RSM APPROACH FOR MODELING AND OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF CHOKEBERRY

Valentina M. Simić[1*], Saša S. Stojičević[2], Dragan T. Veličković[3], Nada Č. Nikolić[2], Miodrag L. Lazić[2], Ivana T. Karabegović[2]

(ORIGINAL SCIENTIFIC PAPER)
UDC 665.52:66.061:582.711.71

[1]College of Applied Studies of Technics and Technology, Kruševac, Serbia
[2]University of Niš, Faculty of Technology, Leskovac, Serbia
[3]College of Agriculture and Food Technology, Prokuplje, Serbia

Optimization of chokeberry (*Aronia melanocarpa*) microwave-assisted extraction (MAE) with different microwave power (300, 450, 600 W), ethanol concentration (25, 50, 75 %) and extraction time (5, 10, 15 min) were analyzed using response surface methodology (RSM). The predicted maximum extractive substances yield (ESY) of 19.2 g/100 g of the fresh plant material was obtained at the optimal condition (525 W, 62.5%, 15 min), while 20.1 g /100 g of the fresh plant material was obtained from laboratory experiments. The most effective process parameter was extraction time. In order to rationalize the production and minimize costs, optimal economic conditions have been proposed, so the extraction parameters were set as follows: 417.5 W, 43.03%, 11.2 min. Under these conditions, the model predicts the ESY of 16.95 g/100 g of the fresh plant material which means that it is possible to obtain 88% of the maximum predicted ESY, but with a significantly reduced price per unit of the final product. The experimental values under optimal conditions agreed with those predicted within a 95% confidence interval, thus indicating the suitability of RSM in optimizing MAE of chokeberry.

Keywords: Chokeberry, Microwave-assisted extraction, Response surface methodology, Optimization

Introduction

Chokeberry (*Aronia melanocarpa*) is a bushy plant belonging to the *Rosaceae* family and originates in Canada and North America. It was first transferred to Germany and Russia, and nowadays it is cultivated in different parts of central Europe. It is namely consumed as a fresh fruit or processed in the form of juice, jam, tea, liqueur and wine [1]. Chokeberry was used in European and North American traditional folk medicine as an antisclerotic agent and as a medicament against high blood pressure, while native Americans have used chokeberry for the treatment of cold [1,2]. The chemical composition of berries (the extract or freshly pressed juice) indicates that chokeberry is a good source of nutrients containinga high level of polyphenols, namely phenolic acids, proanthocyanidins, anthocyanins, flavonols, and flavanones [1-5]. The contribution of the phenolic compound content to an intensive antioxidant activity has already been reported [6]. Chokeberries are one of the richest plant sources of anthocyanins mainly containing cyanidin glycosides, while phenolic acids are represented by chlorogenic and neochlorogenis acids [7]. Polyphenols of chokeberry were reported to have the antioxidant, antiradical [1,5,9], antimutagenic [10] and antiinflammatory activity [11,12]. They might also have blood pressure lowering properties [2], reduce total cholesterol, LDL cholesterol and triglyceride level [1] which all contribute to significant cardioprotective effects. A great number of investigations indicate that the extracts of this plant show the antitumor activity [1,10,13-15], as well as the hepatoprotective [4] and gastroprotective effect [16]. At the same time, there is no study reporting toxic or unwanted effects due to the consumption of chokeberry fruits, juice and extracts [14].

Actual demands concern more efficient and “green”extraction techniques of bioactive compounds which could provide a better quality of extracts, increase the yield, minimize the extraction time and solvent consumption and avoid potential chemical degradation and modification of bioactive compounds, so they would be suitable for eventual pharmaceutical or food processing use [17]. Among them,there are the ultrasound-assisted extraction (UAE) [18,19,20], microwave-assisted extraction (MAE) [6,21,22], and extraction with pressurized liquids (PLE) [23,24]. Compared with other techniques, MAE showed advantages because it is relatively low cost, requires less extraction time, solvent consumption and could be labeled as “green” according to environmental standards: natural environment is less impacted using reduced quantities of solvents and energy [6,25-27]. At the same time, MAE high extraction efficiency was confirmed along with increased contents of extracted antioxidants in the obtained extracts [24,28]. Numerous investigations have confirmed the benefits of MAE: the extraction of polyphenolic antioxidant compounds from

***Author address:** Valentina M. Simić, College of Applied Studies of Technics and Technology, 36 Kosančićeva St., 37000 Kruševac, Serbia
E-mail: valentinasimic70@yahoo.com
The manuscript received: March, 13, 2018.
Paper accepted: March, 29, 2018.

peanut skins [21], phenolic compounds from wine lees [22], tomatoes [6], phenolic acids from citrus mandarin peels [26], bioactive compounds from pine seeds [24], antioxidants from rosemary [29] or polyphenols from grape seed [25].

The MAE extraction efficiency is largely influenced by the following factors,the impact of which can be individual and/or combined: solvent type and concentration, microwave power, temperature, extraction time and solvent to solid ratio [6,30]. The impossibility of classical single variable optimization ("one-variable-at-a-time") to examine both individual and combined interaction of extraction parameters could be overcome by using mathematical modeling techniques. Response surface methodology (RSM) is a couple of both statistical and mathematical techniques. It presents the method that could be applied to determine optimal extraction conditionsby using the effects of some process variables, and their interactions to identify the relationship between the response function and these variables [30]. RSM is used in order to estimate optimum extraction conditionsin the following examples: phenolic compounds from *Morusnigra* leaves [31], phenolic antioxidant from peanut skins [21] and from tomatoes [6], phenolic acids from citrus mandarin peels [26], polyphenols from grape seed [25], marjoram [19] and dry fruit of Andean species *Vaccinum meridionale* [32] and also bioactive compounds from *Lycium barbarum* [33].

