Cyclodextrin-aided green extraction from *Fagopyrum esculentum* Moench – an effective strategy for the sustainable recovery of polyphenols

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As a rich source of polyphenolic compounds, *Fagopyrum esculentum* Moench herb, is a valuable subject for a numerous industries, such as pharmaceutical, chemical, food, or cosmetic. Given the biological potential that is closely related to the polyphenolic content, this study aimed to develop ecologically suitable cyclodextrin-aided extraction from the aerial parts of *F. esculentum*. To maximize the polyphenolic content, highly-reliable mathematical and statistical method – response surface methodology (RSM) was used for the evaluation of crucial extraction parameters: temperature, extraction time, concentrations of ethanol and two types of cyclodextrins (β-cyclodextrin (β-CD), and (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD)). While temperature and ethanol concentration significantly influenced the total phenolic content (TPC) in both models, the impact of cyclodextrin concentration was observed when HP-β-CD was used. According to the RSM analysis, optimal conditions for the maximization of TPC were noted for the extraction at 80 °C for 40 min, using 50% ethanol and 1.50% HP-β-CD. Thus, under optimized extraction conditions, the measured TPC was 42.16 ± 1.01 mg GAE/g DW, among rutin was the most abundant with content of 29.55 mg/g DW, followed by the quercetin and quercitrin in slightly scarcer content. This study confirmed that cyclodextrin-aided extraction is an ecologically friendly, effective method for the recovery of *F. esculentum* polyphenolics.

Keywords: common buckwheat; ultrasound-assisted extraction; cyclodextrin; extraction optimization; response surface methodology; rutin

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1. INTRODUCTION

For centuries, medicinal plants and their products have built the foundation for the treatment of various diseases. Considering that nearly 80% of the population is still dependent on herbal medicine, the demand for medicinal herbs, including minor grain crops and their products, is currently increasing on the global level (Purkait et al., 2023). Among them, buckwheat, as a member of the genus *Fagopyrum*, family Polygonaceae, has been widely grown mainly in the cold regions, with China and Russia being the main producers (Li, 2019). The most frequently used, common buckwheat (*Fagopyrum esculentum* Moench), has been recognized for significant health benefits, including its potential for the prevention of chronic diseases (Radan et al., 2023). For example, the consumption of this herb or its products has been shown to express anti-inflammatory, hypoglycemic, hypocholesterolemic, and anticancer properties (Giménez-Bastida and Zieliński, 2015). According to the randomized, double-blind, single-center, placebo-controlled clinical trial carried out by (Ihme et al., 1996), the buckwheat herb tea proved its safety and favorable effects against further edema development in chronic venous insufficiency as well. Polyphenolic components have been described as the carriers of the buckwheat's bioactive potential, where rutin, quercetin, hyperin, chlorogenic acid, and catechins, have been commonly identified among various extracts (Hinneburg and Neubert, 2005; Inglett et al., 2010; Radan et al., 2023). Numerous biological effects have been described for these polyphenolics, such as antimicrobial, anti-hypertensive, antioxidant, and anti-inflammatory potential (Ahmed et al., 2014; Noreen et al., 2021).

Not only that, rutin has benefits in the treatment and prevention of cardiovascular diseases, with its potential to lower plasma cholesterol and decrease the fragility of capillaries (Salvamani et al., 2014). Besides the potential pharmaceutical use, it has been shown that it could be also used as a natural pigment, food preservative, and stabilizer, making it an interesting compound in the food and chemical industries (Kim et al., 2005). Furthermore, in the cosmetic industry rutin showed promising effects as a component of suncare products as well, due to its potential to scavenge free radicals and absorb UV radiation (Hinneburg and Neubert, 2005). On the other hand, quercetin was reported to evince neuroprotective, antineoplastic, anti-inflammatory, anti-allergic, and antihistamine effects (Rakha et al., 2022; Zalpoor et al., 2022).

