https://doi.org/10.15255/KUI.2025.009
KUI-35/2025
Original scientific paper
Received February 18, 2025
Accepted April 12, 2025

Statistical Modelling of Ultrasound-assisted Extraction of Bioactive Compounds from Yarrow (*Achillea millefolium* L.)

N. Vasiljević,[a,b*] *M. Tomić*,[a,c] *S. Panić*,[b] *T. Ljubinac*,[a] and *V. Mićić*[a]

[a] University of East Sarajevo, Faculty of Technology Zvornik, Karakaj 34A, 75 400 Zvornik, Republic of Srpska, Bosnia and Herzegovina
[b] Faculty of Technology, University of Novi Sad, Boulevard cara Lazara 1, 21 101 Novi Sad, Serbia
[c] Engineering Academy of Serbia, Kneza Miloša 9/IV, 11 000 Beograd, Serbia

Abstract

This study presents the statistical modelling of ultrasound-assisted extraction (UAE) of (poly)phenolic compounds from yarrow flowers (*Achillea millefolium* L.) using a central composite design (CCD). The effects of ethanol content in the solvent (21.36–88.64 %v/v, including extremes), solid-to-solvent ratio (1 : 9.77–60.23 w/v, including extremes), and initial solution pH (1.95–12.05, including extremes) on extraction efficiency of total (poly)phenols, flavonoids, and anthocyanins were examined. The results showed that the responses were most significantly influenced by the solid-to-solvent ratio and ethanol content in the solvent, while the effect of pH of the initial solution was weak. Regression analysis yielded highly reliable models with coefficients of determination (R^2) close to unity. The highest contents of total (poly)phenols (≈30.31 mg GAE/g) and flavonoids (≈12.21 mg CTH/g) were achieved at a high solid-to-solvent ratio (1 : 60.23 w/v) and a medium ethanol content (55 %v/v). In contrast, the highest anthocyanin content (≈0.157 mg Cy3G/g) was achieved at the highest ethanol content (88.64 %v/v) and a medium solid-to-solvent ratio (1 : 35 w/v). Regardless of the response, the highest yields were achieved in a neutral environment, though acceptable results could also be achieved under alkaline conditions. ANOVA analysis confirmed the proposed quadratic models had high statistical significance ($p < 0.0001$ for all responses), validating their use in predicting extraction efficiency under varying conditions.

Keywords

ANOVA, ultrasound-assisted extraction (UAE), (poly)phenols, yarrow flowers, flavonoids

1 Introduction

Polyphenolic compounds are a potent natural source of antioxidants, and are increasingly used in the production of bio-based products, including food additives, cosmetics, pharmaceuticals and dietary supplements.[1] Yarrow (*Achillea millefolium* L.) is a plant rich in these compounds. It typically grows in meadows and along roadsides, and is recognisable by its delicate leaves, hairy stems, and a height ranging from 20 to 90 cm.[2,3] A member of the *Asteraceae* family, yarrow thrives in the temperate regions of Eurasia and North America, and has a long history in traditional medicine.[4]

Numerous bioactive compounds have been identified in yarrow, including phenolic acids, flavonoids, terpenoids, acetylcholine, sterols, thiamin, tannins, coumarins, lignans, alkadiamides, polyacetylenes, sesquiterpenes, amino acids, ascorbic acid, and various sugars.[3,5–9] Its essential oil is known to contain α-terpineol, piperitone, pinene, borneol, piperitone, 1,8-cineole, *p*-micin, artemisia ketone, bornyl acetate, camphor, linalyl acetate, D-limonene, and sabinene.[4,5,10–14] Yarrow is well-known in traditional medicine and is used to treat wounds, headaches, inflammation, digestive issues, respiratory infections, and as a mild sedative.[2–4] Due to its stimulant, gastroprotective, antispasmodic, analgesic, anti-inflammatory, and antimicrobial effects, yarrow has potential applications in the pharmaceutical industry.[9] Its astringent, antioxidant and antimicrobial activities also support its use in the cosmetics industry.[9,12] Conventional extraction methods such as percolation, maceration, and Soxhlet extraction are often time-consuming and require large volumes of solvent to achieve high yields of bioactive compounds.[5,15,16] In response, green extraction techniques have been developed allowing shorter processing and retention times, faster heat and mass transfer, improved product quality, and reduced solvent use. In addition, green extraction reduces energy consumption and lowers environmental impact.[17,18] One widely adopted green method is ultrasound-assisted extraction (UAE).[19–22] Ultrasound has both mechanical and thermal effects on the extraction of plant material.[23] Thermally, ultrasound energy is converted to heat and then absorbed by plant tissues. Mechanically, it induces acoustic cavitation, which leads to the splitting of plant cells.[23,24] In fact, acoustic cavitation is a phenomenon where the passage of waves through a liquid leads to the formation and growth of bubbles in the liquid, which eventually burst. Due to the bursting of the bubbles, at the molecular level, the temperature rises to 5000 K and the pressure rises to 50 MPa. Such a sharp increase in temperature and pressure causes the disintegration of plant cell walls and easier diffusion of dissolved substances into the solvent.[25] Initially, extraction studies often used the "one factor at a time" experimental design to evaluate the influence of factors (process parameters) on a certain response (*e.g.*, yield, content of a certain component in the product...). However, this method is time-consuming, expensive due to the large number of experiments required, and does not account for

* Corresponding author: Nebojša Vasiljević, MSc in Chem. Eng.
Email: nebojsa.vasiljevic@tfzv.ues.rs.ba

interactive effects among the selected parameters.[26] The response surface methodology (RSM) offers a more efficient alternative. RSM is a set of mathematical techniques used in data processing, which aim to determine how process parameters affect the response, and then to determine the mathematical model that best fits the obtained data.[27] RSM provides a large amount of relevant data while reducing the time required to conduct experiments.[28] The main types of RSM are factorial design (FD), optimal designs (D-optimal, I-optimal), Box-Behnken Design (BBD), Doehlert Design and Central Composite Design (CCD).[27] Among them, the Central Composite Design, developed in 1951 by Box and Wilson, is especially popular.[29] CCD consists of: 1. factorial design, which includes basic combinations of factors at high (+1) and low (−1) levels; 2. central points, which include the mean values of the factors (0) to assess replicability, and 3. axial points, which include points at a specific distance from the centre (usually $\pm\alpha$), to assess the quadratic effects of the factors.[30]

This work investigates the influence of solids-to-solvent ratio, ethanol content in the extraction solvent, and initial solution pH on the ultrasound-assisted extraction (UAE) of (poly)phenols from yarrow flowers (*Achillea millefolium* L.). By observing the interactions of process parameters and statistical analysis, this study aims to determine their effects on the extraction of (poly)phenolic compounds and identify the optimal extraction conditions.

2 Experimental

Statistical modelling of ultrasound-assisted extraction (UAE) of (poly)phenolic compounds from yarrow flowers (*Achillea millefolium* L.) was conducted. The study evaluated the total (poly)phenol, flavonoid, and anthocyanin content in the extracts as response variables. Further is a brief overview of the reagents used, methods, process conditions, and design of the experiments.

