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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF ANTHOCYANINS FROM *ARONIA MELANOCARPA* BY-PRODUCT FROM FILTER-TEA FACTORY

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Abstract: Chokeberry (Aronia melanocarpa) fruits represent good source of polyphenolic compounds, particularly anthocyanins. In this research extraction of chokeberry fine cut (processed plant material obtained after cutting, grinding, sifting and fraction collecting in the herbal filter tea factory) was investigated. Ultrasound-assisted extraction (UAE) of dried and grounded chokeberry fruits were optimized using response surface methodology (RSM). Experiments were carried out according to a three level, three variable face-centered cubic design (FCD) with ultrasonic power (X_1: 72-216 W), temperature (X_2: 30-70°C) and extraction time (X_3: 30-90 min) as independent variables. Experimental results were fitted to a second-order polynomial model where multiple regression analysis and analysis of variance were used to determine fitness of the model and optimal conditions for maximizing the extraction of total extractable compounds (TEC) and total anthocyanins (TA). Three-dimensional surface plots were generated from the mathematical models. The optimal conditions for the UAE of TEC and TA were: X_1 = 184 W, X_2 = 70°C, X_3 = 86.1 min and X_1 = 208.8 W, X_2 = 70°C, X_3 = 51.9 min, respectively. Generated model predicted values of the TEC and TA to be 40.40 mg/ml and 2.73 mg C3G/ml, respectively. Experimental validation was performed and close agreement between experimental and predicted values was found.
Keywords: Aronia melanocarpa, anthocyanins, response surface methodology

1. INTRODUCTION

Aronia melanocarpa is a member of the Rosaceae family and originates from North America, but more recenlty it is cultivated in Eastern Europe (Benvenuti et al. 2004). This fruit is highlighted with respect to its food-coloring properties (Birdle and Timberlake, 1997) and as a suitable food supplement for strengthening human immunity system (Jakobek et al., 2007). Chokeberries have high content of polyphenolic compounds, particularly anthocyanins. Four cyanidin glycosides are responsible for dark red color of these fruits (3-O-galactoside, 3-O-glucoside, 3-O-arabinoside and 3-O-xyloside) (Oszmianski and Sapis, 1998). It was reported that red pigment fraction of black chokeberry has a potent antioxidative activity in both in vitro and in vivo systems (Matsumoto et al., 2004).

Ultrasound-assisted extraction (UAE) has already showed many advantages comparing to conventional solvent extraction in significant reduction of extraction time and enhancement of process efficiency, i.e. increasing in yields of desirable group(s) of compounds. Improvement of solvent extraction from plant material by ultrasound is due mainly to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into plant material and the intracellular product release by disrupting the cell walls (Mason et al., 1996). Significant enhencement of the mass transfer from damaged cells into crude extract comes as a final result.

Optimization of the extraction parameters represents another improvement of the process. In that purpose, response surface methodology (RSM) was applied. Comparing to one-factor-at-a-time optimization, RSM is able to overcome main disadvatages such as determination of interactive effects of variables, and reduction in time and cost. Its main objective is to simultaneously optimize levels of these variables to attain the best system performance (Bezerra et al., 2008).

In this study, ultrasonic power (72-216 W), temperature (30-70°C) and extraction time (30-90 min) were investigated UAE parameters. They were optimized using face-centered cubic design (FCD) and RSM in order to obtain highest yields of total extractable compounds (TEC) and total anthocyanins (TA) in liquid extracts.

2. MATERIALS and METHODS

2.1. Materials

Dried and grounded fruits of A. melanocarpa (fraction which passed through 0.315 mm sieve) were obtained from Fructus (Bačka Palanka, Serbia) as by-product from filter-tea industry. All other chemicals and reagents were of analytical grade.

2.2. Sample preparation and determination of total extractable compounds (TEC)

All experimental runs in face-centered cubic design (FCD) were performed by mixing 10 g of powdered chokeberry fruits and 100 ml of 50% ethanol. Extraction process was carried out in sonication water bath (EUP540A, Euinstruments, France) at 40 kHz frequency. Independent variables were controlled by the instrument. After extraction was finished, crude extracts were filtered through filter paper under vacuum. Certain volume of liquid extract was evaporated under vacuum and dried at 105°C during 2 hours. After that, the mass of dry extract was measured and TEC yield was expressed as mg/ml of liquid extract. Residual liquid extract was collected into glass flasks and stored at 4°C until the analysis.