Embracing the great potential of buckwheat's bioactive compounds in various industries, optimal extraction techniques must be employed, covering not only the highest yield and quality of the final product but also choosing the sustainable methods following green chemistry principles (Radan et al., 2024). Proper extraction method is of the key importance for both qualitative and quantitative composition of bioactive compounds (Azmir et al., 2013). While being usually used on the small research and manufacturing level, traditional methods such as Soxhlet extraction and maceration have certain disadvantages, related to low reproducibility, low production efficiency, and a demand for a significant amount of time (Pilkington et al., 2014). Thus, ultrasound-assisted extraction (UAE) stands out as a proper, sustainable, and more feasible method, capable of providing high quality products, with a significant reduction in solvent and time consumption (Pandey et al., 2018; Pilkington et al., 2014). This particular method potentiates the solvent's mass transport into the treated plant material and simultaneously facilitates the recovery of bioactive compounds by applying the acoustic cavitation effect that increases the contact surface between plant cells and solvent (Azwanida, 2015). Additionally to selecting the optimal extraction method, the green chemistry principles embolden the use of eco-friendly, safe, and non-toxic solvents as well (Alibante et al., 2021). Polyphenolic compounds are usually extracted using various organic solvents, which raise some toxicological and environmental concerns (Cai et al., 2018). However, despite being the most green solvent of all, pure water proved to be significantly less potent in extracting bioactives from buckwheat, mainly due to the poor solubility of main polyphenolics – quercetin and rutin (Çelik et al., 2015; Chemat et al., 2019; Hinneburg and Neubert, 2005; Radan et al., 2023). For example, (Zieliński and Kozłowska, 2000) revealed that 80% methanol as an extraction solvent provided a 64 times greater yield of polyphenolics from buckwheat and four times more powerful antioxidant potential than pure water. Thus, the mixture of water with diverse portions of ethanol accouter to provide multiple benefits – adequate environmental status, non-toxic behavior, and satisfactory potential for the extraction of quercetin glycosides (Hinneburg and Neubert, 2005).

For the enhancement of bioactives' solubility and bioavailability, a novel, cutting-edge trend of cyclodextrin-based extraction proved to be the green and safe alternative to the overuse of organic, toxic solvents (Bozinou et al., 2021). Some research revealed the beneficial use of cyclodextrins (CDs) for the solubility improvement of rutin and quercetin as well (Carlotti et al., 2011; Nguyen et al., 2013; Paczkowska et al., 2015; Savic et al., 2016). The specific cone structure of these molecules, with primary and secondary hydroxyl functional groups facing outwards, provides improved solubility in polar solvents, embracing hydrophobic molecules encapsulated into the cone cavity at the same time (Alibante et al., 2021). The β-cyclodextrin (β-CD), which has the approved GRAS (generally recognized as safe) status, is the most commonly used natural CD due to its low price and accessibility, while its modified molecule – (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) has been described as the more water-soluble and safer than its precursor (Cai et al., 2018). This straightforward approach proved to be simple and easily implemented at both laboratory and industrial scales (Bozinou et al., 2021).

However, as the final yield of bioactive compounds is closely dependent on several factors, such as extraction temperature, time of the extraction process, and the concentration of the used solvent, their optimization is the essential step (Cai et al., 2018; Radan et al., 2024). While being time-consuming and incompetent to evaluate the interactions between the observed factors, the conventional "one-factor-at-a-time" optimization approach has been widely replaced with more sophisticated and accurate mathematical and statistical techniques such as response surface methodology (RSM) (Radan et al., 2023). The RSM has been applied to a great extent for the optimization of bioactives' extraction (Mudrić et al., 2020; Setyani et al., 2023; Weremfo, Abassah-Oppong, et al., 2023). This particular method investigates the relative importance of each varied factor on the dependent variables (monitored responses) and provides information on the most optimal conditions for the maximized desired outcomes (Yu et al., 2019).

Therefore, hypothesizing that CDs may improve the recovery of poor-soluble valuable bioactive compounds from the *F. esculentum* Moench aerial parts, and reduce the ethanol content and duration of the extraction process, this study aimed to reveal the novel, environmentally friendly, and cost-effective extraction method for the maximum recovery of buckwheat's polyphenolics. In that manner, the extraction temperature, extraction time, ethanol concentration, simultaneously with the concentration of CDs – β-CD and HP-β-CD, were varied, and their individual and interactive influence on the polyphenols content have been analyzed by the RSM approach. This is the novel method for the extraction of *F. esculentum* polyphenols, using a green extraction technique and potent and safe complexation agents. To the best of the authors' knowledge, the optimization of the environmentally-friendly UAE method of bioactive compounds from common buckwheat, by using the RSM modeling approach evaluating several crucial factors on the final yield of polyphenols, including the influence of two CDs, has not been investigated so far.

2. MATERIALS AND METHODS

2.1. Plant material

The dried aerial parts in the flowering stage of *F. esculentum* Moench (control number: 01540120), were provided from the Production sector of the Institute for Medicinal Plants Research "Dr. Josif Pančić", Belgrade, Serbia. The plant material was grinded using the industrial mill and then sieved through a 5-sieve set of standardized pore size according to the Yugoslavian Pharmacopoeia, 2000 (*V. Yugoslavian Pharmacopoeia*, 2000) Yug. V). The fraction of 0.75–2 mm particle size was selected for further experiments.

2.2. Chemicals

Both CDs – β-CD and HP-β-CD, were provided by Acros Organics (Geel, Belgium). Ortho-phosphoric acid and acetonitrile were of analytical grade, and purchased from Sigma Aldrich (St Louis, MO, USA), while the following standards: rutin, quercetin, and quercitrin, were provided from Extrasynthese (Genay, France). The Milli-Q water purification system (Millipore, Molsheim, France) was used to provide the ultrapure distilled water used for the preparation of the mobile phase.

