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Research Article

Brewing Conditions Optimization of *Berberis vulgaris* L. Tea by Assessing Antioxidant Activity and Phenolic Content Using Box-Behnken Design

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Abstract: *Berberis vulgaris* is a wild fruit with valuable antioxidant and phenolic content but limited dietary use. This study aimed to optimize the brewing conditions of instant *B. vulgaris* tea to maximize its antioxidant activity and Total Phenolic Content (TPC). A Box-Behnken design was employed to evaluate the effects of brewing temperature (60-90 °C), time (5-15 minutes), and liquid-to-solid ratio (1-3 g/mL) on tea quality. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and TPC were measured as response variables. Results showed that the liquid-to-solid ratio significantly affected DPPH, while temperature had the most influence on TPC. Optimization using response surface methodology identified ideal conditions as 90 °C, 13.69 minutes, and a 1 g/mL ratio. Under these conditions, DPPH and TPC values were significantly higher than those reported in previous studies. The findings demonstrate that *B. vulgaris* tea, when properly brewed, can serve as a potent source of natural antioxidants. This study provides a scientific basis for promoting the nutritional use of wild fruits and supports further product development using *B. vulgaris*.

***Keywords*:** optimization, *Berberis vulgaris*, fruit tea, *B. vulgaris* tea

1. Introduction

Berberis spp. are plants belonging to the family Berberidaceae, usually thorny and deciduous or evergreen shrubs. About 500 species of this genus are distributed over a wide geographical area, including Central and Southern Europe, Western Asia, Northwest Africa and the northeast of the United States of America [1-3]. These plants are characterized by their yellow woody stems, long and short dimorphic shoots, and small, oval fruits that turn blue or red as they ripen, usually fruiting in late summer and autumn [2].

The fruits of Berberis species are consumed fresh or dried and used in the production of fruit juice, syrup, beverages, confectionery and similar products [1-2]. In addition, leaves and fruits are used as flavouring or additives in food applications, especially in tea production [2, 4, 5]. However, the information in the literature on the direct integration of these plants into food products is still limited.

The rich biological activity of Berberis species lies at the heart of their attractiveness. Antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic and anti-metabolic syndrome effects reveal the potential of these plants in both food and pharmaceutical industries [6-9]. For example, in the USA, extracts of *B. vulgaris* of the Berberidaceae

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family are used as botanical dietary supplements [10]. While its fruits and leaves are utilised in different industrial fields, ornamental species are used in landscaping and medicinal species are traditionally used in the treatment of liver, kidney, urinary tract, stomach and bronchial diseases as well as neurological disorders such as hypertension and epilepsy [3, 7, 11].

These effects are based on the bioactive compounds contained in Berberis species. The plant shows anti-inflammatory and immunosuppressive effects with protoberberine and bisbenzyl-isoquinoline alkaloids (berbamine, tetrandrine, chondocurine, etc.) [2, 6, 11, 12]. *Berberis vulgaris*, which is also a strong antioxidant source, contains catechin and similar phenolic compounds and shows protective effect against reactive oxygen species [7-11]. Reactive oxygen species are formed during metabolism and play a role in the formation of many chronic diseases such as diabetes, cancer and cardiovascular diseases. Therefore, the discovery and utilisation of natural antioxidant sources are of great importance for human health.

Nowadays, plant-based teas stand out as a practical, effective and economical method for the intake of such natural compounds. Tea is a beverage traditionally originating from China and widely consumed worldwide [13-14]. Not only tea plants but also leaves, flowers and fruits of many plants are used in tea making [15-17]. Such herbal teas contain many health beneficial components such as phenolic compounds, vitamins and polysaccharides, especially tannins and catechins [13-18]. By using hot water, these components can be effectively dissolved and functional beverages can be obtained.

In this study, tea was produced from *B. vulgaris* fruit and its antioxidant activity and total phenolic content were analysed. In light of the data obtained, tea brewing conditions were optimized using Response Surface Methodology (RSM) and Box-Behnken design. Optimization is a multivariate statistical approach that aims to maximise the performance of a system, process or product and is more time, cost and resource efficient than traditional univariate methods [19-21]. This research is the first study to perform the optimization of tea production and brewing parameters from *B. vulgaris* fruit on a scientific basis.

