

Optimisation of Poly Phenolic Extract of Tea Waste with Complex Formation by β -cyclodextrin (β -CD)

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ABSTRACT: Agricultural waste is considered a low-cost source of antioxidant compounds. Moreover, due to environmental pollution, toxicity, and undesirable organoleptic properties associated with conventional organic solvent extraction methods, the need for alternative and more efficient techniques is evident. Therefore, optimizing the extraction of bioactive compounds from tea waste has garnered attention. In this study, an inclusion complexation method using β -cyclodextrin was employed. In order to optimize the extraction process, response surface methodology based on a central composite design was applied, using two independent variables: β -cyclodextrin concentration (A) (0–5%) and temperature (B) (25–60°C). The optimal extraction conditions were determined to be a β -cyclodextrin concentration of 2.91% and an extraction temperature of 30.12°C. Under these conditions, the highest extraction yield, total phenolic content, and antioxidant inhibition percentage were evaluated at 9121.16 mg/g and 98.61%, respectively. Therefore the use of inclusion complex formation with beta-cyclodextrin can be considered a suitable method for extracting tea waste extract while preserving bioactive compounds.

Keywords: Antioxidant Activity, Beta-cyclodextrin (β -CD), Bioactive, Phenolic Compound, Tea Wastage.

Introduction

Three different kinds of tea are generally consumed: fermented (black tea), non-fermented (green tea), and semi-fermented (oolong)—all made from the young leaves of the plant *Camelia sinensis*. Following the harvesting process, tea waste, including stems and wood, is segregated and stored in tea production factories before tea processing to be subsequently used as fertilizer. Tea waste

denotes materials that diminish the quality of dry tea and, thereby, decrease its market value. A portion of this waste, resulting from human error or the rubbing stage, inadvertently progresses to the next phases of tea production, leading to an increase in dry waste. Tea waste peaks during the grading and drying stages, as well as in the autumn. The Iranian Tea Organization reports that Iran produces approximately 25,000 to 30,000 tons of black tea annually; about 10% of this quantity is discarded as waste by sorting machines,

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which is of low value in the global market. Tea and its waste contain beneficial compounds that can be utilized in a variety of industries following extraction. These compounds include flavanols, flavonols, phenolic acids, amino acids, organic acids, caffeine, etc. Additionally, tea waste may contain catechin, a type of antioxidant, and its derivatives, including galocatechin, catechin gallate, galocatechin gallate, epigallocatechin, and epigallocatechin gallate (Arayanfar *et al.*, 2012). Tea compounds, particularly antioxidant extracts from tea waste, are utilized in controlling the microbial load in food. Humans have historically pursued methods to extend the shelf life of food items by addressing microbial factors and preventing fat oxidation. Phenolic compounds serve as effective alternatives to chemical preservatives. Plant essential oils exhibit antibacterial effects because of their hydrophobic qualities, which enable them to penetrate phospholipids in bacterial and microbial cell membranes, breaking down their structures and increasing their permeability. This ultimately results in cell death by causing the leakage of ions and other cellular contents. Since tea polyphenolic compounds and tea waste are water-soluble, they can be extracted by simple methods such as aqueous extraction (soaking), alcoholic extraction (ethanol), supercritical fluid extraction, ultrasound, microwave extraction, and complexion addition (Esmailzadeh Kenari *et al.*, 2022). When it comes to the extraction of bioactive compounds from plants, all efforts are focused on minimizing energy consumption and maximizing speed while preserving the beneficial bioactive compounds of plants. Growing concerns over environmental impacts, safety, toxicity, and undesirable organoleptic qualities of extracts obtained using organic