2.3. Ultrasound-assisted extraction of bioactive compounds

Recovery of polyphenols from *F. esculentum* dried aerial parts was obtained by the UAE method in the ultrasonic water bath (Bandelin Sonorex, Berlin, Germany). The 1.0 g of sieved plant material was mixed with 20 mL of CD aqueous ethanolic solution in a 50 mL glass flask (1:20 w/v), and exposed to the sonification under constant power and frequency (320 W and 35 kHz, respectively). To reveal the optimal extraction conditions for the maximized polyphenolic content, four crucial parameters according to the literature (Cai et al., 2018; Mudrić et al., 2020) were varied, including extraction time (10–70 min), temperature (20–80 °C), ethanol concentration (0–80%), and the concentration of used CD (0–1.6%). Looking into perspective that this study aimed to maximize total phenolic content (TPC) under the most possible "green" extraction conditions, including the reduction of ethanol concentration and duration of extraction process, and taking into account that cyclodextrins have been attributed with significant potential to enhance the solubility of poorly dissolved bioactives (Pinho et al., 2014), the influence of CD was evaluated simultaneously with time, temperature, and solvent concentration. As the difference between the solubility of β-CD and HP-β-CD can be expected (Cai et al., 2018), two separate experimental protocols were applied for each CD. Obtained mixtures were filtered through filter paper, and properly stored at 4 °C until further experiments.

2.4. Chemical characterization of *F. esculentum* extracts

2.4.1. Total phenolic content

The TPC in the obtained *F. esculentum* extracts was measured spectrophotometrically by the Folin-Ciocalteu (FC) method (Waterman and Mole, 1994) with modifications related to the volume of diluted extract, sodium carbonate solution, and FC reagent described in the study of (Radan et al., 2024). After 2 h of incubation at room temperature and hidden from light, the absorbance was measured at 765 nm. All experiments were performed in three repetitions, and the results were expressed as a mean value of milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW) of *F. esculentum* herb (mg GAE/g DW).

2.4.2. HPLC analysis

Quantification of the main polyphenols from the optimal *F. esculentum* extract (extract with the highest TPC) was performed by HPLC analysis on Agilent Technologies 1260 Series with the Lichrospher RP-18 (250 × 4.0 mm) analytical column, with particle size 5 µm, and DAD detector, following the method described in the study of (Radan et al., 2023). After the UV detection at 270 nm and 340 nm, the identification of the most abundant polyphenols – rutin, quercetin, and quercitrin, was carried out by comparison of obtained UV spectra and retention time, with the same parameters corresponding to the reference standards. The final content of polyphenolics, presented as milligrams per gram of dry weight of *F. esculentum* herb (mg/g DW), was calculated using the generated calibration curves for each compound ($R^2 > 0.99$).

2.5. Experimental design using Response surface methodology (RSM)

The RSM was employed for the optimization of the UAE recovery of polyphenols from *F. esculentum* herb, with applied central-composite design for the evaluation of the impact of

Table 1. Central-composite design of four independent variables at five levels, using β-CD as a complexation agent, and experimentally observed values of TPC as an investigated response.

	Independent variables				*Dependent variable*
Run	X_1: Temperature (°C)	X_2: Time (min)	X_3: EtOH concentration (%)	X_4: β-CD concentration (%)	TPC (mg GAE/g DW)
1	35	55	20	0.4	15.11
2	65	25	60	0.4	35.83
3	50	40	40	1.6	28.81
4	50	40	40	0.8	28.83
5	50	40	40	0.8	33.57
6	35	55	20	1.2	17.56
7	50	40	40	0	31.82
8	35	55	60	0.4	30.35
9	35	55	60	1.2	30.56
10	50	40	40	0.8	32.43
11	65	55	60	0.4	35.09
12	35	25	20	0.4	15.41
13	20	40	40	0.8	21.74
14	65	55	20	1.2	25.03
15	50	10	40	0.8	31.39
16	50	70	40	0.8	29.83
17	80	40	40	0.8	36.51
18	65	25	20	0.4	24.75
19	35	25	60	1.2	29.62
20	50	40	0	0.8	12.23
21	50	40	40	0.8	30.69
22	65	55	20	0.4	28.44
23	50	40	40	0.8	30.60
24	50	40	80	0.8	29.93
25	65	25	60	1.2	34.32
26	35	25	60	0.4	30.99
27	35	25	20	1.2	19.73
28	65	25	20	1.2	27.10
29	50	40	40	0.8	30.89
30	65	55	60	1.2	34.24

β-CD – β-cyclodextrin; TPC – Total phenolic content.