The aim of this study was to examine MAE as a method convenient for the extraction of bioactive compounds from chokeberry, evaluation of individual and combined effects of principal extraction parameters (ethanol concentration, microwave powerand extraction time) and the optimization of the process parameters in order to achieve the maximum extractive substances yield (ESY)by using RSM.

Experimental

Material

Commercially available frozen fruits of *A. melanocarpa* were obtained from "Fungo-jug" Leskovac, Serbia. The plant material originated from Poland and was harvested throughout September 2013.

Determination of total dry matter

Frozen chokeberries were milleddirectly before MAE, while the determination of the total dry matter was realized by drying at 105 °C to constant mass. The obtained result was 75.97±3.2%.

Microwave assisted extraction

The MAE was performed in a microwave oven ("SAMSUNG", Type M1712N, Malaysia) modified according to the scheme given by Karabegović and coworkers [30]. The aim of the mechanical and electrical modification of the microwave oven was to construct an open extraction system under atmospheric pressure with the ability to regulate the power of the microwave and to ensure the effect of constant-power microwaves throughout the entire extraction process.

The milled plant material (50 g) was subjected to MAE with aqueous ethanol solutions (250 cm^3, 25, 50 and 75%) as a solvent. The extraction was carried out for 5, 10 and 15 minutes at different microwave power (300, 450 and 600 W). After the completion of the extractions, the liquid extracts were separated from the exhausted plant material under vacuum on the Büchner funnel through Whatman filter paper (No. 2) and evaporated to a dry rotary vacuum evaporator (800 rpm) at 40 °C.

Determination of the extractive substances yield

The evaporated extracts were dried to a constant mass at 105 °C. The total extractive substances yield, expressed as the mass of extracting substances (g) in 100 g of the plant material, was calculated from the weight of the extracts and the weight of the fresh plant material.

Experimental design

For determining the effect of the process parameters on the extractive substances yield (depended parameter, Y), the experiments were designed using the 3^3 full factorial experiment design. The data of preliminary experiments were used to define the range of independent parameters (variables): microwave power (300, 450, 600W, X_1), ethanol concentration (25, 50, 75%, X_2), and extraction time (5, 10, 15 min, X_3). Each of the experiments was performed in triplicate.

Response surface methodology

In order to estimate the influence of the microwave power, ethanol concentration and extraction time on the ESY (dependent parameter, Y) and to define the optimal combination of these process parameters, RSM was used. Obtained mean values of the determined ESY were applied for the regression analysis. Design-Expert software was used in the prediction of optimal conditions and in the analysis of experimental data which were fitted to the second-order polynomial model given in equation (1):

$$Y= \beta_o + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j \qquad (1)$$

where *Y* is the predicted response (extractive substances yield g/100 g of the fresh plant material), βo, βi, βii, and βij are regression coefficients for intercept, linear, quadratic and interaction terms, respectively and Xi and Xj are the actual levels of the independent variables.

The experimental design, regression coefficient calculations and a graphical analysis of experimental data (response surfaces and contour plots) were performed by using the statistical software Design Expert (Trial version 7.0.0, STAT-EASE Inc.).The analysis of variance (ANOVA) was applied in order to estimate the adequacy and statistical significance of the fitted model, as well as regression coefficients and individual independent parameters and their interactions.

Results and discussion

The influence of extraction parameters on the extractive substances yield

ESY in chokeberry extracts obtained by MAE largely depends on the extraction conditions, which is illustrated by the results shown in Figure 1.

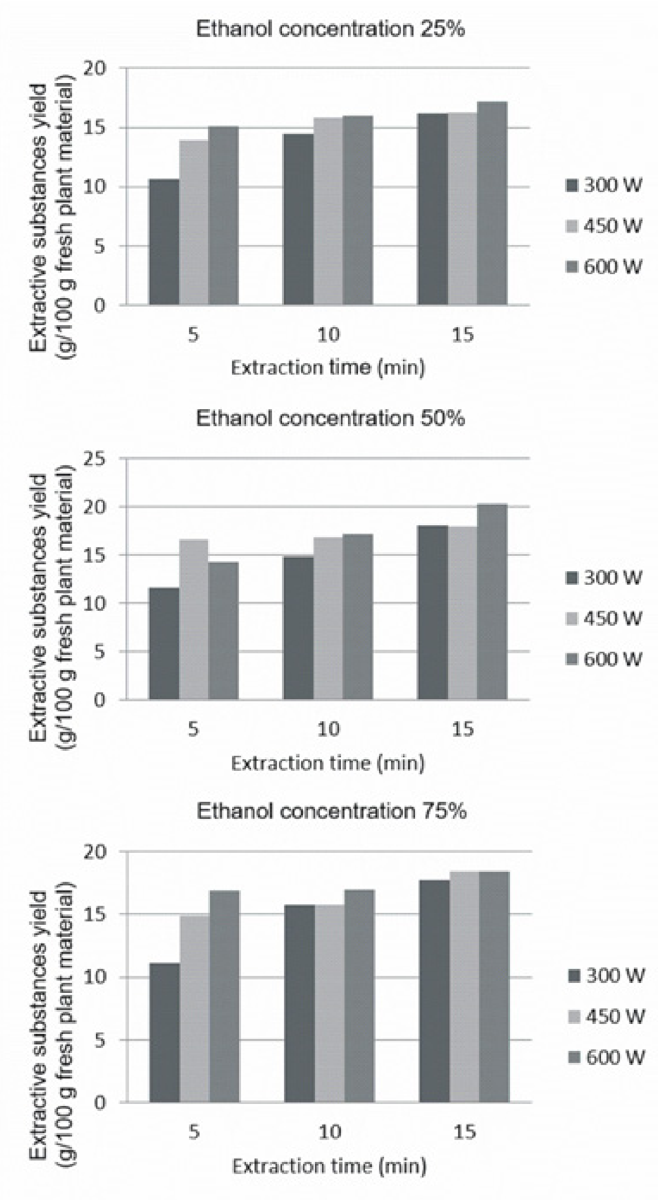

Figure 1. The extractive substances yield from chokeberry obtained under different microwave power, extraction time and ethanol concentration by the microwave assisted extraction