solvents in food systems have raised the need for efficient aqueous extraction methods (Kalantari *et al.*, 2018). The use of microwaves is among the most efficient and rapid techniques, as they interact with the water within the cell, producing significant heat that compromises the cell wall. This process facilitates the release of desired compounds by breaking down the cellular structure (G-mohammadi & Zamani, 2017; Alara *et al.*, 2017). Water is a solvent commonly utilized for the extraction of bioactive compounds; however, it exhibits limited efficiency in extracting certain compounds. Beta-cyclodextrin (β -CD) can address this limitation by removing essential plant residues from plant cells. Cyclodextrins are cyclic oligosaccharides composed of glucose subunits linked by α -1 and α -4 glucopyranose bonds. These compounds are classified as generally recognized as safe (GRAS) and are utilized across the food, pharmaceutical, drug delivery, and chemical industries, as well as agricultural and environmental engineering. Their effectiveness in forming inclusion complexes with polyphenolic compounds enhances stability, solubility, and bioavailability. Cyclodextrins possess a conical spatial structure characterized by a hydrophilic surface and a hydrophobic cavity, enabling them to dissolve in water and encapsulate nonpolar compounds (Basilio *et al.*, 2013). In another study, β -CD was used as an additive to enhance the aqueous extraction of peppermint polyphenols. First, the concentration of β -CD was examined for its effect on polyphenol extraction performance. Subsequently, the extraction process was optimized through response surface methodology; β -CD concentration and temperature were regarded as process variables, while total polyphenols, total flavonoids, iron-reducing power, and anti-

radical activity served as response variables. The optimization results indicated that a β -CD concentration between 1.02 and 1.15 mmol, along with a temperature of 80°C, yielded the most favorable outcomes regarding the total extracted flavonoids. The study concluded that β -CD-assisted polyphenol extraction from the peppermint plant provides extracts with enhanced polyphenolic composition and superior antioxidant properties, presenting this method as a viable alternative to solvent extraction (Athanasiadis *et al.*, 2022). Kalantari *et al.* (2018) employed a green extraction method with water as the solvent and β -CD solution as the extraction medium. They evaluated the optimization of non-flavonoid polyphenolic compounds from pomegranate peel as a natural source for extracting bioactive compounds. The extraction process involved ultrasound and the formation of an inclusion complex with β -CD, utilizing response surface methodology based on the Box-Behnken design. This approach considered three independent variables: temperature, time, and β -CD concentration. Their findings demonstrated that the combination of β -CD and ultrasound enhanced the efficiency of extracting non-flavonoid polyphenolic compounds from pomegranate peel. Response surface methodology (RSM) comprises mathematical techniques that analyze the relationship between one or more response variables and multiple independent variables in order to identify the optimal relationship. RSM designs seek to optimize the output variable influenced by multiple independent variables. RSM is advantageous in the food industry as it allows for the simultaneous examination of variable effects during optimization. Therefore, this study employs this method to investigate the efficacy of various

factors in optimizing the extraction process (Jabrouiti and Ghofrani, 2014). A literature review showed that a few studies have employed ethanol and β -CD simultaneously to enhance the hydroalcoholic extraction of polyphenolic compounds from tea waste. As a result, this study investigates the optimization of extraction conditions for polyphenolic compounds from tea waste. It examines the effect of factors such as temperature, time, and β -CD concentration on the efficiency of the inclusion complex method, as well as its impact on enhancing the extraction efficiency of active compounds and phenolic content in the resulting extracts.

Materials and Methods

- *Materials*

Green tea waste, comprising leaves, stems, branches, pods, and bran, was collected from the factory of Ghazal Tea Co. in Lahijan, Guilan Province, Iran, in May. The Folin-Ciocalteu reagent, 80% ethanol, beta-cyclodextrin (β -CD), Whatman filter paper, and Falcon 50-ml tubes were acquired from Sigma-Aldrich (Germany).

- *Methods*

- *Sample preparation*

The green tea waste was initially rubbed by hand and subsequently placed in a Memmert oven (model D10383, Germany) at 105°C for 2 hours. The dried waste was subsequently ground with an electric grinder (Pars Khazar, Iran), packaged in ziplock bags, and stored at 4°C.

- *Extraction*

First, a magnetic stirrer (Ajand Pajouhan-e Toos, Iran) was employed to mix the dried powder of tea waste with a 0 to 5% β -CD solution in 80% ethanol at a

1:10 ratio for 3 hours at temperatures ranging from 25 to 60°C. Subsequently, the mixture was subjected to ultrasonic treatment in a bath (Bekker, model vClean1-L27, Iran) for 20 minutes at a frequency of 28 kHz without heat application. After the extract was filtered using Whatman filter paper and a vacuum pump, it was centrifuged for 15 minutes at 7000 rpm. Finally, the extract was dried using freeze-drying to yield tea extract powder.