different extraction conditions. Four process variables, including extraction time, temperature, ethanol concentration, and the concentration of CD were varied at five levels, resulting in 30 runs with 5 central points, per each used CD (β-CD or HP-β-CD) (Tables 1 and 2). The relationship between the varied factors (independent variables) and the TPC as the tracked response is explained with Equation (1):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (1)$$

where Xi and Xj represent the independent variables, Y the dependent variable, while β_0, β_i, β_{ii}, and β_{ij}, stand for the intercept, linear, quadratic, and interactive regression coefficients, respectively. Design-Expert 13 program (Stat-Ease, Minneapolis, Minnesota, USA) was used for the central-composite experimental design and statistical analysis, and analysis of variance (ANOVA) was used to evaluate the models' significance. The adequacy of the model was observed by the evaluation of coefficient of determination (R^2), root-mean-square error (RMSE), lowest mean absolute deviation (MAD), models' *p* values, and finally, the lack-of-fit testing. In order to verify the capability of the model's prediction, the validation analysis was performed in triplicates, under optimized conditions.

3. RESULTS AND DISCUSSION

Exploring the opportunities of the *F. esculentum* herb's polyphenolics in various industries, such as pharmaceutical, food, chemical, and cosmetic, this study investigated the optimal extraction method for the maximized recovery of bioactive compounds, respecting the green chemistry principles. Being the main carriers of the common buckwheat's bioactive potential, the recovery of polyphenols was potentiated through extraction optimization using a highly competent mathematical and statistical method – RSM.

3.1. Total phenolic content in the obtained extracts

Following the matrix provided by the RSM, thirty separate extractions for each model were performed, varying crucial factors that may affect the yield of total phenolics. As presented in Tables 1 and 2, the obtained results revealed that the TPC ranged from 12.23 to 36.51 mg GAE/g DW when β-CD was used, while the HP-β-CD provided the content of 13.35–39.67 mg GAE/g DW. These results are slightly higher than the obtained TPC range of 9.87–25.87 mg GAE/g DW in our previous study, when the conventional method for extraction (maceration), was used for the recovery of bioactives from the same plant material (Radan et al., 2023). Given that the mass transport of the solvent into the treated plant material is potentiated by the generation of ultrasound in the UAE (Azwanida, 2015), the superior yield obtained in this study is not surprising. The highest yield (39.6730 mg GAE/g DW) was accomplished by the extraction at 80 °C for 40 min, using 40% ethanol solution with the addition of 0.8% HP-β-CD, while lower TPC was obtained with β-CD under the same conditions, which could be related to a greater solubility of hydroxypropyl derivate (Cai et al., 2018). A similar observation was reported by (Diamanti et al., 2017) when TPC

Table 2. Central-composite design of four independent variables at five levels, using HP-β-CD as a complexation agent, and experimentally observed values of TPC as an investigated response.

	Independent variables				*Dependent variable*
Run	X_1: Temperature (°C)	X_2: Time (min)	X_3: EtOH concentration (%)	X_4: HP-β-CD concentration (%)	TPC (mg GAE/g DW)
1	35	55	20	0.4	16.34
2	65	25	60	0.4	35.99
3	50	40	40	1.6	33.61
4	50	40	40	0.8	29.14
5	50	40	40	0.8	31.56
6	35	55	20	1.2	17.11
7	50	40	40	0	29.48
8	35	55	60	0.4	30.13
9	35	55	60	1.2	30.92
10	50	40	40	0.8	33.10
11	65	55	60	0.4	34.05
12	35	25	20	0.4	17.54
13	20	40	40	0.8	22.57
14	65	55	20	1.2	27.91
15	50	10	40	0.8	30.35
16	50	70	40	0.8	33.45
17	80	40	40	0.8	39.67
18	65	25	20	0.4	24.83
19	35	25	60	1.2	28.74
20	50	40	0	0.8	13.35
21	50	40	40	0.8	32.55
22	65	55	20	0.4	27.29
23	50	40	40	0.8	32.12
24	50	40	80	0.8	31.07
25	65	25	60	1.2	37.55
26	35	25	60	0.4	28.76
27	35	25	20	1.2	20.04
28	65	25	20	1.2	26.83
29	50	40	40	0.8	32.21
30	65	55	60	1.2	37.40

HP-β-CD – HP-β-cyclodextrin; TPC – Total phenolic content.

obtained after polyphenolic extraction from pomegranate fruit with HP-β-CD (71.70 mg GAE/ g DW) was significantly higher than by using the β-CD-aided extraction (58.70 mg GAE/g DW). On the other hand, the poorest TPC of 12.23 mg GAE/g DW was obtained with 0.8% β-CD, using pure water (0% ethanol) for 40 min at 50 °C. Slightly higher TPC was noted under the same conditions using HP-β-CD (13.35 mg GAE/g DW).

3.2. RSM analysis

3.2.1. Model fitting

As polyphenols represent the carriers of common buckwheat's bioactivity, the optimization of UAE was focused on the maximization of TPC in both models. The individual influence of each varied independent variable, as well as the influence of their interactions were evaluated by RSM technique based on the obtained results on the TPC, and the summary of statistically significant factors for each model is presented in Table 3.