By increasing the microwave power and extraction time, as well as the changes in the concentration of ethanol, the extraction yield varies from 11.1 to 20.3 g/ 100 g of the fresh plant material. These values are in agreement with the results of some authors stating that chokeberry ESY of 14.2% can be obtained by using 70% ethanol, at the temperature of 70 °C, after 3 hours of the extraction time and in the ratio of solvents and the plant material 1:10 [34]. In the investigation of Wangensteen and associates, four different cultivated types of aronia were examined: "Moscow", "Hugin", "Nero" and *A. prunifolia* and ESY with the 80% boiling ethanol solution were in the range from 9.4 to 19.7% [35].

The solvent system used for the extraction is a substantial factor affecting the extraction yield from the plant matrix.Hence the plant material is a complex mixture of different classes of phenolic compounds,actual investigations deal with the extractive efficiency of numerous solvents, but methanol, acetone and their water mixtures are the most often used for the phenolic compounds extraction (according to phenols solubility and polarity of the solvent) [36]. In case of MAE, a chosen solvent needs to have the ability to absorb the microwave energy, too [37]. Methanol and acetone are appropriate extragents, but they are not suitable for food and pharmaceutical application because of their toxicity. Therefore, ethanol has been frequently used as more suitable for the reasons considering the environmental impact and safe food production [38].Irrespective of the applied microwave power and the duration of the extraction, with the increase in the concentration of the ethanolic solution from 25% to 50%, ESY increases and reaches the maximum value of 20.3 g/100 g of the fresh plant material (600 W, 15 min). Further increase in the concentration of the ethanolic solution to 75% causes a decrease in EYS for about 1% (when the extraction time is 10 min) and 10% (when the extraction time is 15 min). The tendency to increase the extraction yield by increasing the ethanol concentration to a certain maximum, and a subsequent decline in the yield value by the continuing increase of the ethanol concentration was confirmed in the MAE of ginseng [39]and the plant *Limnophila aromatic* [40].

In the MAE of anthocyanins from blackberry fruit, the effect of the ethanol concentration on the extraction yield was examined by varying this value in the range from 0 to 100%. The obtained results indicate thatthe increase of this process parameter up to 40% contributes to the increase in the extraction yield, too (which at this value reaches its maximum). Further increase of the ethanol concentration results in a decrease in the extraction yield, and this drastically after reaching the value of 80% [41].The increase in the proportion of ethanol in the aqueous solution to 80% (v/v) increased the yield of flavonolignane silbinin, while further increase resulted in a decrease in the extraction yield of this compound in the MAE of the Silybummarianum. The authors' explanation was that the presence of a certain amount of water can improve the mass transfer process by increasing the relative polarity of the solvent, thereby increasing its dissolution power. Also, efficient swelling of the plant material is ensured, which increases the surface for the interaction between solvents and soluble substances. But a higher proportion of water in this binary dissolution system can cause the increased thermal stress in terms of accelerated solvent warming due to more efficient absorption of water by microwaves [42].

In the case of MAE of chokeberry, ESY enhancement was achieved by the increase of the microwave power with the maximum value at 600 W (15 min). Karabegović and coworkers accomplished the similar observation with the MAE of cherry laurel fruit, the yield of which increased by increasing the microwave power (300-600 W) and the extraction time (10-30 min) [43]. In our investigation, the increase in the microwave power from 300 W to 600 W had the greatest effect on the extraction yield at the extraction time of 5 min (increasement of 1.52 times), while this trend is less pronounced in the extraction time of 10 min and 15 min with the increase of 7.6% and 3.9%, respectively. Dhobi and associates examined the effect of the microwave power on the extractive substances yield with the conclusion that the extraction efficiency was improved by increasing the power of the microwave (from 200-800 W) during a shorter extraction time, and a significant increase in the yield was found by engaging the power of 600 W [42]. This effect of the microwave power on the extraction yield is explained by the fact that a higher power of microwaves accelerates the extraction because direct effects of the microwave energy on biomolecules (ionic conduction and dipole rotation) are in function of this process parameter. Higher power causes a better distribution of the microwave energy (total volume) within the solvent and plant material, thereby generating more intense molecular motion and heating. Generally, the higher the power of the microwave, the faster and the more electromagnetic energy is transferred to the extraction system making the extraction more efficient. An increase in power from 100 to 400 W (at constant values of other extraction parameters) caused an increase in ESY in the MAE of the blackberry fruit. The maximum yield was reached at about 400 W, but the further increase in the microwave power influenced the reduction of the extractive yield [41].