- **Tests**

- **Measurement of polyphenolic compounds**

In order to measure polyphenolic compounds, the total amount was first determined using the Folin–Ciocalteu method. For calibration curve plotting, gallic acid was used at various concentrations. Then, 0.2 mL of the prepared powder solution was mixed with 0.8 mL of 7.5% sodium carbonate solution and 2 mL of Folin–Ciocalteu reagent, followed by centrifugation. In the next step, the absorbance of the samples was measured at a wavelength of 765 nm using a spectrophotometer after 30 minutes of incubation at room temperature. The total phenolic content in the extract was calculated based on gallic acid using the equation obtained from the calibration curve, expressed as milligrams of gallic acid per gram of extract (Charoen et al., 2015).

- **Measurement of scavenging activity of free radicals (DPPH)**

In order to measure the free radical scavenging activity, DPPH was used as a stable radical compound. For this purpose, 20 mg of the prepared samples were dissolved in 10 mL of water. Then, 1 mL of the sample was mixed with 0.2 mL of ethanolic DPPH solution (0.01 mM). After

60 minutes of incubation in the dark, the absorbance changes were read at a wavelength of 517 nm. Finally, the percentage of DPPH free radical inhibition was calculated using the following formula (Mishra et al., 2012):

$$\text{DPPH free radical scavenging} = \frac{\text{Control light absorption} - \text{Sample light absorption}}{\text{Control light absorption}} \times 100$$

(Equation 1)

- **Total polyphenol optimization**

This study determined the total concentration of tea waste polyphenols, including galukatchin (GA), epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). The effects of optimized temperatures on the extraction of these compounds were examined, and results indicated the highest concentrations of total catechins and caffeine content, expressed as gram percentages of dry matter, at specific temperatures. The Design Expert software was employed to formulate the treatments, followed by the selection of the optimal treatment by RSM. A central composite design featuring five levels and five replication points was used to optimize the extraction process. This design included consistent β-CD concentration and stirring temperature at the central point. The independent variables were temperature and β-CD concentration, each assessed at five levels, while the dependent variables comprised total polyphenol concentration and DPPH antioxidant capacity test. Finally, quadratic polynomial equations were fitted through regression analysis on the dependent variables in the specified model.

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{12}AB$$

(Equation 2)

In this equation, Y denotes the desired responses, which include total polyphenol and DPPH levels, while β_n signifies the regression coefficients. The coefficients indicate both the binomial linear effects and the interactive effects of the process variables. Moreover, A and B represent the independent variables of the process.

- **Model fitting**

Modeling entails the formulation of quantitative relationships between process variables and target responses derived from experimental data. The best model was selected based on the significance of the F test at $P < 0.05$, the non-significance of the lack of fit, R^2 and adjusted R^2 values, and the coefficient of variation. The analysis of variance (ANOVA) Table indicates that the fitted model was significant for all studied parameters. To examine the most influential parameters, terms that did not exhibit significant F test results were excluded from the model, while those demonstrating significant differences were retained. Ultimately, the parameter with the highest sum of squares was identified as the most influential (Noshad *et al.*, 2011).

Results and Discussion

- **Effect of inclusion complex formation on total polyphenol content in the final extract**

The linear analysis of variance Table indicates that β -CD concentration (A) at $P < 0.05$ and stirring temperature (B) at $P < 0.005$ significantly affect total polyphenol content. Analysis of the coefficients for β -CD concentration and stirring temperature revealed that temperature (B) exerts a more substantial effect than β -CD concentration (A) on the concentration of phenolic compounds. Additionally, the quadratic effect of β -CD concentration (A^2) demonstrated a

significant and negative impact on phenolic compounds. Parameters lacking a significant effect were excluded from the model. Considering the parameters with notable effects, the overall equation of the quadratic model for the response of total polyphenol concentration is presented as Equation (3).

$$Y_1 = 8455.04 + 396.84A - 516.82B - 839.54A^2 \quad (\text{Equation 3})$$

The coefficient of determination (R^2) for the predicted Table corresponding to the above response was 0.9079, while the P-value for the lack-of-fit test was 0.4953. The lack-of-fit test assessed the adequacy of the selected model in accurately explaining and interpreting the observed data, determining if a more complex model is warranted. A time model is considered statistically significant at the 95% confidence level if its lack-of-fit P-value is greater than 0.05 (tayefe *et al.*, 2020); therefore, it can be concluded that the model presented in the above equation provides a good fit for the desired response. Figure 1 presents the effects of the independent variables on total polyphenol content, as assessed through the inclusion complex method, as a three-dimensional response surface.