Table 3. RSM model fitting of β-CD- and HP-β-CD-aided extractions.

β-CD-aided extraction		**HP-β-CD-aided extraction**	
TPC		TPC	
	p-value		p-value
Model	< 0.0001	**Model**	< 0.0001
X_1	< 0.0001	X_1	< 0.0001
X_3	< 0.0001	X_3	< 0.0001
X_1X_3	0.0042	X_4	0.0174
$(X_1)^2$	0.0715	$(X_3)^2$	< 0.0001
$(X_3)^2$	< 0.0001		
Lack-of-fit - not significant		**Lack-of-fit - not significant**	
R^2 = 0.9522		**R^2 = 0.9501**	
R^2 adj = 0.9422		**R^2 adj = 0.9421**	
R^2 pred = 0.9145		**R^2 pred = 0.9119**	

TPC – total phenolic content; X_1 – Extraction temperature (°C); X_3 – Ethanol concentration (%); X_4 – CD concentration (%); $p > 0.10$ – not significant; $0.05 < p \leq 0.10$ – moderately significant; $0.01 < p \leq 0.05$ – significant; $p < 0.01$ – highly significant; β-CD – β-cyclodextrin; HP-β-CD – hydroxypropyl-β-cyclodextrin; R^2 – coefficient of determination; R^2 adj – adjusted coefficient of determination; R^2 pred – predicted coefficient of determination.

Based on the obtained results, the total polyphenols recovered by both β-CD and HP-β-CD-aided extraction from aerial parts of *F. esculentum* were appropriately described with highly significant quadratic regression models ($p < 0.0001$). Additionally, a statistically non-significant parameter of lack-of-fit confirmed the adequacy of the models. Strong predictive capability and robustness were confirmed by high values of coefficients of determination (0.9522 and 0.9501 for the β-CD and HP-β-CD-aided extraction models, respectively), indicating a good fitting between the experimentally obtained and predicted values, which is graphically shown in Figure 1.

3.2.2. Effect of UAE extraction parameters on the TPC

As noticeably presented in Table 3, the recovery of polyphenols from *F. esculentum* herb by the UAE method in both models was highly influenced by the linear terms of the temperature and ethanol concentration with a positive effect, as well as the quadratic term of the solvent concentration ($p <$ 0.0001). Also, the β-CD-aided extraction model revealed the moderately significant negative influence of the quadratic effect of temperature ($p < 0.1$), as well as the highly significant interaction term between ethanol concentration and temperature ($p < 0.01$). Under studied experimental conditions, the influence of CD concentration was statistically significant ($p <$ 0.05) only when HP-β-CD was used, with the positive influence in the linear term. In addition, variations in extraction time did not show a significant impact on polyphenol extraction in either model. Predicted regression models for TPC are presented by the following equations based on the statistically significant independent variables, for β-CD- (Eq. (2)) and HP-β-CD-aided extraction (Eq. (3)):

$$TPC_{\beta\text{-CD}}\ (\text{mg GAE/g DW}) = 30.5996 + 3.5419X_1 + 5.1366X_3 - 1.2224X_1X_3 - 0.5471(X_1)^2 - 2.5587(X_3)^2 \tag{2}$$

$$TPC_{\text{HP-}\beta\text{-CD}}\ (\text{mg GAE/g DW}) = 30.8955 + 4.0194X_1 + 5.0454X_3 + 0.8270X_4 - 2.5500(X_3)^2 \tag{3}$$