2.1 Materials and methods

Dried yarrow flowers were purchased from a local health food store. The crushed plant material was extracted using ethanol solutions of varying concentrations. After UAE, the spent drug was separated from the newly formed extract by vacuum filtration. Total polyphenol, flavonoid, and anthocyanin contents in the extracts were analysed spectrophotometrically using a Shimadzu UV-1800 spectrophotometer (Cole Parmer, USA).

Total (poly)phenols were determined using the Folin-Ciocalteu method at 765 nm following the procedure of *Singleton and Rossi*; flavonoids were quantified using $AlCl_3$ complexation at 510 nm, according to *Zhishen et al.*, and anthocyanins were measured by the pH differential method at 520 and 700 nm, as outlined by *Giusti and Wrolstad*.[31–33] The Folin-Ciocalteu reagent (Carlo Erba, Germany) was used for the determination of total phenols, with sodium carbonate (Lach:ner, Czech Republic) providing the necessary basic environment. Aluminum chloride (Lach:ner, Czech Republic) was used to form complexes with flavonoids, assisted by sodium hydroxide (Lach:ner, Czech Republic) and sodium nitrite (Zorka Šabac, Serbia). For the determination of anthocyanins, an acetate buffer (pH = 4.5) and a potassium chloride buffer (pH = 1.0) were used. Results were expressed in mg gallic acid equivalents (GAE)/g of dry plant material for polyphenols, mg catechin hydrate (CTH)/g of dry plant material for flavonoids, and mg cyanidin-3-glucoside (Cy3G)/g of dry plant material for anthocyanins. Throughout the remaining text, only the abbreviated units, mg GAE/g, mg CTH/g, and mg Cy3G/g, will be used to avoid unnecessary repetition.

2.2 Design of experiments

To determine the responses as efficiently as possible, response surface methodology (RSM) was applied. In our case, it was used to fit a second-order quadratic model to the data, thereby identifying optimal response conditions. Given that we wanted to include extreme values of process variables in the study, the Central Composite Design (CCD) was chosen as one of the most common types of RSM. Statistical analysis was conducted using Design-Expert 13 (trial version), Stat-Ease Inc, USA. Responses in the study were influenced by three independent variables: ethanol content in solvent – X_1 (35–75 %v/v), solid-to-solvent ratio – X_2 (1 : 20–1 : 50 w/v), and initial solution pH– X_3 (4–10). All extractions were performed in an ultrasonic bath at room temperature for 30 min.

The design included 15 different process condition combinations and 5 replicates at the central point to assess variability, totalling 20 experiments. Table 1 shows the values of the independent variables used for the extraction of (poly)phenolic compounds from yarrow flowers.

Table 1 – Values of independent variables used for RSM* modelling for the ultrasound-assisted extraction of yarrow flowers
Tablica 1 – Vrijednosti neovisnih varijabli koje se primjenjuju za RSM* modeliranje za ultrazvučnu ekstrakciju cvjetova stolisnika

Independent variable		Unit	Level				
Coded	Uncoded		−1.68	−1	0	+1	+1.68
X_1	Ethanol content in solvent	%v/v	21.36	35	55	75	88.64
X_2	Solid-to-solvent ratio	w/v	1 : 9.77	1 : 20	1 : 35	1 : 50	1 : 60.23
X_3	Initial solution pH	–	1.95	4	7	10	12.05

*Study type: Response Surface, Subtype: Randomised, Design Model: Quadratic.

The input and output variables are related through a second-order polynomial equation, as shown in Eq. (1).[34]

$$Y = \beta_0 + \sum_{i=1}^{n}\beta_0 X_i + \sum_{i=1}^{n-1}\sum_{j=2}^{n}\beta_{ij} X_i X_j + \sum_{i=1}^{n}\beta_{ii} X_i^2 \quad (1)$$

Y is the response value, n is the number of independent variables in the model, X_i and X_j are the independent variables, β_0 is a constant coefficient, and β_i, β_{ii} and β_{ij} represent the coefficients for the linear, quadratic, and interaction terms, respectively, in the quadratic model. The values of the β coefficients were obtained by regression analysis using the least squares method on the experimental data. The magnitude of these coefficients measures the influence of each term on the response and allows predicting the behaviour of the system.

The adequacy of the developed model was evaluated by calculating the lack of fit, the coefficient of determination (R^2), F-value, and the p-value. The F-value is the ratio of the mean squared deviation between groups to the mean squared deviation within the group. The p-value is derived from the F-value and the degrees of freedom. A high F-value combined with a low p-value indicates a significant difference between the groups. Analysis of variance (ANOVA) was also used for regression analysis and to generate contour plots.

3 Results and discussion

The results of the study are presented in Table 2. In addition to the actual values (measured using a spectrophotometer), the table also includes predicted values that fit more closely with the proposed model.

The measured values in Table 2 were analysed using the ANOVA method to determine the polynomial coefficients for the response surface model. For each point of the regression model, an F-value and a p-value were determined, with a confidence level greater than 95 % (*i.e.*, a p-value greater than 0.05 is not statistically significant).

To evaluate the adequacy of the model after eliminating parameters that had no significant effect, and to assess its ability to accurately predict responses under various pro-

Table 2 – Measured and predicted values for all responses
Tablica 2 – Izmjerene i predviđene vrijednosti za sve odzive

Std	Run	Factor			Response					
		X_1/%v/v	X_2/w/v	X_3/–	Total (poly)phenol content / mg GAE/g		Flavonoid content / mg CTH/g		Anthocyanin content / mg Cy3G/g	
					Actual	Predicted	Actual	Predicted	Actual	Predicted
14	1	55	35	12.05	16.82	17.33	10.15	10.52	0.1181	0.1119
10	2	88.64	35	7	15.80	16.38	7.12	7.06	0.1894	0.1967
2	3	75	20	4	12.64	13.64	6.12	6.42	0.1523	0.1442
11	4	55	9.77	7	11.77	11.24	5.03	5.09	0.0744	0.0902
17	5	55	35	7	19.30	19.37	9.21	9.34	0.0608	0.0823
3	6	35	50	4	28.36	28.98	9.53	9.85	0.0718	0.0782
5	7	35	20	10	8.37	9.43	5.34	5.27	0.0842	0.0875
4	8	75	50	4	21.08	19.67	8.90	8.99	0.2021	0.1945
15	9	55	35	7	19.12	19.37	9.30	9.34	0.0713	0.0823
7	10	35	50	10	28.64	27.29	11.33	11.06	0.0985	0.1024
13	11	55	35	1.95	19.27	19.26	9.78	9.37	0.0877	0.0999
16	12	55	35	7	19.80	19.37	8.43	9.34	0.0737	0.0823
1	13	35	20	4	18.30	17.83	7.06	7.06	0.0608	0.0461
9	14	21.36	35	7	21.26	21.18	6.65	6.68	0.0608	0.0595
20	15	55	35	7	17.12	19.37	9.14	9.34	0.1041	0.0823
6	16	75	20	10	14.00	13.03	6.87	6.58	0.1449	0.1343
18	17	55	35	7	19.30	19.37	10.47	9.34	0.0818	0.0823
8	18	75	50	10	25.65	25.78	12.12	12.15	0.1570	0.1674
19	19	55	35	7	21.64	19.37	9.50	9.34	0.1029	0.0823
12	20	55	60.23	7	30.31	31.33	12.21	12.12	0.1549	0.1451