The results demonstrated that the concentration of total polyphenols in the final extract diminished as temperature increased within the studied range of 25 to 60°C. Applying higher temperatures seems to soften plant tissue, disturb bonds between phenolic compounds and proteins or polysaccharides, and increase the solubility of phenolic compounds. This phenomenon enhances mass transfer and elevates the extraction rate of these compounds at higher temperatures (Gan & Latiff, 2011). However, as the temperature rises within the examined range, the self-

polymerization reaction of the compounds results in a reduction of polyphenol compound concentration in the extracted solution (Ya-Qin *et al.*, 2009). Another study investigated the effects of temperature and time on the extraction of phenolic compounds from the peel of citrus fruits. The results revealed that increasing the extraction temperature to approximately 40°C enhanced the extraction of these compounds. However, a decline in the amount extracted was noted at extended durations and elevated temperatures (Ya-Qin *et al.*, 2009). Some other studies on the extraction process of phenolic compounds reported a decline in extraction rates at higher temperatures. All of these studies attributed this phenomenon to the self-polymerization reactions of phenolic compounds (Manthey & Grohman, 1995; Pinelo *et al.*, 2005; Yalmiz & Toledo, 2004). Wang *et al.* (2010) found that both the polyphenol content and antioxidant activity of the extract decline at temperatures exceeding 80°C due to decreased stability and thermal decomposition, resulting in diminished antioxidant activity. In this study, increasing the β -CD concentration within the specified range (0 to 5%) initially resulted in a rise in total polyphenols by approximately 3%. However, further increases in β -CD concentration led to a decrease in the total polyphenol content (Kalantari *et al.*, 2009). Shalmashi and Amani (2020) reported that the increased capacity to dissolve the soluble substances in the tea sample was responsible for the positive effect of increasing the solvent content to tea sample ratio on the weight percentage of extraction efficiency. Proper optimization of this factor is crucial, as a low value will result in a low capacity for dissolving substances, which will lead to a low extraction efficiency. Conversely, a high value will necessitate high energy to

concentrate the resulting extract. Kalantari *et al.* (2019) stated that the observed increase is due to the formation of an inclusion complex via the lipophilic cavity of β -CD with hydrophilic polyphenols, enhancing their solubility in water (Del Valle, 2004). Most polyphenolic compounds are non-polar aromatic substances with low solubility in polar solvents like water; therefore, these compounds exhibit a very low extraction rate in water-based methods. Other studies in this area have demonstrated the interaction between β -CD and polyphenolic compounds, attributed to the infiltration of β -CD into the cell membrane of these compounds, facilitating binding to target sites. The biochemical and biological properties of the guest molecules are altered, leading to increased permeability, solubility, and bioavailability of these compounds (Pralhad & Rajendrakumar, 2004). The β -CD concentration necessary to form an inclusion complex with bioactive compounds from plant sources depends on the maximum solubility of these compounds in water (El Darra *et al.*, 2018). Similar studies have attempted to increase the extraction efficiency of polyphenolic compounds from the peach pulp (Rajha *et al.*, 2014), the apple pulp (Parmar *et al.*, 2015), different grape varieties (Korompokis *et al.*, 2017), red grape and its pulp (Ratnasooriya *et al.*, 2012), and *Sideritis scardica*; all these studies consistently demonstrated that β -CD improved the efficiency of extracting polyphenolic compounds. In this study, the highest total polyphenol extraction rate (9018.749 mg/ml) was related to the treatment that used the 2.918% concentration of β -CD at a temperature of 30.126°C.