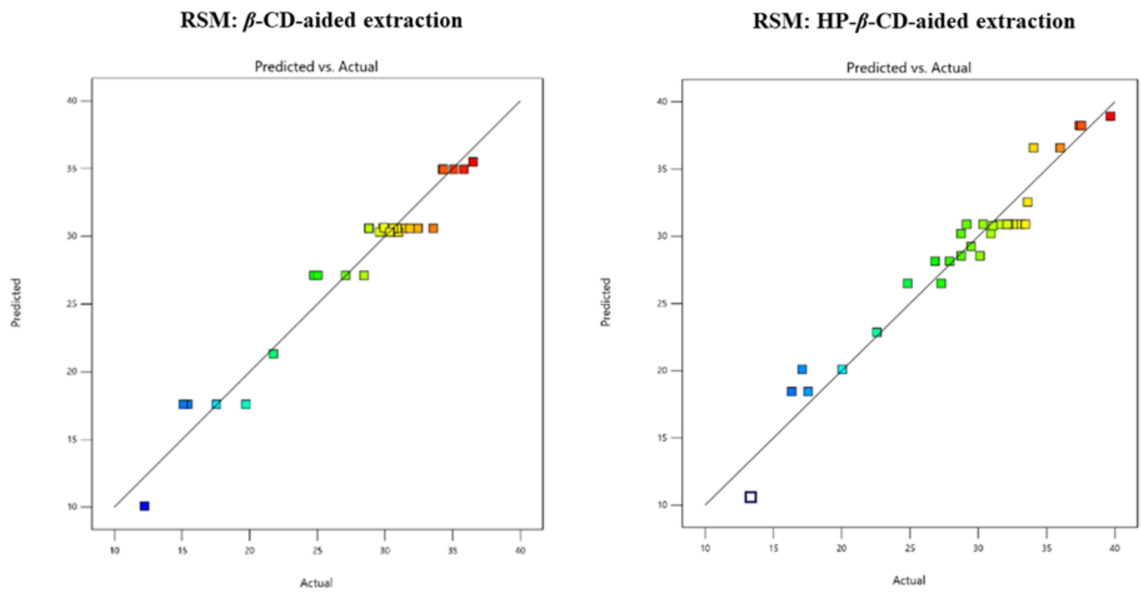

Fig. 1. Plots of experimental versus predicted values for each response in the created RSM models.

Based on the obtained results, it can be observed that the temperature highly influenced the yield of total polyphenols. In both models, the increase of the extraction temperature resulted in the following augmentation of TPC (Figures 2A1 and 2A2), which can be explained by the improvement of phenolic solubility, extraction, and diffusion rate, as well as the reduction of surface tension under higher extraction temperatures (Prasad et al., 2011). Moreover, the UAE method provides additional temperature-augmented compound solubility, as the heating evokes an increase in the amount of cavitation bubbles favoring the diffusivity of the solvent (Chemat et al., 2017). However, under relatively high-temperature conditions, polyphenols could be degraded due to their insufficient stability, resulting in a decrease in the total yield (Durling et al., 2007). This particular outcome was revealed only for a model with β-CD that indicated negative quadratic influence ($(X_1)^2$) of extraction temperature on the polyphenols recovery. Notably, while the formation of inclusion complexes with CDs and polyphenols may be improved by the increasing temperature, at 50–60 °C the decomposition of the formed inclusion complexes may happen (Cai et al., 2018). Thus, as the positive effect of CDs may be weakened with the temperature rise (Del Valle, 2004), these results may indicate the supremacy of HP-β-CD-formed inclusion complexes on the stability endurance compared with β-CD.

Ethanol concentration also revealed the significant positive linear (X_3) and negative quadratic $(X_3)^2$ influence in both CD models (Figures 2B1 and 2B2), highlighting the importance of identifying the optimal solvent's concentration to achieve the best TPC. While the increasing ethanol content to certain values leads to the reduction of the dielectric constant of the solvent, and subsequently to the better attraction of the dissolved fraction, i.e. improved solubility (Pompeu et al., 2009), higher concentrations may lead to dehydration of the plant material and cause the lower solubility and decreased recovery of polyphenolic compounds (Kumar et al., 2021).

While the linear influence of temperature and ethanol content revealed a positive impact, their interaction (X_1X_3) showed a negative influence on the polyphenols recovery in the β-CD-aided extraction model, which is presented in Figure 2C. These results implied that the extraction temperature exerted a positive effect more evident at lower concentrations of ethanol, and vice versa – the ethanol content presented a significantly greater impact at low extraction temperatures. Other studies confirmed the negative influence of this particular interaction on the extraction of polyphenols from different plant materials as well (Ilaiyaraja et al., 2015; Mašković et al., 2024; Radan et al., 2024; Živković et al., 2019).

Lastly, as CDs are described as powerful agents for the improvement of poorly soluble bioactive compounds, the positive linear influence of CD concentration (X_4) was observed in the HP-β-CD model, which is presented in Figure 2D. Therefore, this model predicted that recovery of polyphenols may be enhanced by the increasing HP-β-CD content. This can be explained by the fact that with the increase of CD molecules' amount, the probability of successful interactions between poorly-soluble bioactive compounds and the cavity of CDs is improved as well (Radan et al., 2023).

3.3. Model validation

As the RSM approach was used for the optimization of the UAE extraction of polyphenolic compounds from *F. esculentum* herb, the obtained analysis pointed out that the TPC can be maximized under the following conditions: extraction temperature (X_1) of 80 °C, extraction time (X_2) of 30 min, ethanol concentration (X_3) of 50%, and β-CD concentration (X_4) of 0.80% (Table 4). Slightly different optimal conditions were provided for the HP-β-CD-aided extraction, emphasizing the influence of CD used in this model: extraction temperature (X_1) of 80 °C, extraction time (X_2) of 40 min, ethanol concentration (X_3) of 50%, and HP-β-CD concentration (X_4) of 1.50%. The validation step is crucial for strategic decision-making and possible industrial application, as it confirms the process's reliability (Mayer and Butler, 1993a). Thus, to validate the accuracy of both models, a new set of experiments was conducted under the predicted optimal conditions, in three repetitions.