2. Material and method

2.1 *Collection and preparation of B. vulgaris samples*

The *Berberis vulgaris* to be used within the scope of the research was obtained from the local markets of Bayburt province. In this context, the samples provided were brought to Bayburt University Food Engineering Laboratory and kept under appropriate conditions until analyses were conducted. Before analysis, measles samples were dried in an oven at 50 °C for 2 days in a controlled manner. Dried fruits were turned into powder using a grinder.

2.2 *DPPH radical scavenging activity*

The antioxidant activity of teas was assessed following the procedure outlined by Kumaran et al. [22]. 100 µl of tea was mixed with 2 ml of 0.2 mM DPPH. The prepared sample was placed in a dark environment at room temperature for 30 minutes. After this period, its absorbance was measured at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference antioxidant in this analysis. Absorbance values were measured using different concentrations of Trolox solutions (e.g. in the range of 10-100 µmol/L) and a standard curve was constructed. The absorbance values of the tea samples were compared with the values corresponding to this standard curve, and the results were expressed as mg Trolox Equivalent (mg TE/g dry matter).

2.3 *Determination of total phenolic compounds*

Teas' TPC was gauged using Folin Ciocalteu reagent as per [23]. 1 ml of diluted tea sample (1 : 10, v/v) was combined with Folin Ciocalteu reagent (5 ml, diluted 1 : 10 with distilled water) and aqueous Na_2CO_3 (4 ml, 7.5%). The resulting mixture was left at room temperature for 60 minutes, and its absorbance was colorimetrically measured at 765 nm. Total phenol values are denoted as milligrams of Gallic Acid Equivalent (mg GAE) per gram of dry matter (mg GAE/g dry matter).

2.4 *Design of experiments and statistical analysis*

In *B. vulgaris* fruit, three independent variables (liquid-solid ratio, brewing temperature, and time) were taken as factors, and the brewing process was carried out. 200 ml of pure water was used as a solvent during the brewing stage. For this purpose, a 3-factor, 3-level Box-Behnken experimental design was used. The factors and their levels used for brewing conditions in the study are presented in Table 1. The Box-Behnken method, consisting of 15 experimental studies, was employed for response surface methodology to examine both main effects and interactions (Table 2). Optimum brewing conditions, maximum total phenolic compound, and antioxidant activity were determined using response surface methodology (MINITAB 18.1.1.0).

Table 1. Factors and their respective levels

Code	Factors	Factor		
		-1	0	1
X_1	Brewing temperature (°C)	90	75	60
X_2	Brewing duration (min.)	5	10	15
X_3	Liquid-solid ratio (g/mL)	1	2	3

Table 2. Designing experiments for RSM and the experimental data for tea samples

Analysis	X_1	X_2	X_3	Y_1	Y_2
1	90	10	1	78.75	488.24
2	90	15	2	43.92	536.67
3	90	10	3	29.79	405.30
4	90	5	2	45.08	352.09
5	75	5	3	29.90	325.99
6	75	10	2	43.48	403.78
7	75	15	1	68.26	412.43
8	75	5	1	72.00	427.65
9	75	10	2	47.44	345.04
10	75	15	3	29.69	398.97
11	75	10	2	45.18	350.08
12	60	15	2	44.50	313.02
13	60	10	1	70.57	416.26
14	60	10	3	28.51	346.44
15	60	5	2	43.83	299.39

X_1, Brewing temperature (°C); X_2, Brewing duration (min.); X_3, Liquid-solid ratio (g/mL)
Y_1, DPPH (mg TE/g dry matter); Y_2, TPC (mg GAE/g dry matter)

3. Results

The regression analysis yielded quadratic equations as outlined in Table 3. For sensory, colorimetric, and physicochemical outcomes, a regression coefficient (R^2) value of at least 70% is considered suitable [24]. In this study, the quadratic equations demonstrated robust regression coefficients (R^2 > 87%), and the lack of fit in the regression models was statistically insignificant ($P > 0.05$).

Table 3. Quadratic equations derived from regression analysis

Dependent variables	Model	R^2	Lack of harmony
DPPH	$Y_1 = 94.6^* + 0.072\ X_1 + 1.18\ X_2 - 38.95\ X_3^* + 0.00203\ X_1X_1 - 0.0595\ X_2X_2 + 6.09\ X_3^*X_3^* - 0.0061\ X_1X_2 - 0.1149\ X_1X_3^* + 0.176\ X_2X_3^*$	99.43	0.342
TPC	$Y_2 = 1{,}095 - 12.5\ X_1^* - 39.6\ X_2 - 189\ X_3 + 0.0706\ X_1^*X_1^* - 0.276\ X_2X_2 + 31.9\ X_3X_3 + 0.570\ X_1^*X_2 - 0.22\ X_1^*X_3 + 4.41\ X_2X_3$	87.53	0.397