2.3. Determination of total anthocyanins (TA)

Total anthocyanins (TA) were determined by single pH method (Fuleki et al., 1968). Potassium chloride buffer, pH 1.0 (0.0025 M) was used. Briefly, 400 µl of sample (diluted liquid extract) was added in 3.6 ml of potassium chloride buffer and absorbance was measured against a blank probe at 510 and 700 nm. Absorbance (A) was measured using VIS-spectrophotometer (6300, Jenway, United Kingdom) and calculated as:

$$A = (A_{510} - A_{700})_{pH1.0} \quad (1)$$

Anthocyanin concentration in the extract (TA) was calculated and expressed as cyanidin-3-glycoside equivalent (C3G):

$$TA = \frac{A \cdot MW \cdot DF \cdot 1000}{M_a} \quad (2)$$

where A is obtained from Eq. (1), MW is a molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor of the samples and M_a is the molar absorptivity of cyanidin-3-glucoside (26.900 M·cm^{-1}). Results were expressed as mg of cyanidin-3-glucoside equivalents (C3G) per ml of liquid extract (mg C3G/ml).

2.4. Design and statistical analysis

Face-centered cubic design (FCD) with three variables on three levels was applied in order to determine optimal extraction conditions for TEC and TA as responses. Natural and coded values of independent variables (ultrasonic power – X_1, temperature – X_2 and extraction time – X_3) on all experimental runs are presented in Table 1. A second-oder polynomial regression model was used for mathematical modelling of the extraction system (Baş and Boyaci, 2007):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i + \sum \sum_{i<j=1}^{3} \beta_{ij} X_i X_j \quad (3)$$

where Y represents the response, X_i and X_j are the process variables affecting the response and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively. Multiple regression analysis, analysis of variance (ANOVA) and optimization were realized using the Design-Expert v.7 Trial (Stat-Ease, Minneapolis, Minnesota, USA).

3. RESULTS and DISCUSSION

Experimental results of TEC and TA obtained in all runs are presented in Table 1, and they varied from 20.46 to 40.26 mg/ml and from 1.82 to 2.73 mg C3G/ml, respectively. The lowest yield for both responses were on the same experimental point (X_1=216 W; X_2=30°C; X_3=30 min), while conditions where TEC and TE were highest were X_1=216 W; X_2=70°C; X_3=90 min and X_1=144 W; X_2=70°C; X_3=60 min, respectively.

Significant regression coefficients generated by the Eq. (3) and coefficients of multiple determination (R^2) for investigated models are presented in the Table 2. Particularly high values of R^2 indicated that suggested mathematical model fits well to the experimental data. ANOVA was used to determine statistical significance for the model and the lack of fit. According to low p-value for the model (<0.05) and high p-value for the lack of fit (>0.05), adequacy of the model was confirmed. Suggested model predicts good reproducibility due to relatively low values of coefficients of variation (CV<10%).

Table 1 Face-centered cubic design with observed and predicted values of the TEC and TA

Run	X_1 Ultrasonic power [W]	X_2 Temperature [°C]	X_3 Extraction time [min]	TEC [mg/ml]		TA [mg C3G/ml]	
				observed	predicted	observed	predicted
1	144 (0)	50 (0)	60 (0)	23.12	25.25	2.10	2.08
2	144 (0)	50 (0)	60 (0)	24.30	25.25	1.99	2.08
3	144 (0)	50 (0)	60 (0)	24.28	25.25	2.01	2.08
4	72 (-1)	50 (0)	60 (0)	20.78	21.34	1.88	1.95
5	216 (1)	50 (0)	60 (0)	27.74	25.15	2.29	2.15
6	72 (-1)	30 (-1)	30 (-1)	20.70	20.57	1.92	1.90
7	72 (-1)	30 (-1)	90 (1)	23.46	22.77	2.10	2.04
8	144 (0)	30 (-1)	60 (0)	24.02	24.09	2.10	2.15
9	216 (1)	70 (1)	30 (-1)	36.32	37.52	2.59	2.66
10	216 (1)	70 (1)	90 (1)	40.26	40.89	2.55	2.59
11	144 (0)	70 (1)	60 (0)	39.90	37.81	2.73	2.61
12	72 (-1)	70 (1)	30 (-1)	31.76	32.26	2.37	2.42
13	72 (-1)	70 (1)	90 (1)	34.14	33.90	2.23	2.18
14	144 (0)	50 (0)	30 (-1)	26.50	24.19	2.09	1.94
15	144 (0)	50 (0)	90 (1)	26.68	26.97	1.88	1.97
16	216 (1)	30 (-1)	30 (-1)	20.46	21.20	1.82	1.88
17	216 (1)	30 (-1)	90 (1)	25.12	25.13	2.21	2.18