Table 3 – ANOVA results for the quadratic model of the Response Surface Method for all responses in the process of ultrasound-assisted extraction of yarrow flowers

Tablica 3 – Rezultati ANOVA za kvadratni model metode odzivne površine za sve odzive za ultrazvučnu ekstrakciju cvjetova stolisnika

Source	*df*	Total (poly)phenol content		Flavonoid content		Anthocyanin content	
		F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Model	9	33.33	< 0.0001	31.25	< 0.0001	13.70	0.0002
X_1	1	13.98	0.0039	0.6044	0.4549	82.06	< 0.0001
X_2	1	245.29	< 0.0001	207.83	< 0.0001	13.09	0.0047
X_3	1	2.26	0.1633	5.56	0.0401	0.6289	0.4462
X_1X_2	1	6.59	0.0280	0.0804	0.7825	0.5978	0.4573
X_1X_3	1	15.26	0.0029	6.58	0.0281	4.74	0.0545
X_2X_3	1	11.33	0.0072	15.60	0.0027	0.5338	0.4818
X_1^2	1	0.3155	0.5867	38.36	0.0001	13.66	0.0041
X_2^2	1	3.34	0.0975	3.43	0.0939	8.15	0.0171
X_3^2	1	1.05	0.3303	2.30	0.1604	3.63	0.0860
Lack of fit	3	0.8981	0.5455	0.3133	0.8858	0.7823	0.6029
Fitting statistics		R^2 = 0.9677 Adj. R^2 = 0.9387 Pred. R^2 = 0.8440 AP = 21.9737		R^2 = 0.9657 Adj. R^2 = 0.9348 Pred. R^2 = 0.8976 AP = 18.6288		R^2 = 0.9250 Adj. R^2 = 0.8575 Pred. R^2 = 0.6805 AP = 12.7967	

**df* – degrees of freedom, Adj. R^2 – Adjusted R^2, Pred. R^2 – Predicted R^2, AP – Adequate Precision

cess conditions, the adjusted R^2 and predicted R^2 were analysed. The results of the ANOVA analysis are presented in Table 3.

The lack-of-fit analysis (Table 3) confirmed the validity of the model for all responses. In each case, the *p*-value was significantly greater than 0.05, indicating that the models were adequately fitted to the experimental data. The adequate precision (signal-to-noise ratio) for all three responses exceeded 4, suggesting that the model was suitable for navigating the design space.

The very high R^2 values for the total (poly)phenol, flavonoid and anthocyanin contents in the extracts, as shown in Table 3, indicate that the generated model effectively explains response variability, and demonstrate a strong correlation between the independent variables and the corresponding responses. The proposed models were able to explain 96.77 % of the variation in total (poly)phenol content, 96.57 % of the variation in flavonoid content, and 92.50 % of the variation in anthocyanin content in the extracts.

By eliminating points in the model that do not have a significant impact on the responses, a reduced regression model was obtained. ANOVA analysis of this reduced model yielded adjusted R^2 values of 0.9387 for total (poly) phenols, 0.9348 for flavonoids, and 0.8575 for anthocyanins in the extracts. These values are very close to the R^2 values, indicating that even the reduced regression models could effectively explain the variations. Subsequently, the predictive performance of the regression model for some other experimental results was assessed. The predicted R^2 values for total (poly)phenols and flavonoids in the extracts were 0.8440, and 0.8976, respectively, confirming that the models were capable of providing good predictions even with different experimental results. However, the predicted R^2 for total anthocyanins (0.6805) was considerably lower than R^2 value (0.925), suggesting that while the model fit the experimental data well, its predictive accuracy was limited.

The regression coefficients, along with the *p*-values for the terms in the proposed quadratic model for all three responses, were obtained through ANOVA analysis. The results are presented in Table 4.

These results were used to generate the regression equation and create the contour diagrams.

Fig. 1 shows the correlation between actual and predicted data. Predicted *vs* actual data (Fig. 1) show that there is a linear relationship between the experimental data (all values are close to the line $y=x$) and the predicted data. This implies that the responses are significantly affected by each component of the model (process variables), *i.e.,* there is a "goodness of fit".[35]

The studentised residual represents the difference between the actual and predicted values, divided by the standard error of that difference. Fig. 2 shows the correlation between studentised residuals and predicted data. It can be observed that the residuals are distributed around zero, without a negative pattern (*e.g.,* systematic increase or decrease, or grouping of data. From Fig. 2, it is evident that all data points lie within the upper and lower limits, indicating that the process is not influenced by any external factor and that the experimental procedure is stable.[36]

Table 4 – Regression coefficients and p-values for all responses
Tablica 4 – Regresijski koeficijenti i p-vrijednosti za sve odzive

Variable	Total (poly)phenol content			Flavonoid content			Anthocyanin content		
	Coded	Uncoded	*p*-value	Coded	Uncoded	*p*-value	Coded	Uncoded	*p*-value
Constant	+19.37	+24.15204	< 0.0001	+9.34	+3.33240	< 0.0001	+0.0823	+0.083402	< 0.0001
X_1	−1.43	−0.091840	0.0039	+0.1128	+0.195753	0.4549	+0.0408	−0.001452	< 0.0001
X_2	+5.98	+0.160869	< 0.0001	+2.09	+0.114147	< 0.0001	+0.0163	−0.002973	0.0047
X_3	−0.5741	−2.69008	0.1633	+0.3421	−1.24715	0.0401	+0.0036	+0.003291	0.4462
X_1X_2	−1.28	−0.004267	0.0280	−0.0537	−0.000179	0.7825	+0.0046	+0.000015	0.4573
X_1X_3	+1.95	+0.032458	0.0029	+0.4862	+0.008104	0.0281	−0.0128	−0.000214	0.0545
X_2X_3	+1.68	+0.037278	0.0072	+0.7488	+0.016639	0.0027	−0.0043	−0.000096	0.4818
X_1^2	−0.2086	−0.000521	0.5867	−0.8748	−0.002187	0.0001	+0.0162	+0.000041	0.0041
X_2^2	+0.6788	+0.003017	0.0975	−0.2614	−0.001162	0.0939	+0.0125	+0.000056	0.0171
X_3^2	−0.3801	−0.042230	0.3303	+0.2141	+0.023793	0.1604	+0.0084	+0.000928	0.0860

*Coded – Coded regression coefficients, Uncoded – Uncoded regression coefficients