With the prolongation of the extraction process (5 to 15 min) there was the increase of ESY, irrespective of other extraction parameters values. The increase in extraction time has the most pronounced effect in the case of the extraction with 50% ethanol solution, in the sense of increasing ESY from 22.9% (prolonging the MAE time of 5-15 min). Otherwise, the higher value of the microwave power contributed to the yield increase of 1.15 times at the 10 minute extraction duration and 1.12 when the extraction time was 15 minutes. With a shorter exposure to microwaves, a trend of decreasing yields was observed with an increase in power from 450 W to 600 W, by approximately 14%. The yield of the extractive substances was increased by the longer duration of the MAE of flavonolignan – silybinin and after reaching the maximum further increase in the extraction time did not improve the efficiency of the process[42]. The increase in the extraction time to 3 min (in the interval of testing from 1 to 9 min) influenced the growth of the yield of extractive substances in the MAE of anthocyanins from the blackberry, while by prolonging the duration of the process, the yield of the extraction decreased [41].

Table 1. Experimental design matrix and results

	Design matrix						Response	
	Coded factors			Uncoded factors			Extractive substances yield (g/100 g fresh plant material)	
Run	Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	Ethanol concentration, % (X_1)	Microwave power, W (X_2)	Time, min (X_3)	Experimental	Predicted
1	0	0	0	50	450	10	16.9	16.94
2	0	-1	0	50	300	10	14.9	15.73
3	0	0	-1	50	450	5	16.6	14.97
4	1	0	0	75	450	10	15.8	17.51
5	-1	-1	-1	25	300	5	10.7	12.44
6	0	1	1	50	600	15	20.3	19.37
7	-1	0	1	25	450	15	16.3	18.34
8	1	-1	1	75	300	15	17.7	19.02
9	0	0	0	50	450	10	19.4	16.94
10	1	1	-1	75	600	5	16.9	17.50
11	-1	1	0	25	600	10	16.0	17.58
12	0	0	0	50	450	10	15.9	16.94
13	0	-1	1	50	300	15	18.1	18.45
14	1	1	0	75	600	10	17.0	18.72
15	-1	0	-1	25	450	5	13.9	14.40
16	-1	-1	0	25	300	10	14.5	15.16
17	1	0	1	75	450	15	18.4	19.48
18	-1	1	1	25	600	15	17.2	18.80
19	0	0	0	50	450	10	15.4	16.94
20	0	1	-1	50	600	5	14.3	16.93
21	0	0	0	50	450	10	14.9	16.94
22	1	-1	-1	75	300	5	11.1	13.58
23	1	-1	0	75	300	10	15.8	16.30
24	1	1	1	75	600	15	18.4	19.94
25	-1	1	-1	25	600	5	15.1	16.36
26	1	0	-1	75	450	5	14.9	15.54
27	0	-1	-1	50	300	5	11.6	13.01
28	0	0	1	50	450	15	18.0	18.91
29	0	0	0	50	450	10	17.7	16.94
30	-1	-1	1	25	300	15	16.2	17.88
31	0	1	0	50	600	10	17.2	18.15
32	0	0	0	50	450	10	18.1	16.94
33	-1	0	0	25	450	10	15.8	16.37

RSM modeling and optimization

The influence of three process parameters of extraction (independent variables) on ESY of chokeberry obtained by MAE was analyzed using RSM. The process parameters, their coded and uncoded values, as well as the experimentally obtained and predicted (by the developed model) ESY values (dependent variable-response) are presented in Table 1. ESY was in the range of 11.1 to 20.3 g of extractive substances (ES)/100 g of the fresh plant material.

By using a multiple non-linear regression analysis of the experimental data obtained with 33 design experiments, a quadratic polynomial equation was proposed fordescribing the influence of independent variable on ESY:

$$Y_{ESY}=16.94+0.57X_1+1.21X_2+1.97X_3+0.067X_1X_2+0.13X_1X_3-0.75X_2X_3-0.81X_1^2-0.59X_2^2-0.14X_3^2 \quad (2)$$

The statistical significance of the model, the extraction parameters (independent variable), the interaction between the extraction parameters and the deviation from the model was verified using F-test and p-values. The analysis of variance (ANOVA) for the proposed quadratic modelis given in Table 2.

Table 2. Analysis of variance (ANOVA) for quadratic model

Source of variation	Sum of squares	Deegres of freedom (df)	*F*-value	*p*-value [a]
Model	119.84	9	10.21	**< 0.0001**
X_1- Ethanol concentration	5.89	1	4.52	**0.0461**
X_2-Microwave power	26.40	1	20.25	**0.0002**
X_3-Time	70.01	1	53.71	**< 0.0001**
X_1X_2	0.053	1	0.04	0.8417
X_1X_3	0.21	1	0.16	0.6901
X_2X_3	6.75	1	5.18	**0.0340**
X_1^2	4.66	1	3.57	0.0733
X_2^2	2.49	1	1.91	0.1820
X_3^2	0.14	1	0.11	0.7455
Residual	26.07	20		
Lack of fit	2.49	17	1.30	0.0584
Cor total	0.58	3	0.190	
CV = 7.11%	R^2 = 0.9013	AdjR^2 = 0.8409	Predicted R^2 = 0.7788	

[a]*p* < 0.01 very significant; 0.01≤ *p* < 0.05 significant; *p* ≥ 0.05 not significant

A small *p*-value (p<0.0001) and a large F-value point to the significance of the model developed for optimizing the operational conditions of chokeberry MAE in order to achieve the maximum ESY. Small p-values of individual extraction parameters, their squares or interactions indicate the statistical significance of the parameters in the context of their effect on response. All individual factors are statistically significant, as well as the interaction of the microwave power and extraction time. Extraction time is the most important factor on the ESY (F=53.71 and p<0.0001), which agrees with the value of the coefficient for X_3=31.97 in the regression equation of the polynomial. The coefficient absolute value of any member of the polynomial equation directly points to the extent of its influence on the response, while the sign of the coefficient (positive or negative) shows this influence orientation [44]. Namely, if the coefficient value of the member of the polynomial is greater, than its impact on the response is higher, and the positive sign of the coefficient means that the factor increases the response value.