Obtained regression coefficients provide information about the influence of process parameters on investigated response. Linear terms of all independent variables exhibited positive effects on TEC, whilst only ultrasonic power and temperature had significant effects in case of TA. For both responses, temperature effects were the most notable which is in accordance with previous reports (Fan et al., 2008). Elevated temperature has multiple effects on mass transfer from plant material to crude extract, increasing the diffusivity of extraction solvent into plant cells and enhancing the solubility of extractable compounds (Cacace and Mazza, 2003). Moreover, it reduces the solvent viscosity and surface tension. Positive linear effects of ultrasonic power on both responses could be explained by the increased disruption and softening of the plant tissues. Stronger ultrasonic waves weaken the cell walls and enhace the release of bounded compounds. Positive effect of extraction time indicates that longer period is needed for the final equilibrium between solute from the plant matrix and the crude extract. On the other hand, prolonigng the extraction time could cause degradation of polyphenolic compounds due to oxidation and temperature damage (d'Alessandro et al., 2012).

Table 2. Significant regression coefficients for the predicted quadratic model for TEC and TA

Total extractable compounds: R^2=0.96; CV=7.03%				
β_0	β_1	β_2	β_3	β_{22}
25.2482	1.9060	6.8620	1.3920	5.7007
Total anthocyanins: R^2=0.91; CV=5.65%				
β_0	β_1	β_2	β_{23}	β_{22}
2.0766	0.0962	0.2309	-0.0946	0.3060

Temperature exhibited positive quadratic effects on TEC and TA. This indicate that extractable compounds from A. melanocarpa (including anthocyanins) seem to need more energetic conditions to be delivered from the plant material (Silva et al., 2007). Interaction between temperature and extraction time had significant negative influence on the extraction of anthocyanins. It was confirmed that prolonged extraction on elevated temperature produces irreversible degradation of anthocyanins. Influence of investigated UAE conditions on TEC and TA was accomlished with response surface plots (Figure 1). Ultrasonic power and extraction time expressed rather linear influences while temperature effects where more influental comparing to other variables.

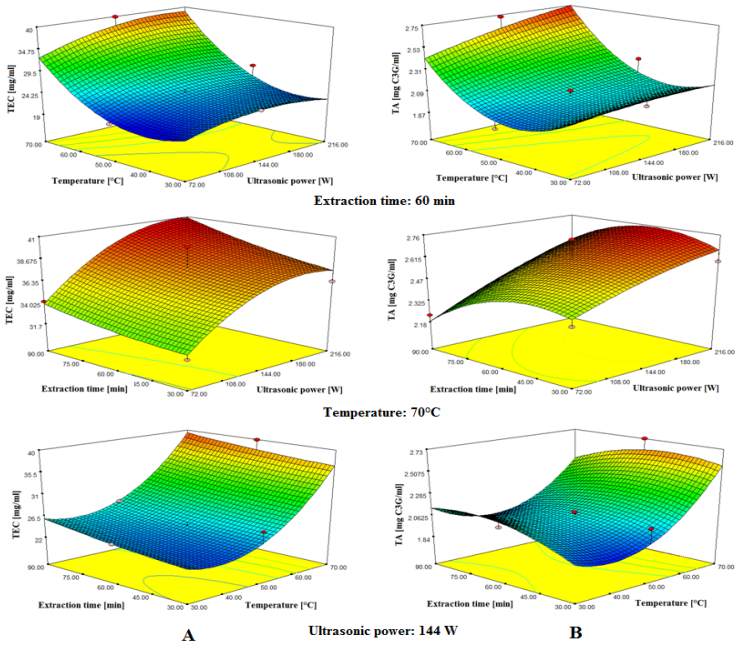

Figure 1. Three-dimensional contour plots for (A) TEC and (B) TA

Due to the most significant influence of the temperature and the highest experimentally obtained TEC and TA on the higher level of temerature (1), it was expected that this temperature will be optimal for extraction of TEC and TA. The best combination of UAE parameters for the maximization of TEC extraction were: X_1 = 184 W; X_2 = 70°C; X_3 = 86.1 min; and using the Eq. (3) it was predicted that TEC obtained on these conditions would be 40.40 mg/ml. In case of anthocyanins extraction, optimized parameters were X_1 = 208.8 W; X_2 = 70°C; X_3 = 51.9 min; with expected TA yield of 2.73 mg C3G/ml. Reduction of extraction time is beneficial due to preventing the decomposition of anthocyanins on elevated temperature and providing the faster and cheaper production. Liquid extracts obtained at determined optimal conditions could be utilized as a feed in the production of anthocyanin-rich A. melanocarpa extracts using the spray drying process. For that purpose, drying parameters such as inlet temperature, flow and adequate carriers have to be investigated.

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