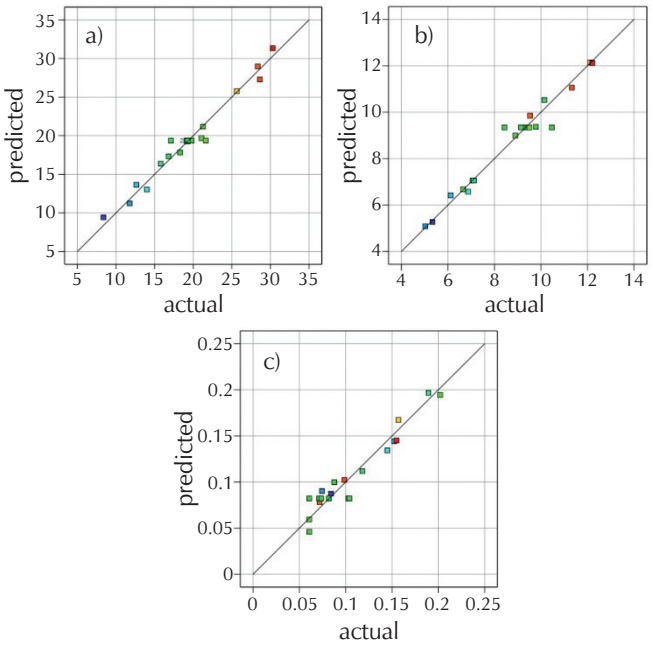

Fig. 1 – Predicted *vs.* actual data. Model validation for: a) (poly) phenols, b) flavonoids, and c) anthocyanins.
Slika 1 – Predviđeni u odnosu na stvarne podatke. Validacija modela za: a) (poli)fenole, b) flavonoide i c) antocijane.

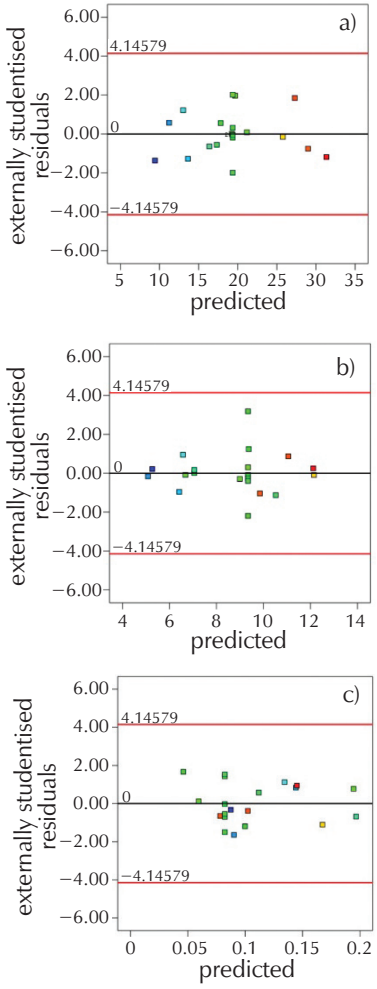

Fig. 2 – Studentised residuals *vs* predicted. Model validation for: a) (poly)phenols, b) flavonoids, and c) anthocyanins.
Slika 2 – Studentizirani reziduali u odnosu na predviđene. Validacija modela za: a) (poli)fenole, b) flavonoide i c) antocijane

3.1 Influence of independent variables on the content of total (poly)phenols

The relationships between the independent variables and the response ((poly)phenol content in the extract) were examined based on the quadratic model (Eq. (1)). ANOVA analysis revealed that the content of total (poly)phenols in the extract has a significant influence ($p < 0.05$) on the following parameters: ethanol content in the solvent (X_1), solid-to-solvent ratio (X_2), mutual interaction of the ethanol

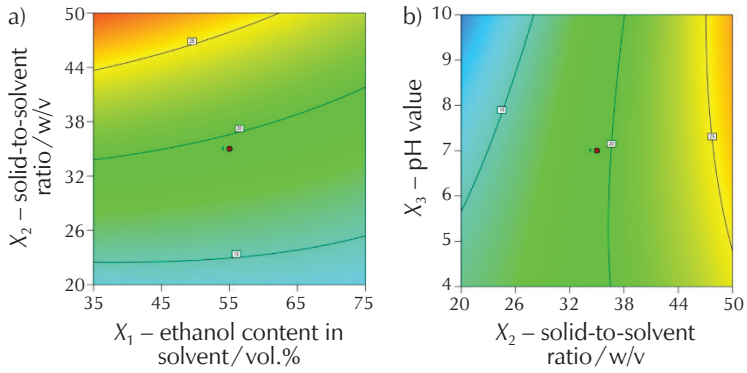

Fig. 3 – Contour diagrams of the content of (poly)phenols for: a) mutual interaction of X_1 and X_2, and b) mutual interaction of X_2 and X_3

Slika 3 – Konturni dijagrami sadržaja (poli)fenola za: a) međusobnu interakciju X_1 and X_2 i b) međusobnu interakciju X_2 and X_3

content in the solvent and the solid-to-solvent ratio (X_1X_2), mutual interaction of the ethanol content in the solvent and initial solvent pH value (X_1X_3), and the mutual interaction of the solid-to-solvent ratio and pH value (X_2X_3). Using Table 4, and retaining only those terms that have an impact on the response, the regression equation takes the following form (Eq. (2)):

$$Y = 24.15204 - 0.09184 \cdot X_1 + 0.16087 \cdot X_2 - \\ - 0.04267 \cdot X_1X_2 + 0.032458 \cdot X_1X_3 + 0.037278 \cdot X_2X_3 \quad (2)$$

For a clear visual representation of the effect of process parameters on the output value, contour diagrams were constructed (Fig. 3).

Fig. 3a illustrates the interaction of the solid-to-solvent ratio (X_2) and the content of ethanol in the solvent (X_1) on the total (poly)phenol content in the extracts, while the initial solvent pH (X_3) is held constant. It was observed that the response value was lowest at a low solid-to-solvent ratio, regardless of the ethanol content in the solvent (<15 mg GAE/g). Increasing the solid-to-solvent ratio resulted in a corresponding increase in the response value, with this increase being more pronounced at a low ethanol content in the solvent. Thus, increasing the solid-to-solvent ratio from 1 : 20 w/v to 1 : 50 w/v using a solvent with low ethanol content (35–45 %v/v) raised the response value from < 15 mg GAE/g of sample to > 25 mg GAE/g of sample. In contrast, using solvents with higher ethanol content (70–75 %v/v) led to an increase in the response value from < 15 mg GAE/g of sample to only 20 mg GAE/g of sample. This indicated that the solid-to-solvent ratio significantly influenced the extraction of (poly)phenols, as it was the main driving force for mass transfer (the difference in concentration between the solid and liquid phases).[37] Increasing the solid-to-solvent ratio increased the contact surface area between the plant material and the solvent, thereby accelerating the transfer of target compounds into the extraction medium and enhancing extraction efficiency.[38] On the other hand, the use of solvents with high ethanol content leads to rapid dehydration of plant cells; this causes aggregation of small structures within the cells (such as organelles or membrane fragments), which hinders the diffusion of compounds into the solvent.[39]

Fig. 3b depicts the influence of the pH value (X_3) and the solid-to-solvent ratio (X_2) on the response value at the mean value of ethanol content in the solvent (X_1). It is evident that the total (poly)phenol content increased with an increasing solid-to-solvent ratio. The total (poly)phenol content was lowest at a low solid-to-solvent ratio and pH values ranging from 7.5–10 (< 15 mg GAE/g). With a further increase in the solid-to-solvent ratio, the effect of pH became less pronounced, and at the highest solid-to-solvent ratio, pH had almost no influence on the response value. As shown in Fig. 3b, pH is not as dominant a factor as the other process parameters. Few studies have highlighted the negative influence of low pH on (poly)phenol extraction as clearly as this one.[40]

3.2 Influence of independent variables on the content of flavonoids

ANOVA analysis identified the parameters that significantly affect ($p < 0.05$) the extraction of flavonoids from the yarrow flower: solid-to-solvent ratio (X_2), pH value (X_3), mutual interaction of ethanol content in the solvent and pH value of initial solvent (X_1X_3), mutual interaction of the solid-to-solvent ratio and pH value of initial solvent (X_2X_3), and the square of the ethanol content in the solvent (X_1^2). Using Table 4, the abbreviated regression equation for the flavonoid content in the extract is presented by Eq. (3).

$$Y = 3.33240 + 0.114147 \cdot X_2 - 1.24715 \cdot X_3 + \\ + 0.008104 \cdot X_1X_3 + 0.016639 \cdot X_2X_3 - 0.002187 \cdot X_1^2 \quad (3)$$

Contour plots for the flavonoid content in the extract, as the Response, are shown in Fig. 4.