The value of the coefficient of determination R^2=0.9013 suggests that the model is adequate and that 90.13% of the variation in the response values could be explained by the model.The value of R^2 adj=0.8409 is greater than 0.75 which suggests that the values of the fitted model are reliable and confirm the statistical significance of the model. The coefficient of variation CV = 7.11% is relatively small and as this value is less than 10%, it means that the fit model is reliable and reprodu-cible to optimize the ESY of chokeberry MAE.

The regression equation, namely the response function (its geometric interpretation is the response surface) is graphically represented by three-dimensional response surface plots and as two-dimensional contour surface plots (using the statistical software Design-Expert). In this way, the visualization of the relations between the response and each independent variable (factor), or between the response and the interaction of any two parameters examined (the interaction of the two-process parameters at the same time when the third parameter is fixed), was performed. This approach enables the determination of optimum values of independent variable, on which the dependent variable will have a maximum response [43]. The influence of extraction parameters (the concentration of ethanol, the microwave power and extraction time) on ESY in chokeberry extracts is shown in Figure 2.

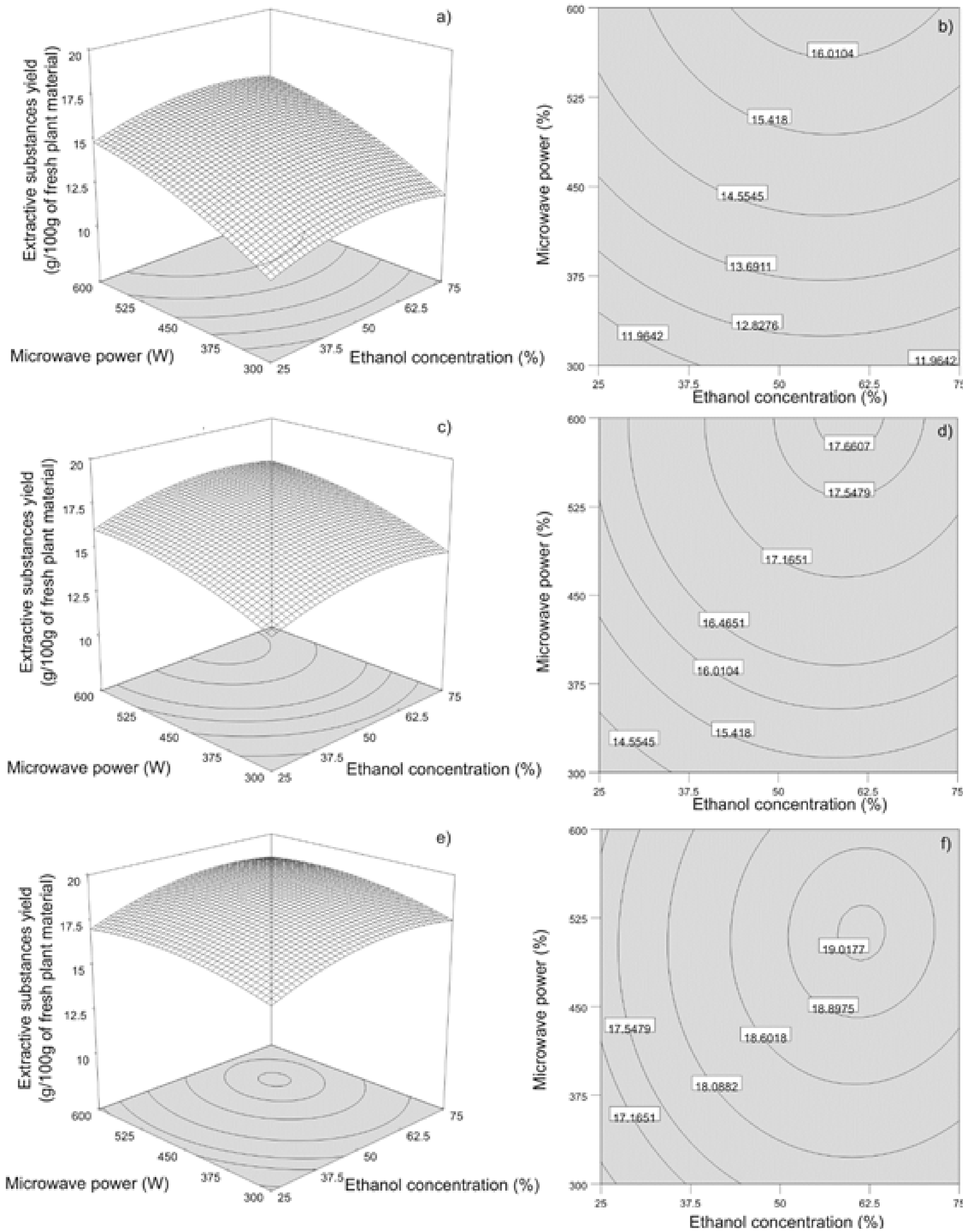

Figure 2. 3D response surface plots and two-dimensional plots for the extractive substances yield as a function of the microwave power, ethanol concentration and extraction time of 5 min (a, d), 10 min (b,e) and 15 min (c,f)

By analyzing graphs in Figure 2., there is a conclusion that irrespective of the microwave power and ethanol concentration, ESY increases with the increase in the extraction time from 5 to 15 min. At the same time, irrespective of time, by increasing the concentration of ethanol and the microwave power,the ESY increases, but in the case of the ethanolic concentration effect, after reaching the maximum value, the ESY decreases slightly. The microwave power has a greater influence on the increase of the ESY, comparing to the ethanol concentration. Also, the increase in the microwave power has the most pronounced effect on the ESY at the shorter extraction time (5 min).