* Significant at $P < 0.05$; Y: Dependent variables; X_1, Brewing temperature (°C); X_2, Brewing duration (min.); X_3, Liquid-solid ratio (g/mL)

3.1 *Influence of brewing parameters on DPPH activity*

Liquid-solid ratio (g/mL) was statistically identified as the most effective factor on DPPH radical scavenging activity in *B. vulgaris* tea, while temperature and brewing time did not show significant effects ($p > 0.05$). The highest DPPH value of 78.75 mg TE/g dry matter was obtained under the conditions of 90 °C, 10 min and 1g/mL liquid-solid ratio. The response surface contour plot presented in Figure 1 shows the effects of these brewing variables on the antioxidant capacity. Although increased temperature and prolonged brewing time can increase the extraction of antioxidant compounds up to a point, many bioactive phytochemicals (especially phenolics) are thermolabile and degrade at elevated temperatures or prolonged exposure. This model, supported by previous research, suggests that extraction efficiency generally peaks at a certain time and temperature, then plateaus or decreases depending on the matrix and solvent used [25].

Our findings show that the temperatures and times applied in this study were sufficient to extract antioxidants at almost maximum levels. However, the liquid-solid ratio seems to play a limiting role in this process. A clear negative correlation was observed: as the liquid-solid ratio decreased, DPPH values increased. This trend may indicate that antioxidant compounds reach solubility thresholds in the solvent system used and that a denser solid content leads to a higher concentration of antioxidants in the resulting infusion. It is known that increasing the solvent volume can facilitate extraction by increasing the solubility and mass transfer of bioactives, but excessive dilution can reduce the concentration of compounds in the final product [26-27].

Comparable observations were reported by Nguyen and Chuyen [25], who studied the effects of brewing parameters, including temperature, liquid-solid ratio and time, on the antioxidant capacity and phenolic content of Roselle tea. Their results showed that increasing the liquid-to-solid ratio from 8 : 1 to 10 : 1 increased antioxidant extraction; however, the efficiency began to decrease beyond a certain threshold, similar to our findings. Although previous studies have evaluated *B. vulgaris* extracts using various solvents and plant parts [3-7], few have specifically investigated fruit tea infusions under optimized brewing conditions. Eroglu et al. [3] reported DPPH values ranging from 25.17 to 40.44% in aqueous extracts of *B. vulgaris* fruits. Another study analysing both aqueous and alcoholic extracts at different concentrations (1.25-5 mg/mL) recorded aqueous DPPH levels of 20.55%, 43.02% and 74.71%, respectively [28].

In contrast, the DPPH values observed in our study (28.51-78.75 mg TE/g dry matter) were higher than most literature data, indicating that the use of optimized brewing conditions significantly improved antioxidant extraction. Maintaining moderate temperature and brewing time probably helped to preserve the structural integrity of thermos sensitive antioxidants and thus improved the measurement accuracy. In addition, the low liquid-to-solid ratio may have increased the concentration of bioactive compounds in the infusion. These findings emphasise the need for the

development of more refined and targeted infusion strategies for both domestic and industrial applications.