Fig. 4a shows the influence of the solid-to-solvent ratio (X_2) and the ethanol content in the solvent (X_1) on the flavonoid content in the extracts based on the mean level (0) of the pH value (X_3). As in the case of total (poly)phenol content, an increase in the solid-to-solvent ratio resulted in an increase in the response (from 7 to 11 mg CTH/g at the central values of ethanol in the solvent). On the other hand,

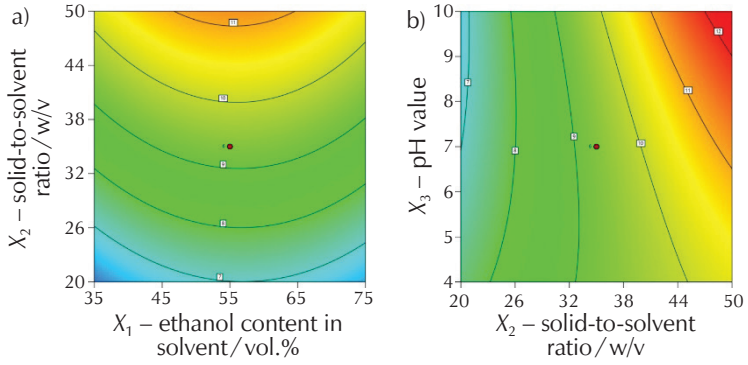

Fig. 4 – Contour diagrams of the flavonoid content for: a) mutual interaction of X_1 and X_2, and b) mutual interaction of X_2 and X_3

Slika 4 – Konturni dijagrami sadržaja flavonoida za: a) međusobnu interakciju X_1 i X_2 i b) međusobnu interakciju X_2 i X_3

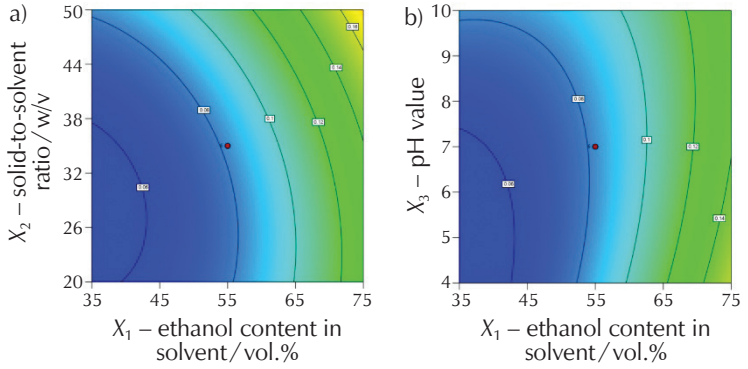

Fig. 5 – Contour diagrams of the anthocyanin content for: a) mutual interaction of X_1 and X_2, and b) mutual interaction of X_1 and X_3

Slika 5 – Konturni dijagrami sadržaja antocijana za: a) međusobnu interakciju X_1 i X_2 i b) međusobnu interakciju X_1 i X_3

an optimal ethanol content in the solvent was observed. It is evident from Fig. 4a that the extraction of flavonoids was best when using 55 %v/v ethanol, and this phenomenon was consistently observed across all solid-to-solvent ratios. The existence of an optimal ethanol concentration in the solvent aligns with findings from some other studies, which also observed that a combination of water and alcohol yields higher extraction efficiency than using pure ethanol as a solvent.[38,41] If the extraction were performed using only water, the high dielectric constant of the solution would hinder efficient extraction. Gradually adding alcohol to the solvent reduces the dielectric constant of the solution, allowing for rapid transfer of flavonoids from the cells to the solvent. However, exceeding the optimal ethanol content in the solvent can lead to cell wall damage and structural changes to proteins, ultimately impeding flavonoid diffusion.[38]

Fig. 4b confirms the results from Fig. 4a, *i.e.*, that an increase in the solid-to-solvent ratio (X_2) results in a higher flavonoid content in the extract. The pH value of the solvent had minimal effect on the output at low solid-to-solvent ratio. On the other hand, its effect of the pH value became more pronounced at a solid-to-solvent ratio of 1 : 50 w/v. At this ratio, the response value increased from 10 to 12.5 mg CTH/g as the pH value increased from 4 to 10.

3.3 Influence of independent variables on the content of anthocyanins in the extract

ANOVA analysis demonstrated that the following parameters had a *p*-value less than 0.05, and as such were considered statistically significant for the extraction of anthocyanins from yarrow flowers: ethanol content in the solvent (X_1), solid-to-solvent ratio (X_2), square of the ethanol content in the solvent (X_1^2), and the square of the solid-to-solvent ratio (X_2^2). Based on Table 4, and by retaining only those factors that are significant, the regression equation for the anthocyanin content in the extract takes the form shown in Eq. (4):

$$Y = 0.008202 - 0.001452 \cdot X_1 - 0.002973 \cdot X_2 + \\ + 0.000041 \cdot X_1^2 + 0.000056 \cdot X_2^2 \quad (4)$$

Contour plots for anthocyanin content in the extract, as the response, are shown in Fig. 5.

From Fig. 5a (which shows the influence of the solid-to-solvent ratio (X_2) and the ethanol content in the solvent (X_1) on anthocyanin content), it can be seen that anthocyanin extraction was most efficient within a specific range of process parameters. Only at high values of the solid-to-solvent ratio (1 : 45–1 : 50 w/v) and a high ethanol content in the solvent (70–75 %v/v) could a significant yield of anthocyanins in the extract be achieved (> 0.16 mg Cy3G/g). Sufficiently high yields (0.10–0.16 mg Cy3G/g can be achieved by using a solvent with over 65 %v/v ethanol, regardless of the solid-to-solvent ratio. Favourable conditions cannot be achieved with other process parameters. Similar conclusions have been drawn in other studies.[42,43] Ethanol exhibits a weaker polar character than water, which makes it more effective in degrading non-polar compounds present in the cell wall and seed, thereby facilitating the release of anthocyanins.[44]

In Fig. 5b, a pronounced decrease in anthocyanin content can be observed with decreasing ethanol content in the solvent from 75 to 35 %v/v in acidic conditions (from 0.14 to 0.06 mg Cy3G/g) compared with a smaller decrease in base medium (from 0.12 to 0.08 mg Cy3G/g). As in the case of total (poly)phenols and flavonoids as responses, the third contour diagram was not processed, as it was dependent on the other two.

