.54 |
| $X_1$ | 569.75* | 3422.45* | 5041.55 | 7787.34* | 151.05* |
| $X_2$ | 0.41* | 1.60* | 0.14* | 20.04* | -0.02 |
| $X_1X_1$ | -1294.04* | -14829.1* | -26238.2* | -26552.8 | -659.75* |
| $X_1X_2$ | -1.83* | 2.50 | 9.84 | -4.78 | -0.10* |
| $X_2X_2$ | -0.0003 | -0.0005 | 0.009 | -0.05* | 0.0001* |
| $R^2$ | 0.97 | 0.96 | 0.95 | 0.98 | 0.98 |

$X_1$ – enzyme dosage (%E/S), $X_2$ – maceration time (min), $R^2$ – coefficient of determination.
*Significant at 95% CI.

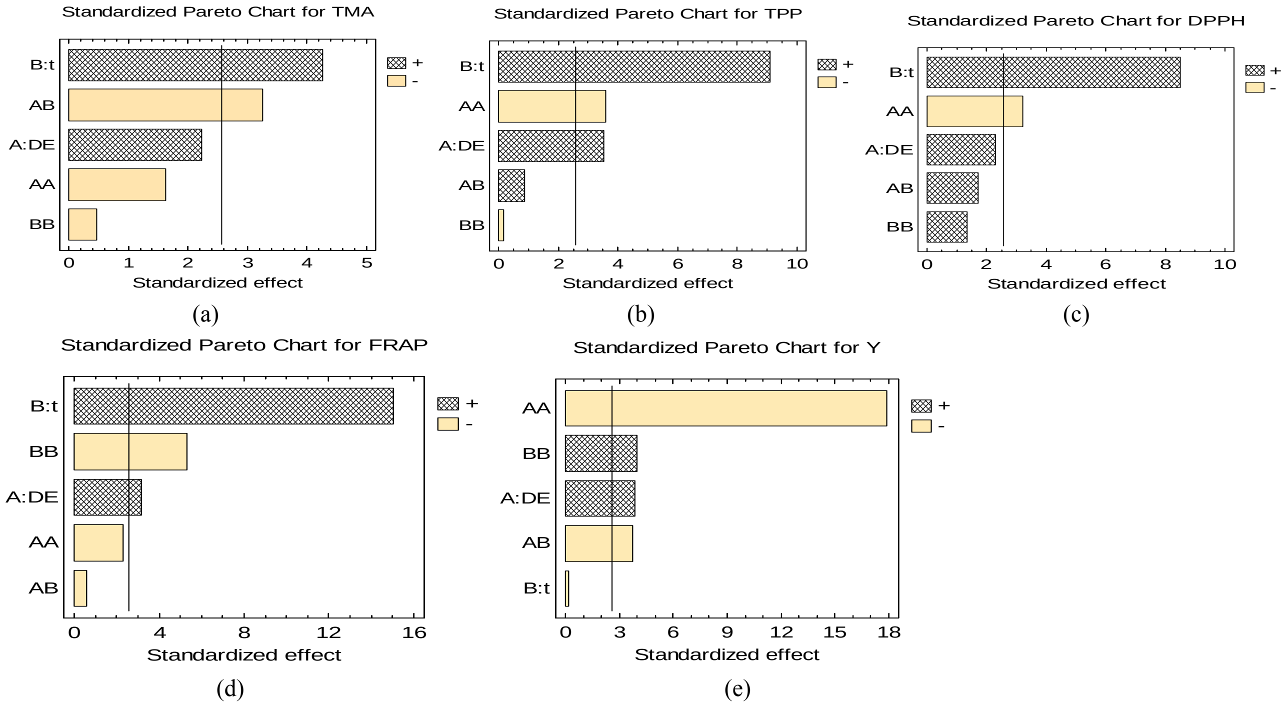


**Fig. 2 Estimated effects of regression model coefficients on TMA (a), TPP (b), DPPH (c), FRAP (d), and juice yield (e).**