- Effects of variables of inclusion complex formation on free radicals (DPPH) in the final extract

The linear term analysis of variance Table indicated that β -CD concentration (A) ($P > 0.01$) and stirring temperature (B) ($P < 0.001$) had a significant effect on the DPPH content. Analysis of the coefficients for β -CD concentration and stirring temperature also revealed that stirring temperature (B) exerted greater effects than β -CD concentration (A) on DPPH content. The results also demonstrated the significant quadratic effect of β -CD concentration (A) ($P < 0.001$) and the significant interactive effects of β -CD concentration (A) and stirring temperature (B) ($P > 0.01$) on the DPPH content (Table 3). Parameters that exhibited no significant effect were excluded from the model. The quadratic effect of β -CD concentration (A^2) was significant and positive, and the interactive effect of β -CD concentration and stirring temperature (AB) was significant and negative. Consequently, considering the parameters with a significant effect, the general equation of the quadratic model for the DPPH response is presented as follows:

$$Y_2 = + 87.7889 + 2.7249 A - 7.3092 B - 4.029175 AB - 8.1828 A^2$$

(Equation 4)

The coefficient of determination (R^2) for the predicted model was 0.9275, and the P-value for the lack-of-fit test was 0.6112. Consequently, the model presented in the above equation demonstrated a good fit for the desired response (Table 3). Figure 2 presents the effects of the independent variables on DPPH content, as assessed through the inclusion complex method, as a three-dimensional response surface.

The effects of temperature on antioxidant properties mirrored those

observed for total polyphenols; as stirring temperature increased, antioxidant properties decreased. The optimal temperature was determined to be 126.30°C . Numerous studies have indicated that phenolic compounds significantly influence antioxidant activity, attributed to their strong reductive capacity and their ability to donate hydrogen to active radicals like DPPH (preire *et al.*, 2009). Vazquez *et al.* (2012) examined the effect of temperature, ethanol concentration, and extraction time on the isolation of antioxidants from oak. Their findings showed that temperature and ethanol concentration were the sole independent variables significantly affecting DPPH antioxidant activity. As observed in the previous Figure, an increase in β -CD concentration initially enhanced the antioxidant property, which subsequently decreased after reaching the optimal value of 2.918. The increase in antioxidant activity of the extract at higher concentrations can be explained by the phenolic compound content, as existing evidence suggests a positive correlation between phenolic compounds and the antioxidant properties of plants (Jamshidi *et al.*, 2010). The free radical scavenging activity diminished at β -CD concentrations ranging from 3% to 4.26777%, with the optimal concentration identified as 2.918. A possible saturation state at higher concentrations may result in minimal effects on the free radical scavenging rate; therefore, a critical concentration of phenolic compounds is adequate for effective free radical scavenging (Ling *et al.*, 2014). The maximum DPPH free radical scavenging activity achieved during the extraction process was 96.238%. Pereira *et al.* (2009) demonstrated a direct relationship between the concentration of phenolic compounds and antioxidant activity at low

concentrations. Conversely, at high concentrations, the DPPH radical inhibition curve resembled a straight line, consistent with the findings of this study. Aboutalebian *et al.* (2016) described the free radical scavenging method as an efficient approach for assessing the antioxidant activity of extracts. They demonstrated that increasing the extract concentration from 5 to 500 ppm corresponded with enhanced antioxidant activity in scavenging free radicals. Furthermore, the antioxidant activity of the extract surpassed that of synthetic antioxidants, which is consistent with the findings of this study.

- Optimization

In the process of extracting polyphenolic compounds from tea waste, achieving the highest phenolic content, maximum antioxidant activity, and optimal extraction yield were considered the primary objectives of the statistical analysis. Operational conditions were optimized using numerical optimization techniques. Initially, the optimization goals, response levels, and independent variables were defined, and the desirability function approach was employed to determine the best responses.

The optimal extraction conditions were identified as a temperature of 30.12°C and a β -cyclodextrin concentration of 2.91%. Under these conditions, the maximum polyphenol content of 9018.74 mg/mL and optimal free radical antioxidant activity of 96.23% were reported using Design-Expert software. The desirability value obtained under these optimal conditions for the studied variables and responses was 0.999.

Therefore, it can be concluded that the predicted values closely matched the experimentally measured values under real conditions, confirming the validity and accuracy of the fitted RSM model (Table 4).

- RSM model fitting

The quadratic polynomial model was acceptably fitted to the experimental data. R^2 (0.9079-0.9275) and adjusted R^2 (0.8757-0.8422) suggest a strong fit of the adjusted binomial model. The coefficient of variation (CV%) remained within acceptable limits (Table 3). Furthermore, the fitted model exhibited sufficient accuracy for total polyphenols and free radical antioxidant activity, as indicated by the ratio of the range of predicted values to the average predicted error at the designed points, which were 12.1097 and 2351.12, respectively; this ratio should be greater than 4. Figure 3 demonstrates a strong concordance between the experimental data and the predicted and calculated results from the model. Therefore, it can be concluded that the quadratic polynomial model effectively encompassed the experimental and measured data regarding total polyphenols and free radical antioxidant activity.