4 Conclusion

This research confirms that ultrasound-assisted extraction (UAE) is an effective method for extracting bioactive compounds from *Achillea millefolium* L. Statistical modelling and response surface methodology identified the influence of various process parameters on the extraction process. The proposed models were able to explain the observed variations well, even after excluding the regression terms with minimal effect on the response value. Predicted R^2 values were high for (poly)phenols and flavonoids, but not for anthocyanins as response, suggesting that while the model aligns well with the original data, its predictive accuracy is limited. The content of total (poly)phenols and flavonoids in the extracts mostly depended on the solid-to-solvent ratio and the ethanol content in the solvent, while the pH value had no great influence. Increasing the solid-to-solvent ratio had a positive effect on extraction, as it provided more solvent for hydration of the sample matrix, and increased the contact surface between the sample and the solvent, further accelerating mass transfer. Medium values of ethanol in the solvent were optimal for extracting polyphenols and flavonoids, as further increases in alcohol concentration could lead to denaturation of cell wall proteins, which inhibits extraction. Unlike the other responses, extraction of anthocyanins was most effective at a high ethanol content in the solvent; the reason for this is that ethanol exhibits a weaker polar character compared to water, making it more effective in breaking down non-polar anthocyanins present in the cell wall and seeds. The proposed models can be used to optimise the process in real conditions, enabling the production of extracts rich in bioactive compounds with rational costs. Future research should investigate the influence of additional process parameters, such as extraction temperature and duration, on ultrasound-assisted extraction.

List of symbols and abbreviations
Popis simbola i kratica

Adj. R^2 – adjusted coefficient of determination
ANOVA – analysis of variance
AP – adequate precision
BBD – Box-Behnken Design
CCD – central composite design
CTH – catechin hydrate equivalents, used to express flavonoid content
Cy3G – cyanidin-3-glucoside equivalents, used to express anthocyanin content
df – degrees of freedom
GAE – gallic acid equivalents, used to express total (poly) phenol content
p-value – probability value, indicating statistical significance
Pred. R^2 – predicted coefficient of determination
R^2 – coefficient of determination
RSM – response surface methodology
UAE – ultrasound-assisted extraction
vol.% – volume percent, used for expressing solvent composition (*e.g.*, ethanol content)
w/v – weight-to-volume ratio (*e.g.*, 1 : 20 w/v indicates 1 g of solid *per* 20 ml of solvent)
X_1 – ethanol content in the solvent
X_2 – solid-to-solvent ratio
X_3 – pH value of the initial solvent

References
Literatura

1. *L. Lazar, A. I. Talmaciu, I. Volf, V. I. Popa*, Kinetic modeling of the ultrasound-assisted extraction of polyphenols from *Picea abies* bark, Ultrason. Sonochem. **32** (2016) 191–197, doi: https://doi.org/10.1016/j.ultsonch.2016.03.009.
2. *S. Alijani, M. R. Raji, Z. Emami Bistgani, A. Ehtesham Nia, M. Farajpour*, Spermidine-induced improvements in water relations and antioxidant defense enhance drought tolerance in yarrow (*Achillea millefolium* L.), Heliyon **11** (2025) e41482, doi: https://doi.org/10.1016/J.HELIYON.2024.E41482.
3. *A. Grigore, S. Colceru-Mihul, C. Bazdoaca, R. Yuksel, C. Ionita, L. Glava*, Antimicrobial activity of an *Achillea millefolium* L., Proceedings **57** (1) (2020) 34, doi: https://doi.org/10.3390/proceedings2020057034.
4. *E. Algın Yapar, E. H. Gökçe, A. Şahiner, E. İnal, Ş. Ulusoy, E. B. Souto, B. Akanoğlu, M. Kartal*, Phytoactive essential oils-composed water-free organogels: Development, characterization and proof of antibacterial activity, J. Drug Deliv. Sci. Technol. **97** (2024) 105811, doi: https://doi.org/10.1016/J.JDDST.2024.105811.
5. *E. Razghandi, A. H. Elhami-Rad, S. M. Jafari, M. R. Saiedi-Asl, H. Bakhshabadi*, Combined pulsed electric field-ultrasound assisted extraction of yarrow phenolic-rich ingredients and their nanoliposomal encapsulation for improving the oxidative stability of sesame oil, Ultrason. Sonochem. **110** (2024) 107042, doi: https://doi.org/10.1016/J.ULTSONCH.2024.107042.
6. *S. Vitalini, G. Beretta, M. Iriti, S. Orsenigo, N. Basilico, S. Dall'Acqua, M. Iorizzi, G. Fico*, Phenolic compounds from

Achillea millefolium L. and their bioactivity, Acta Biochim. Pol. **58** (2011) 203–209, doi: https://doi.org/10.18388/abp.2011_2266.

