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Optimization of bioactive compounds from chicory roots by ultrasound-assisted extraction

Ultrason destekli ekstraksiyon ile hindiba köklerinden biyoaktif bileşiklerin optimizasyonu

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Abstract

Chicory roots (*Cichorium intybus* L.) are rich in phenolic compounds with strong antioxidant activity, making them valuable for functional food and nutraceutical applications. This study optimized ultrasound-assisted extraction (UAE) of bioactive compounds from dried chicory roots using an acidified hydroethanolic solvent system (80% ethanol containing 1% HCl, v/v) and a Box–Behnken design. Ultrasound amplitude (60–80%), extraction time (3–7 min), and solid-to-liquid ratio (5–15%) were evaluated for their effects on total phenolic content (TPC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. ANOVA indicated that all linear factors significantly affected antioxidant activity ($p \leq 0.05$), with solid-to-liquid ratio contributing 71.58% of total variability. For TPC, amplitude and time were significant, while concentration showed a marginal effect. Interaction effects, particularly between amplitude and concentration, were notable. Optimal conditions were 74.85% amplitude, 15% concentration, and 3 min, yielding 133.79 ± 4.43 mg GAE/100 g TPC and 61.06 ± 3.36% DPPH inhibition. UAE proved to be a rapid and efficient method for enhancing phenolic recovery.

Keywords: Chicory root, Ultrasound-assisted extraction, Process optimization

Öz

Hindiba kökü (*Cichorium intybus* L.), güçlü antioksidan aktivite gösteren fenolik bileşikler açısından zengindir ve fonksiyonel gıda ile nutrasötik uygulamalar için önemli bir hammaddedir. Bu çalışmada, asitlendirilmiş hidroetanolik çözücü sistemi (hacimce %80 etanol ve %1 HCl) ve Box–Behnken tasarımı kullanılarak kurutulmuş hindiba köklerinden biyoaktif bileşiklerin ultrason destekli ekstraksiyonu (UAE) optimize edildi. Ultrases gücü (%60–80), ekstraksiyon süresi (3–7 dk) ve katı:sıvı oranının (%5–15) toplam fenolik madde (TFM) ve 1,1-diphenyl-2-picrylhydrazyl (DPPH) radikal giderme aktivitesi üzerindeki etkileri incelenmiştir. ANOVA sonuçlarına göre tüm lineer faktörler antioksidan aktiviteyi anlamlı düzeyde etkilemiş ($p \leq 0.05$) ve en baskın parametre katı:sıvı oranı olmuştur (%71.58). TFM için güç ve süre anlamlı bulunurken, konsantrasyon sınırda etki göstermiştir. Özellikle güç–konsantrasyon etkileşimi önemli bulunmuştur. Optimum koşullar %74.85 güç, %15 katı:sıvı oranı ve 3 dk olarak belirlenmiş; bu şartlarda 133.79 ± 4.43 mg GAE/100 g TFM ve %61.06 ± 3.36 DPPH inhibisyonu elde edilmiştir. UAE hızlı ve etkili bir ekstraksiyon yöntemidir.

Anahtar kelimeler: Hindiba kökü, Ultrason destekli ekstraksiyon, Proses optimizasyonu

1 Introduction

Chicory (*Cichorium intybus* L.) is widely recognized for its diverse applications in the food industry and herbal medicine. It has been consumed as a vegetable for centuries, with both its leaves and roots traditionally utilized for nutritional purposes [1]. Since the late 16th century, particularly during the Napoleonic era, roasted chicory roots have also been used as a coffee substitute [2]. Chicory roots are industrially utilized for inulin extraction, employed as a coffee substitute following roasting, and serve as animal feed [3–5]. Additionally, chicory extracts are incorporated as additives in both alcoholic and non-alcoholic beverages [6]. Owing to their established appetite-stimulating effects and prebiotic activity, extracts of *Cichorium intybus* are widely included in dietary supplements and specialized nutritional products [1,7]. Beyond inulin, chicory is a rich source of valuable sesquiterpene lactones, primarily lactucine and lactucopicrin [8].