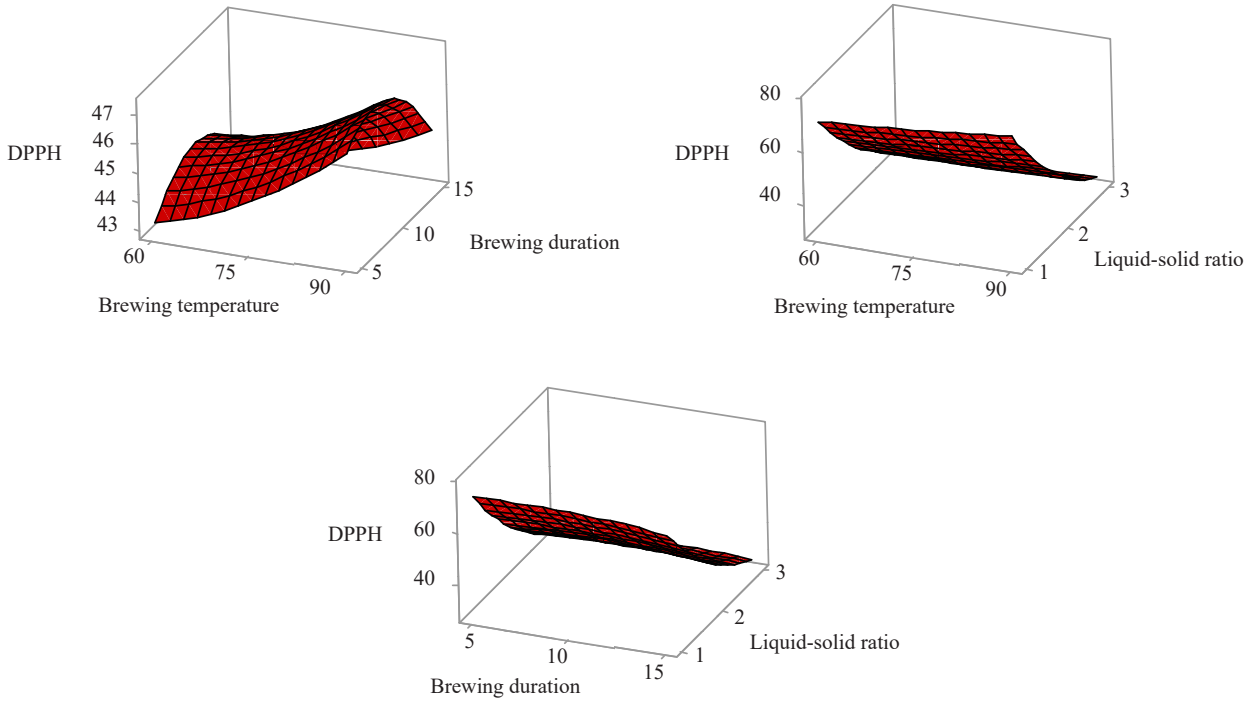

Figure 1. Response surface contour plot of the effect of brewing conditions on DPPH

3.2 *Influence of brewing parameters on TPC*

According to the findings depicted in Table 3, temperature emerged as the primary independent variable exerting the most significant influence on the total amount of phenolic compounds. The response surface contour plot illustrating the effect of brewing conditions on TPC is shown in Figure 2. The figure demonstrates a direct correlation between TPC and both temperature and time, while TPC exhibits an inverse correlation with the liquid-solid ratio. Statistical analysis revealed that the liquid-solid ratio and time did not significantly affect TPC ($P > 0.05$), while the temperature had a marked impact ($P < 0.05$). The highest amount of TPC (536.67 mg GAE/g dry matter) was determined under conditions where the temperature was 90 °C, the time was 15 min and the sample amount was 2 g. According to the data obtained, TPC increases as temperature and time increase, while TPC decreases as the amount increases. This may be due to the fact that similar to DPPH, the phenolic compounds in *B. vulgaris* tea reach their maximum solubility in the given amount of solvent. In addition, some studies have stated that high temperature increases TPC up to a certain level, but above a specific temperature, it decreases due to the degradation of these components [25]. As mentioned above, it is seen that the amount of TPC increases with increasing temperature and time under the brewing conditions used in this study. The total phenolic content of *B. vulgaris* fruit was determined as 148-396.3 microgram GAE/mg dry weight [3]. In their study evaluating the mineral and phenolic component content, as well as the anti-radical activity of three types of Iranian barberry fruits, Rahimi-Madiseh et al. [29] determined the total phenolic content of *B. vulgaris* fruit as 0.54 mg/g. Hoshyar et al. [28] selected the complete phenolic component content of the aqueous extract of *B. vulgaris* fruit, which was 184.1 mg GAE/g dry weight.

The TPC values (in the range of 299.39-536.67 mg GAE/g dry matte) obtained in this study were notably higher than those reported in the literature, underscoring the effectiveness of the applied optimization process. While previous studies have reported total phenolic contents of *B. vulgaris* fruits ranging from 148 to 396.3 µg GAE/mg dry weight, this study achieved a maximum of 536.67 mg GAE/g dry matter. This substantial difference suggests that the optimized brewing temperature and time significantly enhanced the extraction efficiency of phenolic compounds. Additionally, the

use of a lower liquid-to-solid ratio likely increased the concentration of phenolic compounds in the solvent, contributing to the higher TPC values. These findings imply that moderate increases in temperature and duration can facilitate maximum solubility of phenolic compounds without causing thermal degradation. However, it should be noted that factors such as fruit variety, harvest time, and drying methods may also influence the final phenolic content. Future studies focusing on these variables could help standardize phenolic content outcomes and improve reproducibility across different batches of *B. vulgaris* tea.