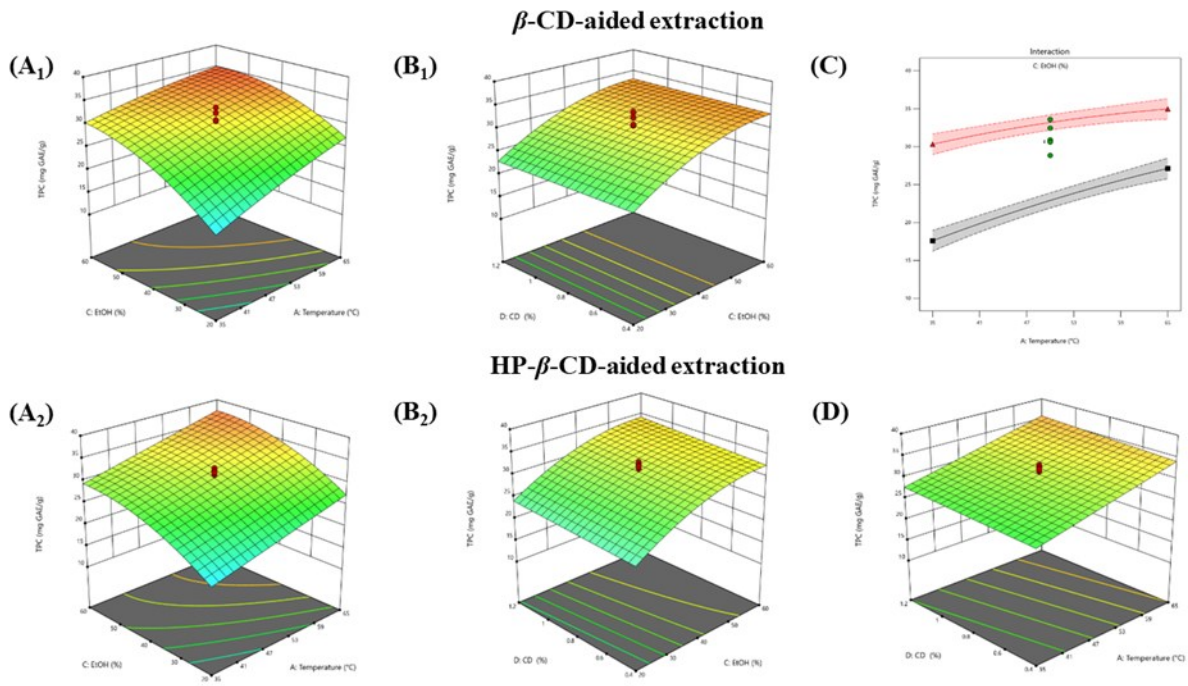

Fig. 2. Response surface plots of β-CD- (the upper half) and HP-β-CD-assisted extractions (the lower half) showing the impact of independent variables on the extraction efficiency of total polyphenols. Independent variables with statistically significant influence: temperature (A1 and A2), ethanol concentration (B1 and B2), interaction between temperature and ethanol concentration (C), and HP-β-CD concentration (D).

Table 4. The optimized conditions for *β*-CD- and HP-*β*-CD-aided extractions.

***Β*-CD-aided extraction**		**HP-*β*-CD-aided extraction**	
Optimal conditions		**Optimal conditions**	
X_1	80 °C	X_1	80 °C
X_2	30 min	X_2	40 min
X_3	50%	X_3	50%
X_4	0.80%	X_4	1.50%
Predicted TPC = 36.20 mg GAE/g DW		**Predicted TPC** = 42.26 mg GAE/g DW	
Validated TPC = 36.19 ± 0.99 mg GAE/g DW		**Validated TPC** = 42.16 ± 1.01 mg GAE/g DW	

β-CD – β-cyclodextrin; HP-*β*-CD – hydroxypropyl-*β*-cyclodextrin; X_1 – Extraction temperature; X_2 – Extraction time; X_3 – Ethanol concentration; X_4 – CD concentration; TPC – total phenolic content.

As the validated responses (36.19 ± 0.99 and 42.16 ± 1.01 mg GAE/g DW for *β*-CD- and HP-*β*-CD-aided extractions, respectively) highly corresponded to the predicted values, it could be concluded that the created models were reliable and accurate.