Chicory has traditionally been utilized in various sectors, including animal feed particularly the bagasse remaining after inulin extraction from the roots and the food industry, where it serves as an ingredient in salads, tea blends, and as a cost-effective alternative to coffee. Recently, there has been growing interest in the extraction of its bioactive components, notably inulin, oligofructose, and sesquiterpene lactones, due to their functional and health-promoting properties [9]. Ethanolic and methanolic extracts of chicory have shown cytotoxic activity against MCF-7 and AML cell lines, an effect attributed to their antioxidant capacity and high phenolic content [10]. The authors of the study

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suggested that these extracts hold potential for use in the protection or treatment of cancer cells. Additionally, enzyme-treated chicory roots yielded extracts capable of inhibiting the growth of skin pathogens in cosmetic formulations subjected to challenge testing [11].

Due to the increasing generation of waste, which poses a threat to the environmental sustainability of food production systems, the comprehensive utilization of fruits and vegetables has become both a responsibility and an opportunity for companies aiming to adopt low-waste technologies in agribusiness [12]. As stated by Chemat et al. [13], a green extraction process should reduce energy consumption, permit the use of alternative solvents and renewable natural materials, and ensure the safety and quality of the resulting extract or product.

Phenolic compounds represent one of the most prevalent classes of phytochemicals that can be recovered from vegetable by-products. Their extraction serves a dual function: significantly reducing the environmental burden associated with agro-industrial waste and enabling the recovery of high-value bioactive compounds with potential nutraceutical applications [14]. In this context, several studies have focused on the green extraction of phenolic compounds from chicory. Cova et al. [15] investigated ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and a combination of both, using either hydroalcoholic solutions or water (including sub-critical water). Their results showed that MAE with sub-critical water and MAE/UAE combinations using ethanol solutions enabled the recovery of up to ~3 g of gallic acid equivalents (GAE) per kg of fresh material in just 15 minutes, compared to 240 minutes required by conventional methods. Similarly, Pradal et al. [16] applied a Box–Wilson central composite design to optimize UAE of polyphenols from chicory and achieved a total phenolic yield of 7.23 mg/g dry matter under optimal conditions: 9.2 minutes of extraction at 60°C, with 37.5% ethanol in the solvent and 100 W ultrasound power.

Therefore, a more comprehensive optimization strategy that simultaneously evaluates process efficiency, energy-saving short extraction durations, and interaction effects among critical variables remains necessary. In this context, the present study aims to optimize ultrasound-assisted extraction of phenolic compounds from dried chicory roots under controlled temperature conditions using response surface methodology (RSM). Ultrasound amplitude (60–80%), extraction time (3–7 min), and solid-to-liquid ratio (5–15%, w/v) were selected as independent variables based on preliminary trials and literature evidence. Total phenolic content (TPC) and antioxidant activity were employed as response variables to evaluate extraction performance. A three-factor Box–Behnken design was applied to develop a quadratic polynomial model and determine the optimal operational conditions. By focusing on short extraction durations and analyzing interaction effects between amplitude and concentration under acidified hydroethanolic conditions, this study contributes to the development of an efficient and energy-conscious UAE process for the recovery of bioactive compounds from chicory roots.

2 Material and method

2.1 Material

Dried chicory roots (*Cichorium intybus* L.) were obtained from a local herbal supplier in Türkiye. The roots were visually inspected to remove damaged or foreign materials and then ground into powder using a laboratory mill. The powdered samples were sieved to obtain a uniform particle size prior to extraction. The dried chicory root had a moisture content of 8.8 ± 0.14% and an ash content of 1.61 ± 0.29%. All reagents were of analytical grade. Ethanol, hydrochloric acid (HCl), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and gallic acid were obtained from commercial sources.