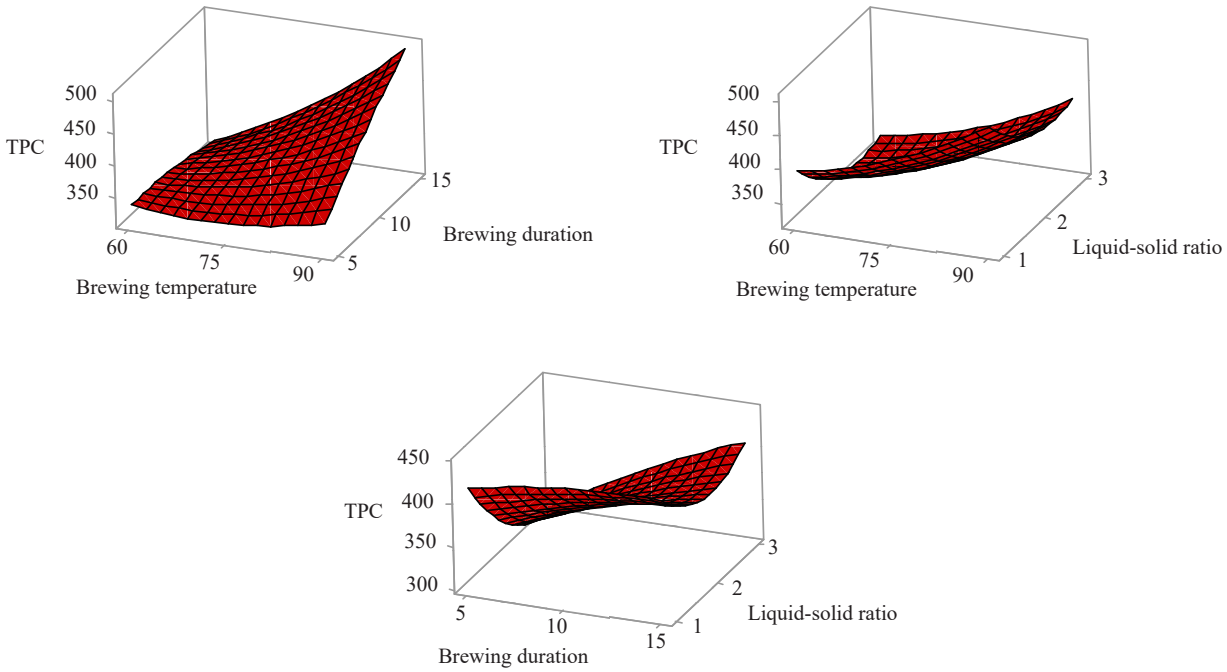

Figure 2. Response surface contour plot of the effect of brewing conditions on TPC

3.3 *Comparison of results obtained under optimum conditions*

The optimization process was carried out by maximizing the amounts of TPC and DPPH. As a result of the optimization process, it was determined that the optimum operating conditions were 90 °C temperature, 13.69 minutes brewing time, and 1 g/mL liquid-solid ratio. The brewing process was carried out under the specified requirements. Consequently, the impact of these conditions on bioactive compounds was assessed, and the findings are outlined in Table 4. Following the optimization, the model-derived values of TPC and DPPH were determined to be 534.51 mg GAE/g dry matter and 73.67 mg TE/g dry matter, respectively. These results closely align well with the expected data.

Table 4. Results of experimental validation

	Predicted value	Experimental value	Error (%)
Response value			
DPPH (mg TE/g dry matter)	74.15	73.67 ± 0.87	0.65
TPC (mg GAE/g dry matter)	536.80	534.51 ± 7.41	0.43

4. Conclusion

The results of this study have significant implications for both scientific research and daily practice. It offers a scientific foundation for researchers to identify the optimal brewing conditions for the most efficient extraction of bioavailable components from *B. vulgaris* fruit. Moreover, the optimization method employed holds potential as a methodological model for future studies on similar plant products. For consumers, the study presents practical insights into preparing *B. vulgaris* tea as a natural source of antioxidants, aligning with current healthy living trends. These findings could aid in the development of functional beverages at both household and industrial scales. In this regard, the study contributes valuable knowledge to the literature by promoting the more efficient use of wild fruits while simultaneously offering potential benefits for public health.

Conflict of interest

The authors declare no competing financial interest.

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