The proposed model predicted that the maximum ESY could be obtained at the microwave power at 525 W, the ethanol concentration at 62.5% after the extraction time of 15 min, in the amount of 19.02 g ES/100 g of the fresh plant material, while the maximum experimentally obtained yield value was 20.1 g of ES / 100 g of the fresh plant material.

From the aspect of the industrial application or economic acceptability of the production, the optimization of extraction process parameters was carried out with the additional criterion: simultaneous minimization of the extraction time, energy and solvent consumption with maximization of the response value (ESY). According to the modified criteria, the optimization of the extraction process based on the cost-effectiveness proposed economic conditions as follows: 43.03% ethanol concentration, 417.5 W microwave power and the extraction time of 11.2 min, which will result with the ESY of 16.95 g/100 g fresh plant material. In the extract obtained under the proposed economic conditions, the ESY would be 16.5% lower. However, the extract could be produced 25% faster with engaged 30% less microwave power and with approximately 25% less ethanol consumption in comparison with the proposed optimal condition of MAE. In this way, the cost of the final product (extract) still containing a considerable amount of bioactive compounds would be significantly reduced by reducingthe energy and solvent consumption.

Conclusions

In the present study, the effects of process parameters on ESY of chokeberry MAE were examined. Greatest ESY can be achieved by the longest duration of the extraction and at the highest microwave power, but the most influencing factor is the extraction time. In order to determine optimal and economic process parameters that will ensure the maximum yield of the microwave extraction, the RMS method was applied. Optimal proposed conditions proposed by the statistical model were: extraction time of 15 min, microwave power of 525 W and ethanol concentration of 62.5%.The application of such conditions would result in the ESY of 19.2 g ES/100 g fresh plant material. Predicted economic conditions that would reduce production costs while simultaneously retaining the relatively high ESY were: 417.5 W microwavepower, 11.2 min of the extraction time and 43.03% ethanol concentration. According to these parameters values, the extraction yield would be 16.95 g/100 g fresh plant material. Based on the value of R^2, p-value, *F*-value and others statistical indicators, it can be concluded that the proposed RMS model is adequate, reliable and statistically significant, and can be successfully applied in the modeling and optimization of the chokeberry microwave assisted extraction.

Acknowledgment

This project was supported by the Ministry of Education,Science and Technological Development of the Republic of Serbia,project OI 172047.

References

[1] S. E. Kulling, H. M. Rawel, Chokeberry (*Aronia melanocarpa*) – A Review on the Characteristic Components and Potential Health Effects, Planta Medica, 74 (2008) 1625-1634.

[2] J. K. Hellström, A. N. Shikov, M. N. Makarova, A. M. Pihlanto, O. N. Pozharitskaya, E. Ryhänen, P. Kivijärvi, V. G. Makarov, P. H. Mattila, Blood pressure-lowering properties of chokeberry (*Aronia mitchurinii*, var. Viking), Journal of Functional Foods, 2 (2010) 163-169.

[3] A. Horszwald, H. Julien, W. Andlauer, Characterisation of Aronia powders obtained by different drying processes, Food Chemistry, 141 (2013) 2858-2863.

[4] S. Valcheva-Kuzmanova, P. Borisova, B. Galunska, I. Krasnaliev, A. Belcheva, Hepatoprotective effect of the natural fruit juice from *Aronia melanocarpa* on carbon tetrachloride-induced acute liver damage in rats, Experimental and Toxicologic Pathology,56 (2004) 195-201.

[5] L. Jakobek, M. Drenjančević, V. Jukić, M. Šeruga, Phenolic acids, flavonols, anthocyanins and antiradical activity of “Nero”, “Viking”, “Galicianka” and wild chokeberries, Scientia Horticulturae, 147 (2012) 56-63.

[6] H. Li, Z Deng, T. Wu, R. Liu, S. Loewen, R. Tsao, Microwave-assisted extractionof phenolics with maximal antioxidant activities in tomatoes, Food Chemistry,130 (2012) 928-936.

[7] J. Oszmianski, A. Wojdylo, *Aronia melanocarpaphenolics* and their antioxidant activity. European Food Research and Technology, 221 (2005) 809-13.

[8] M. Mikulic-Petkovsek, A. Slatnar, F. Stampar, R. Veberic, HPLC–MSn identification and quantification of flavonol glycosides in 28 wild and cultivated berry species, Food Chemistry,135 (2012) 2138-2146.

[9] J. Malinowska, K. Babicz, B. Olas, A. Stochmal, W. Oleszek, *Aronia melanocarpa* extract suppresses the biotoxicity of homocysteine and its metabolite on the hemostatic activity of fibrinogen and plasma, Nutrition, 28 (2012) 793-798.

[10] K. Gąsiorowski, K. Szyba, B. Brokos,B. Kozxllaczyńska, M. Jankowiak-Wzxllodarczyk, J. Oszmiański, Antimutagenic activity of anthocyanins isolated from *Aronia melanocarpa* fruits, Cancer Letters, 119 (1997) 37-46.

[11] M. Bijak, J. Saluk, A. Antosik, M. B. Ponczek, H. M.

Żbikowska, M. Borowiecka, P. Nowak, *Aronia melanocarpa* as a protector of nitration of fibrinogen, International Journal of Biological Macromolecules,55 (2013) 264-268.

[12] M. Naruszewicz, I. Łaniewska, B. Millo, M. Dłużniewski, Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI), Atherosclerosis,194 (2007) 179-184.