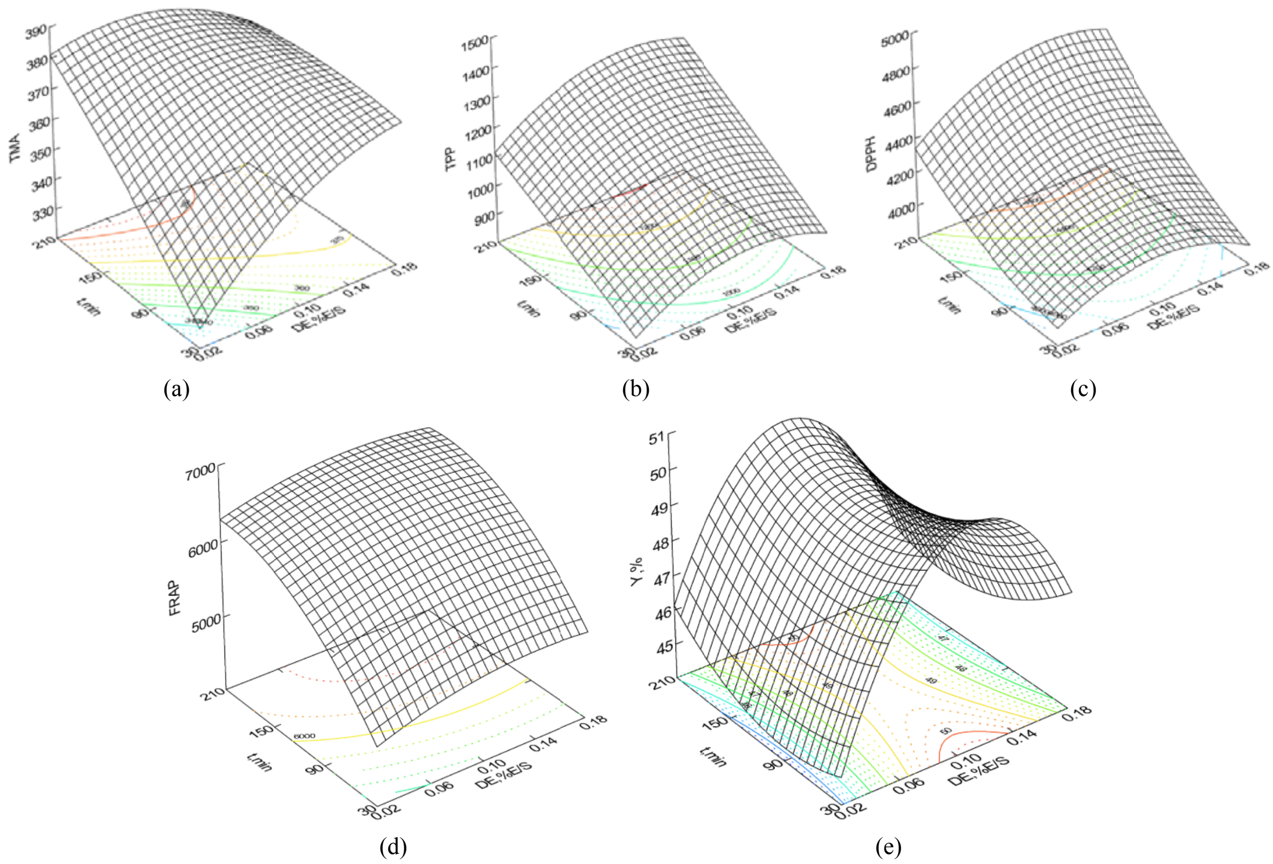


**Fig. 3 Effect of enzyme dosage and maceration time on TMA (a), TPP (b), DPPH (c), FRAP (d), and juice yield (e)**

The criterion established to determine the optimal conditions of enzymatic maceration of blackcurrant mash was to find the conditions leading to high values of total monomeric anthocyanins (TMA > 370 mg CGE/100 g), total polyphenols (TPP > 1100 mg GAE/100 g), and juice yield (Y > 48%). Optimization was carried out by the superposition of the contour plots for predicted TMA, TPP, and juice yield. The optimum area for the enzymatic mash maceration is presented on Fig. 4 (the darkened area). On each point of this area corresponds conditions of enzymatic maceration of blackcurrant mash leading to obtain the juice with high values of TMA, TPP, and Y.

## 4. Conclusions

The effect of conditions of enzymatic maceration of blackcurrant mash on the total monomeric anthocyanins, total polyphenols, antioxidant capacity, and juice yield was investigated. The choice of the preferred conditions was according to survey of other researchers on enzymatic maceration. The linear effect due to the maceration time had the highest impact on TMA, TPP, DPPH, and FRAP. The quadratic effect due to the enzyme dosage had the highest impact on the juice yield. The criterion established to determine

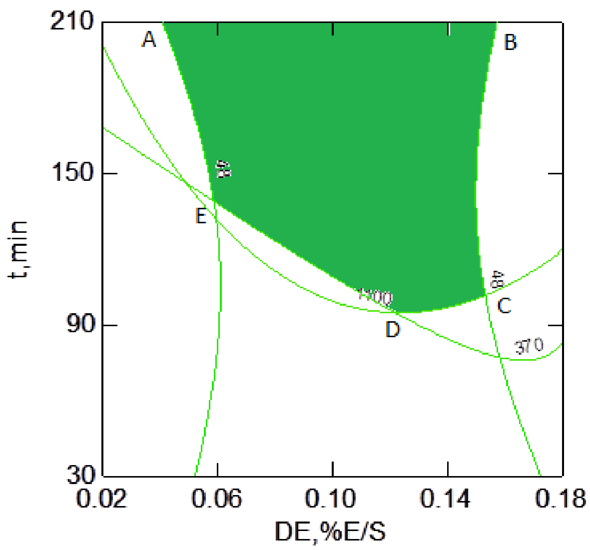


**Fig. 4 Graphical optimization of the enzymatic maceration of blackcurrant mash.**

the optimal conditions of enzymatic maceration of blackcurrant mash was to find the conditions leading to high values of total monomeric anthocyanins, total polyphenols, and juice yield. Maceration time from 120 to 210 min and enzyme dosage from 0.06 to 0.16% E/S could be recommended as the optimal conditions of enzymatic maceration of blackcurrant mash for obtaining juice with high values of TMA, TPP, and Y.

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