3.4. HPLC analysis of the optimal *F. esculentum* extract with maximal TPC

Numerous health benefits have been reported for the use of common buckwheat and its products, such as hypoglycemic, hypocholesterolemic, anti-inflammatory, and anticancer effects (Giménez-Bastida and Zieliński, 2015), which prompted the interest for the formulation of novel dietary products and functional food. Regarding this medicinal plant's pharmaceutical and nutraceutical potential which is derives from polyphenolic compounds, this study aimed to optimize the TPC using CD-aided extraction. As the optimal extract obtained by the HP-*β*-CD-aided extraction model resulted in a higher content of polyphenolic compounds, compared to *β*-CD, it was selected for the final chemical characterization.
The HPLC method revealed that the most abundant compound in the obtained extract was rutin with 29.56 mg/g DW, followed by quercetin and quercitrin, which were detected in smaller quantities (Table 5). Other studies also reported rutin as a main component in the *F. esculentum* extracts (Hinneburg and Neubert, 2005; Radan et al., 2023; Tian et al., 2002; Vollmannová et al., 2021). In fact, obtained rutin yield was similar to the study of (Vollmannová et al., 2021), where *F. esculentum* leaves were treated with 80% methanol for 8 h, resulting in the rutin yield range of 17.742–31.069 mg/g DM. Given the high concentration of methanol, a toxic organic solvent, and the prolonged extraction time used in the study by (Vollmannová et al., 2021), the HP-*β*-CD-aided extraction protocol featuring a significantly shorter extraction time of 40 min and a 50% ethanolic solution opens up new avenue for the application of sustainable practices in the recovery of potent phenolic compounds.
The significance of the evaluated chemical profile lies in the abundance of rutin, a bioactive compound with valuable bioactive potential lying in its' anticancer, anti-inflammation, hepatoprotective, and antioxidant activity (Sofi et al., 2023). Moreover, given the rising rate of non-communicable diseases worldwide, especially hypertension and diabetes, its' biological potential could be the key to the prevention and/or treatment. The mass expansion of these diseases threatens to affect a significant share of the population. Namely, by the year 2025, even 1.56 billion people will be affected only by hypertension, while approximately 642 million people could get diabetes type 2 (Oguntibeju, 2019; Sofi et al., 2023). Hence, the inhibitory potential of rutin towards the diabetes-related enzymes - glucosidase and amylase, as well as the antihypertension-associated effects, such as the improvement of blood circulation, prevention of blood vessels hardening, elimination of toxins, and microcirculation improvement, could be beneficial in the prevention on these threatening diseases (Hou et al., 2017; Jadhav and Puchchakayala, 2012; Sofi et al., 2023). Moreover, the second-abundant polyphenol in the optimal extract – quercetin, has been reported to have positive effects on the regulation of vasoconstriction and heart diastole, resulting in blood pressure lowering (Giménez-Bastida and Zieliński, 2015).
Additionally, the cosmetic, food, and chemical industry potential of rutin should not be unplaced as well, considering the reported benefits (Hinneburg and Neubert, 2005; Kim et al., 2005).

Table 5. The content of individual polyphenolics of *F. esculentum* herb optimal extract.

Rutin (mg/g DW)	**Quercitrin (mg/g DW)**	**Quercetin (mg/g DW)**
29.56	1.42	0.35

4. CONCLUSION

Currently, with the growing demand for medicinal plants and their products in the numerous fields of industry, the development of green and sustainable extraction methods occupies the focus of innovative research. Thus, aiming to contribute to that common goal, this study investigated the optimal extraction conditions for the maximized recovery of *F. esculentum* herb's polyphenols, given their significant bioactive potential. In that manner, the green, feasible, and effective UAE, aided with cutting-edge complexation agents – cyclodextrins, were used for the recovery of bioactive compounds.
Accountable method for the extraction optimization – RSM, which is often used to reduce the time and solvent over excessive consumption, was successfully applied, revealing statistically highly significant models for each used CD ($p < 0.0001$), describing the relationship between the varying factors and the TPC as the dependent variable. Obtained lack-of-fit and coefficients of determination confirmed the models' adequacy, robustness, and strong predictive capability as well. Based on the obtained results of the TPC in *F. esculentum* extracts, and the statistical analysis by the RSM approach, it was concluded that extraction temperature and the content of ethanol had a highly significant impact. Their interaction resulted in a moderately significant negative influence when *β*-CD was used, while the CD content significantly impacted the TPC only when HP-*β*-CD was used, pointing out the differences between used CDs on their potential to preserve the stability of the formed inclusion complexes. Optimal conditions for the total polyphenols maximal recovery in the extracts with *β*-CD

were as follows: 80 °C temperature of the extraction performed for 30 min, using 50% ethanol and 0.80% β-CD, while the HP-β-CD-aided extraction suggested the slightly different optimal conditions: extraction temperature of 80 °C, extraction time of 40 min, ethanol concentration of 50%, and β-CD concentration of 1.50%. Under optimized conditions, the HP-β-CD-aided extract had a superior TPC of 42.16 ± 1.01 mg GAE/g DW, composed of a significant content of rutin, quercitrin, and quercetin.

Overall, the CD-aided extraction revealed a promising approach for the recovery of *F. esculentum* polyphenolic compounds, following the principles of green chemistry and circular economy. In light of the rising industrial demand, this polyphenolic-rich extract, with significant rutin content, could be valuable for the formulation of dietary supplements, functional food, or cosmetic products that could be used in prevention or treatment of various diseases, as well as for the upkeep of the general wellbeing of the modern society.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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