7. *L. Amjad, M. Mohammadi-Sichani*, *M. Mohammadi-Kamalabadi*, Potential activity of the *Achillea wilhelmsii* leaves on bacteria, Int. J. Biosci. Biochem. Bioinforma. **1** (2011) 216–218, doi: https://doi.org/10.7763/ijbbb.2011.v1.39.
8. *G. Niazipoor, M. AghaAlikhani, A. Mokhtassi-Bidgoli, M. Iriti*, *S. Vitalini*, Phytochemical analysis and allelopathic potential of essential oil of yarrow (*Achillea* spp.) ecotypes against redroot pigweed (*Amaranthus retroflexus* L.), Heliyon **10** (2024) e26101, doi: https://doi.org/10.1016/j.heliyon.2024.e2610.
9. *S. Cvetković, A. Ignjatijević, J. Kukić-Marković, S. Vuletić, L. Ušjak, V. Milutinović, D. Mitić-Ćulafić, S. Petrović, B. Nikolić*, Further insights into antimicrobial and cytotoxic potential of *Achillea millefolium* herb methanol and dichloromethane extracts, Ind. Crops. Prod. **225** (2025) 120553, doi: https://doi.org/10.1016/J.INDCROP.2025.120553.
10. *J. Ahmad, M. M. Nadim, A. Malik*, *S. K. Bakshi*, The essential oil composition of *Achillea millefolium* L. cultivated under tropical condition in India, World J. Agric. Sci. **7** (2011) 561–565, url: https://www.idosi.org/wjas/wjas7(5)/7.pdf.
11. *L. Apel, P. Lorenz, S. Urban, S. Sauer, O. Spring, F. C. Stintzing*, *D. R. Kammerer*, Phytochemical characterization of different yarrow species (Achillea sp.) and investigations into their antimicrobial activity, Z. Naturforsch – Section C – J. Biosci. **76** (2021) 55–65, doi: https://doi.org/10.1515/znc-2020-0149.
12. *M. Strzępek-Gomółka, K. Gaweł-Bęben*, *W. Kukula-Koch*, *Achillea* species as sources of active phytochemicals for dermatological and cosmetic applications, Oxid. Med. Cell. Longev. **2021** (2021) 6643827, doi: https://doi.org/10.1155/2021/6643827.
13. *S. T. Chou, H. Y. Peng, J. C. Hsu, C. C. Lin, Y. Shih*, *Achillea millefolium* L. essential oil inhibits LPS-induced oxidative stress and nitric oxide production in RAW 264.7 macrophages, Int. J. Mol. Sci. **14** (2013) 12978–12993, doi: https://doi.org/10.3390/ijms140712978.
14. *Ö. Bariş, M. Güllüce, F. Şahin, H. Özer, H. Kiliç, H. Özkan, M. Sökmen, T. Özbek*, Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan. (Asteraceae), Turk. J. Biol. **30** (2006) 65–73, url: https://journals.tubitak.gov.tr/biology/vol30/iss2/2/.
15. *H. Che, R. Zhang, X. Wang, H. Yu, X. Shi, J. Yi, J. Li, Q. Qi, R. Dong, Q. Li*, Ultrasound-assisted extraction of polyphenols from *Phyllanthi* Fructus: Comprehensive insights from extraction optimization and antioxidant activity, Ultrason. Sonochem. **111** (2024) 107083, doi: https://doi.org/10.1016/j.ultsonch.2024.107083.
16. *S. Rodríguez-Rojo, A. Visentin, D. Maestri, M. J. Cocero*, Assisted extraction of rosemary antioxidants with green solvents, J. Food. Eng. **109** (2012) 98–103, doi: https://doi.org/10.1016/j.jfoodeng.2011.09.029.
17. *F. Chemat, M. A. Vian, G. Cravotto*, Green extraction of natural products: Concept and principles, Int. J. Mol. Sci. **13** (2012) 8615–8627, doi: https://doi.org/10.3390/ijms13078615.
18. *D. Pradal, P. Vauchel, S. Decossin, P. Dhulster, K. Dimitrov*, Kinetics of ultrasound-assisted extraction of antioxidant polyphenols from food by-products: Extraction and energy consumption optimization, Ultrason. Sonochem. **32** (2016) 137–146, doi: https://doi.org/10.1016/j.ultsonch.2016.03.001.
19. *W. Wu, S. Jiang, M. Liu, S. Tian*, Simultaneous process optimization of ultrasound-assisted extraction of polyphenols and ellagic acid from pomegranate (*Punica granatum* L.) flowers and its biological activities, Ultrason. Sonochem. **80** (2021) 105833, doi: https://doi.org/10.1016/j.ultsonch.2021.105833.
20. *J. Yang, N. Li, C. Wang, T. Chang*, *H. Jiang*, Ultrasound-homogenization-assisted extraction of polyphenols from coconut mesocarp: Optimization study, Ultrason. Sonochem. **78** (2021) 105739, doi: https://doi.org/10.1016/j.ultsonch.2021.105739.
21. *S. Zhang, H. Xie, J. Huang, Q. Chen, X. Li, X. Chen, J. Liang, L. Wang*, Ultrasound-assisted extraction of polyphenols from pine needles (*Pinus elliottii*): Comprehensive insights from RSM optimization, antioxidant activity, UHPLC-Q-Exactive Orbitrap MS/MS analysis and kinetic model, Ultrason. Sonochem. **102** (2024) 106742, doi: https://doi.org/10.1016/j.ultsonch.2023.106742.
22. *J. E. Wong Paz, D. B. Muñiz Márquez, G. C. G. Martínez Ávila, R. E. Belmares Cerda, C. N. Aguilar*, Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert, Ultrason. Sonochem. **22** (2015) 474–481, doi: https://doi.org/10.1016/j.ultsonch.2014.06.001.
23. *M. Afroz Bakht, M. H. Geesi, Y. Riadi, M. Imran, M. Imtiyaz Ali, M. J. Ahsan, N. Ajmal*, Ultrasound-assisted extraction of some branded tea: Optimization based on polyphenol content, antioxidant potential and thermodynamic study, Saudi J. Biol. Sci. **26** (2019) 1043–1052, doi: https://doi.org/10.1016/j.sjbs.2018.07.013.
24. *R. E. Ghitescu, I. Volf, C. Carausu, A. M. Bühlmann, I. A. Gilca*, *V. I. Popa*, Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark, Ultrason. Sonochem. **22** (2015) 535–541, doi: https://doi.org/10.1016/j.ultsonch.2014.07.013.
25. *D. Tabio-García, F. Paraguay-Delgado, M. Sánchez-Madrigal, A. Quintero-Ramos, J. C. Espinoza-Hicks, C. O. Meléndez-Pizarro, M. G. Ruiz-Gutiérrez, E. Espitia-Rangel*, Optimisation of the ultrasound-assisted extraction of betalains and polyphenols from *Amaranthus hypochondriacus* var. Nutrisol, Ultrason. Sonochem. **77** (2021) 105680, doi: https://doi.org/10.1016/j.ultsonch.2021.105680.
26. *P. L. Lau, A. C. Affam*, Modeling and optimization of the removal of chlorpyrifos and organics reduction from wastewater by Ag/AgBr/TiO_2 using the central composite design (CCD), Desal. Water. Treat. **175** (2020) 90–98, doi: https://doi.org/10.5004/dwt.2020.24899.
27. *L. López-Salas, X. Expósito-Almellón, I. Borrás-Linares, J. Lozano-Sánchez, A. Segura-Carretero*, Design of experiments for green and GRAS solvent extraction of phenolic compounds from food industry by-products – A systematic review, TrAC **171** (2024) 117536, doi: https://doi.org/10.1016/j.trac.2024.117536.
28. *L. A. Román-Ramírez, J. Marco*, Design of experiments applied to lithium-ion batteries: A literature review, Appl. Energy. **320** (2022) 119305, doi: https://doi.org/10.1016/j.apenergy.2022.119305.
29. *G. E. P. Box, K. B. Wilson*, On the Experimental Attainment of Optimum Conditions, J. R. Stat. Soc. Series B. Stat. Methodol. **13** (1951) 1–45, doi: https://doi.org/10.1111/j.2517-6161.1951.tb00067.x.
30. *J. Wang, W. Wan*, Experimental design methods for fermentative hydrogen production: A review, Int. J. Hydrogen Energy **34** (2009) 235–244, doi: https://doi.org/10.1016/j.ijhydene.2008.10.008.
31. *V. L. Singleton, J. A. Rossi*, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, Am. J. Enol. Vitic. **16** (1965) 144–158, doi: https://doi.org/10.5344/ajev.1965.16.3.144.
32. *J. Zhishen, T. Mengcheng, W. Jianming*, The determination of flavonoid contents in mulberry and their scavenging effects

on superoxide radicals, Food Chem. **64** (1999) 555–559, doi: https://doi.org/10.1016/S0308-8146(98)00102-2.