[13] D. Rugina, Z. Sconta, L. Leopold, A. Pintea, A. Bunea, C. Socaciu, Antioxidant Activities of Chokeberry Extracts and the Cytotoxic Action of Their Anthocyanin Fraction on HeLa Human Cervical Tumor C, Journal of Medicinal Food, 15(8) (2012) 700-706.

[14] M. Kedzierska, B. Olas, B. Wachowicz, R. Glowacki, E. Bald, U. Czernek, K. Szydłowska-Pazera, P. Potemski, J. Piekarski, A. Jeziorski, Effects of the commercial extract of aronia on oxidative stress in blood platelets isolated from breast cancer patients after the surgery and various phases of the chemotherapy, Fitoterapia, 83 (2012) 310-317.

[15] M. J. Bermúdez-Soto, M. Larrosa, J. M. Garcia-Cantalejo, J. C. Espín, F. A. Tomás-Barberan, M. T. García-Conesa, Up-regulation of tumor suppressor carcinoembryonic antigen-related cell adhesion molecule 1 in human colon cancer Caco-2 cells following repetitive exposure to dietary levels of a polyphenol-rich chokeberry juice, The Journal of Nutritional Biochemistry, 18 (2007) 259-271.

[16] M. Matsumoto, H. Hara, H. Chiji, H, T. Kasai, Gastroprotective effect of red pigments in black chokeberry fruit (*Aronia melanocarpa* Elliot) on acute gastric hemorrhagic lesions in rats, Journal of Agricultural and Food Chemistry, 52 (2004) 2226–2229.

[17] I. Ignat, I. Volf, V. I. Popa, A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables, Food Chemistry, 126 (2011) 1821 -1835.

[18] L. Galvan d'Alessandro, K. Kriaa, I. Nikov, K. Dimitrov, Ultrasound assisted extraction of polyphenols from black chokeberry, Separation and Purification Technology, 93 (2012) 42-47.

[19] M. B. Hossain, N. P. Brunton, A. Patras, B. Tiwari, C.P. O'Donnell, A. B. Martin-Diana, C. Barry-Ryan, Optimization of ultrasound assisted extraction of antioxidant compoundsfrom marjoram (*Origanum majorana* L.) using response surface methodology, Ultrasonics Sonochemistry, 19 (2012) 582-590.

[20] I. Karabegović, M. Nikolova, D. Veličković, S. Stojičević, V. Veljkovic, M. Lazić, Comparison of Antioxidant and Antimicrobial Activities of Methanolic Extracts of the Artemisia sp. Recovered by Different Extraction Techniques, Chinese Journal of Chemical Engineering, 19 (3) (2011) 504-511.

[21] T. S. Ballard, P. Mallikarjunan, K. Zhou, S. O'Keefe, Microwave-assisted extraction of phenolic antioxidant compoundsfrom peanut skins, Food Chemistry, 120 (2010) 1185-1192.

[22] J.A. Pérez-Serradilla, M.D. Luque de Castro, Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract, Food Chemistry,124 (2011) 1652-1659.

[23] Z. Solati, B. S. Baharin, H. Bagheri, Supercritical carbon dioxide (SC-CO2) extraction of *Nigella sativa* L. oil using full factorial design, Industrial Crops and Products,36 (2012) 519-523.

[24] A. Liazid, M. Schwarz, R.M. Varela, M. Palma, D.A. Guillén, J. Brigui, F.A. Macías, C.G. Barroso, Evaluation of various extrationtechniques for obtaining bioactive extracts from pine seeds, Food and Bioproducts Processing, 88 (2010) 247-252.

[25] Y. Li, G.K. Skouroumounis, G.M. Elsey, D.K. Taylor, Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols, Food Chemistry,129 (2011) 570-576.

[26] K. Hayat, S. Hussain, S. Abbas, U. Farooq, B. Ding, S. Xia, C. Jia, X. Zhang, W. Xia, Optimized microwave-assisted extractionof phenolic acids from citrus mandarin peels and evaluation of antioxidant activityin vitro, Separation and Purification Technology, 70 (2009) 63-70.

[27] J. Ahmad, T.A.G. Langrish, Optimisation of total phenolic acids extraction from mandarin peels using microwave energy: The importance of the Maillard reaction, Journal of Food Engineering,109 (2012) 162 -174.

[28] J. Song, D. Li, C. Liu, Y. Zhang, Optimized microwave-assisted extractionof total phenolics (TP) fromIpomoea batatasleaves and its antioxidant activity, Innovative Food Science & Emerging Technologies, 12 (2011) 282-287.

[29] S. Rodríguez-Rojo, A. Visentin, D. Maestri, M.J. Cocero, Assisted extraction of rosemary antioxidants with green solvents, Journal of Food Engineering,109 (2012) 98-103.

[30] I. T. Karabegović, S. S. Stojičević, D. T. Veličković, N. Č. Nikolić, M. L. Lazić, Optimization of microwave-assisted extraction and characterization of phenolic compounds in cherry laurel (*Prunuslaurocerasus* L.) leaves, Separation and Purification Technology, 120 (2013) 429-436.

[31] M. Radojković, M. M. Moreira, C. Soares, M. F. Barroso, A. Cvetanovic, J. Švarc-Gajic, S. Morais, C. Delerue-Matos (2018), Microwave-assisted extraction of phenolic compounds from *Morusnigra* leaves: optimization and characterization of the antioxidant activity and phenolic composition, Article in Journal of Chemical Technology and Biotechnology, https://doi.org/10.1002/jctb.5541 (Article in Press).

[32] W. E. Espinosa, L. C. A. Garzón, O. J. Oscar Julio Medina, Microwave-assisted extraction in dry fruit of andean species *Vaccinium meridionale*: Experimental conditions on the recovery of total polyphenols, Ciência e Agrotecnologia, 41(6) (2017) 701-712.