Conclusion

The use of inclusion complex formation with beta-cyclodextrin can be considered a suitable method for extracting tea waste extract while preserving bioactive compounds. In the present study, the optimization of polyphenol extract from tea waste as a source of bioactive compounds was evaluated through the formation of an inclusion complex with beta-cyclodextrin. For optimization, the response surface methodology based on a central composite design was employed, using two independent variables: beta-cyclodextrin concentration and temperature. Under the extraction conditions obtained from the applied optimization method, the highest levels of phenolic compounds and the greatest antioxidant activity were observed.

Table 1. Response values of independent variables in the extraction process

Treatment Number	Independent variables (a)		Response Variables (b)			
	A(%)	B(°C)	Real(y ₁)	Predicted(y ₁)	Real(y ₂)	Predicted(y ₂)
1	2.5	42.5	8000.17	8455.04	92.90	87.79
2	5	42.5	7164.96	7337.17	75.72	75.28
3	4.267	30.125	8927.82	8529.15	93.74	93.67
4	0.732	54.874	7245.38	6701.83	75.49	73.60
5	0.732	30.125	7608.62	7735.47	82.25	80.16
6	2.5	42.5	8665.59	8455.04	90.99	87.79
7	2.5	42.5	8188.80	8455.04	86.65	87.79
8	0	42.5	6000.17	6214.73	65.17	67.57
9	2.5	60	7398.85	7724.14	74.36	77.45
10	2.5	42.5	8820.54	8455.04	83.56	87.79
11	4.267	54.874	7453.67	7495.52	70.86	70.99
12	2.5	25	9023.18	9185.93	94.74	98.13
13	2.5	42.5	8701.35	8455.04	89.36	87.79

a: A and B are β -CD concentrations (%) and temperature (°C), respectively.b: y₁ and y₂ represent total phenol (mg/ml) and DPPH (%), respectively.**Table 2.** Encoded values and levels of independent variables in the extraction optimization process

Independent variables	Symbol	Levels				
		-2	-1	0	1	2
β -CD concentration	A	5	4.27	2.50	0.73	0
Temperature	B	60	54.87	42.50	30.13	25

Table 3. Analysis of variance for model fitting parameters associated with extract response variables

Source	Df	Total phenol content (mg/ml) (y ₁)				DPPH (%) (y ₂)			
		Sum of squares	Average squares	F index	P index	Sum of squares	Average squares	F index	P index
Model	5	8.699	1.740	13.81	0.0016 significant	1037.36	207.47	17.90	0.0007 significant
A- β -CD	1	1.260	1.260	10.00	0.0159	59.40	59.40	5.13	0.0580
B-Temp	1	2.137	2.137	16.96	0.0045	427.40	427.40	36.88	0.0005
A2	1	4.948	4.948	39.26	0.0004	485.31	485.31	41.88	0.0003
B2	1	5897.99	5897.99	0.046	0.8349ns	11.76	11.76	1.01	0.3473ns
AB	1	3.085	3.085	2.45	0.1616ns	64.94	64.94	5.60	0.0498
Remaining	7	8.822	1.260			81.11	11.76		
Lack of fitting	3	3.678	1.226	0.9536	0.4953 ns	27.26	11.59	0.6748	0.6112 ns
Error	4	5.143	1.286			53.86	9.09		
Total score	12	9.581				1118.47	13.46		
R ²			0.9079				0.9275		
Adjusted R ²			0.8422				0.8757		
Coefficient of variation (cv%)			0.6431				0.7515		
Accuracy			12.1097				12.2351		

Table 4. Summary of the predicted and measured values

Responses	Independent variables			
	β -CD concentrations (A)(%)	Temperature (B) (°C)	Predicted Amount	Measured Amount
Phenolic compound concentration (mg/g)	2.91	30.12	9412.11	9121.16
DPPH (%)	2.91	30.12	99.3	98.61