33. *M. M. Giusti, R. E. Wrolstad*, Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy, Curr. Protoc. in Food Anal. Chem. **00** (2001) F1.2.1–F1.2.13, doi: https://doi.org/10.1002/0471142913.faf0102s00.
34. *M. Ganje, S. Gharibi, F. Nejatpour, M. Deilamipour, K. Goshadehrou, S. Saberyan, G. Abdi*, The ANFIS-RSM based multi-objective optimization and modelling of ultrasound-assisted extraction of polyphenols from jamun fruit (*Syzygium cumini*), Ultrason. Sonochem. **113** (2025) 107227. doi: https://doi.org/10.1016/J.ULTSONCH.2025.107227.
35. *T. Belayneh Asfaw, M. Getachew Tadesse, F. Beshah Tessema, H. Woldemichael Woldemariam, A. V. Chinchkar, A. Singh, A. Upadhyay, B. Mehari*, Ultrasonic-assisted extraction and UHPLC determination of ascorbic acid, polyphenols, and half-maximum effective concentration in *Citrus medica* and *Ziziphus spina-christi* fruits using multivariate experimental design, Food Chem. X **22** (2024) 101310, doi: https://doi.org/10.1016/J.FOCHX.2024.101310.
36. *W. M. Farouk, A. Hoballah, Z. M. Omara, F. A. Essa*, Predictive modeling and optimization of tubular distiller operation using response surface methodology under silver nanomaterial infused PCM thickness variations, Case Studies in Therm. Eng. **66** (2025) 105689, doi: https://doi.org/10.1016/J.CSITE.2024.105689.
37. *H. Che, R. Zhang, X. Wang, H. Yu, X. Shi, J. Yi, J. Li, Q. Qi, R. Dong, Q. Li*, Ultrasound-assisted extraction of polyphenols from *Phyllanthi* Fructus: Comprehensive insights from extraction optimization and antioxidant activity, Ultrason. Sonochem. **111** (2024) 107083, doi: https://doi.org/10.1016/J.ULTSONCH.2024.107083.
38. *E. C. Umego, C. Barry-Ryan*, Optimisation of polyphenol extraction for the valorisation of spent gin botanicals, LWT **199** (2024) 116114, doi: https://doi.org/10.1016/J.LWT.2024.116114.
39. *P. Alonso-Vázquez, M. J. Lujan-Facundo, C. M. Sánchez-Arévalo, B. Cuartas-Uribe, M. C. Vincent-Vela, S. Álvarez-Blanco*, Recovery of phenolic compounds from orange juice solid waste by solid-liquid extraction, LWT **203** (2024) 116355, doi: https://doi.org/10.1016/J.LWT.2024.116355.
40. *F. Rubio-Senent, J. Fernández-Bolaños, A. García-Borrego, A. Lama-Muñoz, G. Rodríguez-Gutiérrez*, Influence of pH on the antioxidant phenols solubilised from hydrothermally treated olive oil by-product (alperujo), Food Chem. **219** (2017) 339–345, doi: https://doi.org/10.1016/j.foodchem.2016.09.141.
41. *B. C. G. Gandolpho, A. R. Almeida, G. M. Gandolpho, D. Z. Freitas, O. C. Gasparini, M. H. MacHado, P. L. M. Barreto*, Optimization of brewing waste's (TRUB) phenolic compounds extraction by ultrasound assisted using response surface methodology, Quim. Nova **44** (2021) 1–6, doi: https://doi.org/10.21577/0100-4042.20170688.
42. *H. Kim, Y. S. Jung, N. E. Song, M. Yoo, D. H. Seo, H. S. Kim, T. G. Nam*, Ultrasound-assisted extraction of major anthocyanins in Korean black raspberries (*Rubus coreanus* Miquel) using natural deep eutectic solvents, LWT **199** (2024) 116121, doi: https://doi.org/10.1016/J.LWT.2024.116121.
43. *I. D. Larasati, C. Carrera, H. N. Lioe, T. Estiasih, N. D. Yuliana, Manikharda, H. R. Daniel Ray, M. Palma, W. Setyaningsih*, Anthocyanin extraction from roselle (*Hibiscus sabdariffa* L.) calyces: A microwave-assisted approach using Box-Behnken design, J. Agric. Food. Res. **18** (2024) 101480, doi: https://doi.org/10.1016/J.JAFR.2024.101480.
44. *R. Jaberi, G. Kaban, M. Kaya*, Effects of some extraction parameters on anthocyanin content of barberry (*Berberis vulgaris* L.) and its antioxidant activity, Turk. Tarim. Arast. Derg. **9** (2022) 41–48, doi: https://doi.org/10.19159/tutad.1034137.

SAŽETAK

Statističko modeliranje ultrazvučne ekstrakcije bioaktivnih spojeva iz stolisnika (*Achillea millefolium* L.)

Nebojša Vasiljević,[a,b*] *Milorad Tomić*,[a,c] *Sanja Panić*,[b] *Tijana Ljubinac* [a] i *Vladan Mićić* [a]

U ovom radu provedeno je statističko modeliranje ultrazvučne ekstrakcije (poli)fenolnih spojeva iz cvjetova stolisnika (*Achillea millefolium* L.) pomoću *central-composite* dizajna eksperimenta (CCD). Ispitivan je utjecaj sadržaja etanola u otapalu (21,36 – 88,64 %v/v, uključujući ekstreme), omjera krutine i otapala (1 : 9,77 – 60,23 w/v, uključujući ekstreme) i pH vrijednosti početne otopine (1,95 – 12,05, uključujući ekstreme) na učinkovitost ekstrakcije ukupnih (poli)fenola, flavonoida i antocijanina. Rezultati su pokazali da na odzive najviše utječu omjer čvrste tvari i otapala te sadržaj etanola u otapalu, dok je utjecaj pH početne otopine slab. Modeli dobiveni regresijskom analizom pokazali su visoku pouzdanost, s koeficijentima determinacije (R^2) blizu jedinici. Maksimalni sadržaj ukupnih (poli)fenola (≈ 30,31 mg GAE/g) i flavonoida (≈ 12,21 mg CTH/g) postignut je pri visokom omjeru čvrste tvari i otapala (1 : 60,23 m/v) i srednjem sadržaju etanola (55 %v/v). Za razliku od prethodnog, najveći udio antocijanina (≈ 0,157 mg Cy3G/g) postignut je pri najvišem udjelu etanola (88,64 %v/v) i srednjem omjeru krutine i otapala (1 : 35 w/v). Bez obzira na odziv, najveći prinosi postižu se u neutralnom okruženju, iako se zadovoljavajući prinos može postići i u alkalnim uvjetima. ANOVA analiza pokazala je da predloženi kvadratni modeli imaju visoku statističku značajnost ($p < 0,0001$ za sve odzive), što potvrđuje njihovu valjanost za predviđanje učinkovitosti ekstrakcije u različitim uvjetima.

Ključne riječi
ANOVA, ultrazvučna ekstrakcija (UAE), (poli)fenoli, cvjetovi stolisnika, flavonoidi

[a] *Univerzitet u Istočnom Sarajevu, Tehnološki fakultet Zvornik, Karakaj 34A, 75 400 Zvornik, Republika Srpska, Bosna and Hercegovina*
[b] *Tehnološki fakultet, Univerzitet u Novom Sadu, Bulevar cara Lazara 1, 21 101 Novi Sad, Srbija*
[c] *Akademija inženjerskih nauka Srbije, Kneza Miloša 9/IV, 11 000 Beograd, Srbija*

Izvorni znanstveni rad
Prispjelo 18. veljače 2025.
Prihvaćeno 12. travnja 2025.