[33] M. Mendes, A. P. Carvalho, J. M. C. S. Magalhães, M. Moreira, L. Guido, A. M. Gomes, C. Delerue-Matos, Response surface evaluation of microwave-assisted extraction conditions for *Lyciumbarbarum* bioactive compounds, Innovative Food Science and Emerging Technologies, 33 (2016) 319–326.

[34] S. J. Hwang, W. B. Yoon, O. H. Lee, S. J. Cha, J. D. Kim, Radical-scavenging-linked antioxidant activities of extracts from black chokeberry and blueberry cultivated in Korea, Food Chemistry, 146(1) (2014) 71–77.

[35] H. Wangensteen, M. Bräunlich, V. Nikolić, K.-E. Malterud, R. Slimestad, H. Barsett, Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: antioxidant and enzyme inhibitory effects, Journal of Functional Foods, 7 (2014) 746–752.

[36] J. Tabart, C. Kevers, A. Sipel, J. Pincemail, J. Defraigne, J. Dommes, Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage, Food Chemistry,105 (2007) 1268–1275.

[37] H. Zhang, X. Yang and Y. Wang, Microwave assisted extraction of secondary metabolites from plants: Current

status and future directions, Trends in Food Science & Technology, 22 (2011) 672-688.

[38] P. Denev, M. Ciz, G. Ambrozova, A. Lojek, I. Yanakieva, M. Kratchanova, Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties, Food Chemistry,123 (2010) 1055-1061.

[39] J.-H. Kwon, G.-D. Lee, J. Bélanger, J. R. J. Paré, Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwaveassisted process (MAP TM), International Journal of Food Science & Technology, 38 (2003) 615–622.

[40] Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Lien Huong Huynh, F. E. Soetaredjo, S. Ismadji, Y.-H. Ju, Effect of extraction solvent on total phenol content, total flavonoid, and antioxidant activity of *Limnophila aromatic*, Journal of food and drug analysis,22 (2014) 296–302.

[41] Y. Wen, H. Chen, X. Zhou, Q. Deng, Y. Zhao, C. Zhao, X. Gong, Optimization of the microwave-assisted extraction and antioxidant activities of anthocyanins from blackberry using a response surface methodology, RSC Advances, 5 (25) (2015) 19686-19695.

[42] M. Dhobi, V. Mandal, S. Hemalatha, Optimization of microwave assisted extraction of bioactive flavonolignan – silybinin, Journal of Chemical Metrology, 3:1 (2009) 13-23.

[43] I. Karabegović, S. Stojičević, D. Veličković, N. Nikolić, M. Lazić, Optimization of Microwave-Assisted Extraction of Cherry Laurel Fruit, Separation Science and Technology (Philadelphia), 49(3) (2014) 416-423.

[44] M. Xu, Q. Shao, S. Ye, S. Li, M. Wu, M. Ding, Y. Li, Simultaneous Extraction and Identification of Phenolic Compounds in *Anoectochilusroxburghii* Using Microwave-Assisted Extraction Combined with UPLC-Q-TOF-MS/MS and Their Antioxidant Activities, Frontiers in Plant Science, 8 (2017) 1-11.

Izvod

PRIMENA METODE ODZIVNIH POVRŠINA ZA MODELOVANJE I OPTIMIZACIJU MIKROTALASNE EKSTRAKCIJE ARONIJE

Valentina M. Simić[1], Saša S. Stojičević[2], Dragan T. Veličković[3], Nada Č. Nikolić[2], Miodrag M. Lazić[2], Ivana T. Karabegović[2]

(ORIGINALNI NAUČNI RAD)
UDK 665.52:66.061:582.711.71

[1]Visoka tehničko-tehnološka škola strukovnih studija, Kruševac, Srbija
[2]Univerzitet u Nišu, Tehnološki fakultet, Leskovac, Srbija
[3]Visoka poljoprivredno-prehrambena škola strukovnih studija, Prokuplje, Srbija

Optimizacija mikrotalasne ekstrakcije aronije (*Aronia melanocarpa*) izvedene pri različitim vrednostima mikrotalasne snage (300, 450, 600 W), koncentracije etanola (25, 50, 75%) i ekstrakcionog vremena (5, 10, 15 min) je izvršena primenom metode odzivnih površina (RSM). Predviđeni maksimalni prinos ekstraktivnih supstanci (ESY) od 19,2 g/100 g svežeg biljnog materijala ostvaren je pripredloženim optimalnim uslovima ekstrakcije (525 W; 62,5%; 15 min), dok je eksperimentalno dobijena vrednost iznosila 20,1 g/100 g svežeg biljnog materijala. Najuticajniji ekstrakcioni parameter bilo je ekstrakciono vreme. U cilju racionalizacije i minimiziranja troškova proizvodnje predloženi su optimalni ekonomski uslovi tako da su ekstrakcioni parametric imali sledeće vrednosti: 417,15 W; 43,03%; 11,2 min. Pod ovim uslovima model predviđa ESY od 16,95 g/100 g svežeg biljnog materijala, što znači da je moguće dobiti 88% od makimalno mogućeg ESY uz značajano nižu cenu koštanja po jedinici finalnog proizvoda. Eksperimentalno dobijene vrednosti pod optimalnim uslovima u saglasnosti su sa predviđenim vrednostima u interval pouzdanosti od 95% što ukazuje na adekvatnost RSM pri optimizaciji mikrotalasne ekstrakcije aronije.

Ključne reči: aronija, mikrotalasna ekstrakcija, metoda odzivnih površina, optimizacija