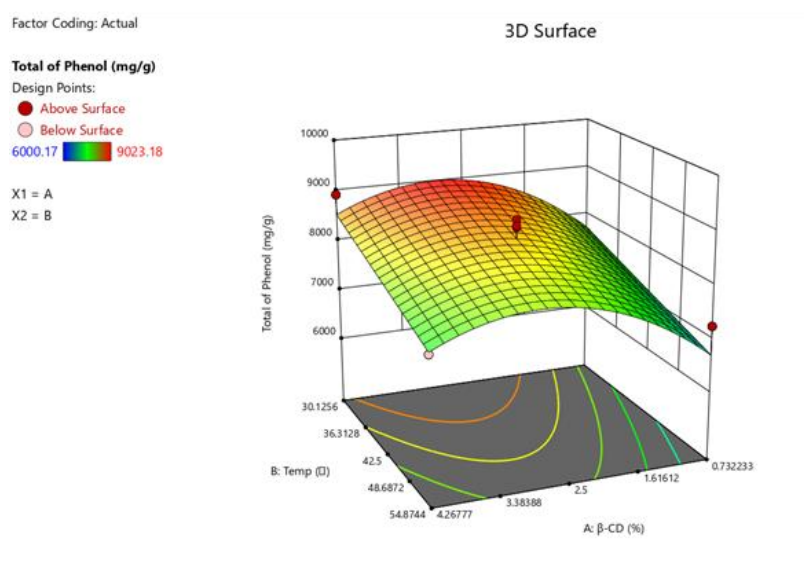


Fig. 1. Effect of studied independent variables on total polyphenol content in final extract measured by the inclusion complex method as a three-dimensional response surface

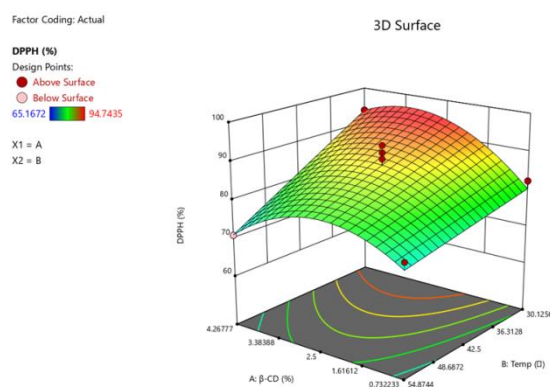


Fig. 2. Effect of independent variables on DPPH assessed by the inclusion complex method in the final extract as a three-dimensional response surface

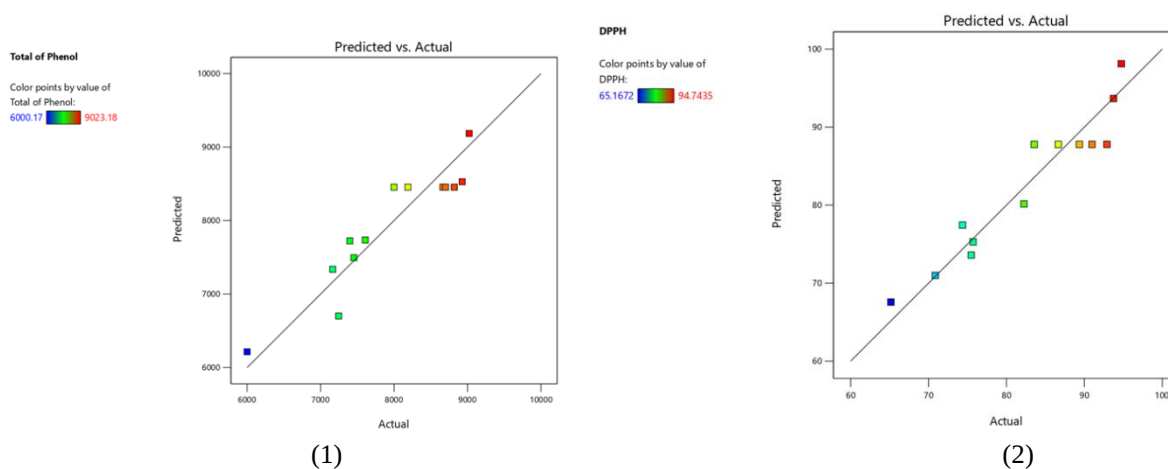


Fig. 3. A comparison of the actual and predicted values for total polyphenols (1) and DPPH (2)

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