2.2 Method

2.2.1 Extraction of chicory root

Extraction was performed using a probe-type ultrasonic processor (UP400S, Hielscher Ultrasonics, Germany) with titanium alloy sonotrode (H22, Dr. Hielscher, GmbH). The ultrasonic system operated at a frequency of 24 kHz with a maximum nominal power output of 400 W. The probe tip diameter was 22 mm. For each extraction, 100 mL of acidified hydroethanolic solvent (80% ethanol containing 1% HCl, v/v) was mixed with the appropriate amount of chicory root powder according to the selected solid-to-liquid ratio. The extraction was carried out in 250 mL glass beakers. An acidified hydroethanolic solvent system was selected because of its effectiveness in improving the solubility and stability of phenolic compounds. The ranges of independent variables were determined based on preliminary trials and literature reports concerning ultrasound-assisted extraction of phenolic compounds from plant materials. Ultrasound amplitude (60–80%), extraction time (3–7 min), and solid-to-liquid ratio (5–15%, w/v) were selected as the independent variables. Temperature was continuously monitored during extraction and maintained at 25 ± 2°C using an ice bath when necessary. Therefore, temperature was not considered as an independent variable in the experimental design.

2.2.2 Experimental design

A three-factor, three-level Box–Behnken design (BBD) was applied to evaluate the effects of independent variables on extraction efficiency. The coded and actual values of the variables are presented in Table 1.

A second-order polynomial model was used to describe the relationship between independent variables and response variables:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Y represents the predicted response (TPC or antioxidant activity), β_0 is the intercept, β_i, β_{ii}, and β_{ij} are regression coefficients, X_1, X_2, and X_3 are coded independent variables. Each experimental run was performed in triplicate. The extracts were filtered and stored at −18°C in airtight containers until analysis.

Table 1. Box Behnken design

Trial Sequence	Amplitude	Concentration	Duration
1	70	5	7
2	60	15	5
3	70	10	5
4	80	10	3
5	70	15	7
6	60	10	7
7	70	15	3
8	60	10	3
9	80	10	7
10	70	5	3
11	70	10	5
12	60	5	5
13	70	10	5
14	80	5	5
15	80	15	5

2.2.3 Extract analysis

2.2.3.1 Determination of total phenolic content

Total phenolic content (TPC) was analyzed using a modified Folin–Ciocalteu procedure [17]. Briefly, 100 µL of sample was mixed with 0.75 mL of 10% (v/v) Folin–Ciocalteu reagent and allowed to stand at room temperature for 5 min. Subsequently, 0.75 mL of Na_2CO_3 solution (75 g/L) was added, and the mixture was vortexed. After incubation in the dark at room temperature for 90 min, absorbance was measured at 725 nm using a spectrophotometer. Gallic acid served as the reference standard, and a calibration curve was prepared using different concentrations of gallic acid solutions. TPC values were calculated from this curve and expressed as mg gallic acid equivalents (GAE) per 100 g on a dry weight basis.

2.2.3.2 Color determination

The color characteristics of chicory extracts were measured using a Konica Minolta CR400 colorimeter (Japan). The instrument was calibrated with distilled water prior to analysis. Liquid samples were then transferred into a cuvette, and the L*, a*, and b* color parameters were recorded.

2.2.3.3 Determination of antioxidant activity

Free radical scavenging activity assay was performed according to Blois' method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [18]. The method is based on the ability of the extracts to give a proton or electron to lighten the color of the purple DPPH solution. The decrease in absorbance of the reaction mixture is an indication of high free radical scavenging activity. After taking 100 µL each of the sample extracts prepared at different concentrations, 3.9 mL of 0.1 mM DPPH solution (in 80% ethanol) was added. After vortexing, the solution was kept in the dark under room conditions for 30 minutes and the absorbance was read at 517 nm at the end of the time. Instead of the sample, 100 µL of 80% ethanol was used as a control under the same conditions. % DPPH radical scavenging activity was calculated by the formula given below [19]:

$$\% \text{ inhibition of DPPH activity} = \left[\frac{(Ac-As)}{Ac}\right] \times 100 \quad (2)$$

Where A_c is the absorbance of control, and A_s is the absorbance of the sample.

2.2.3.4 Total soluble solids content

Brix values of chicory extracts were measured with a refractometer (Hanna, Germany). Each sample was measured in 3 parallel measurements.

2.2.3.5 pH analysis

Calibrated pH meter and pH measurements of the samples were made using Hanna pH meter.

2.2.3.6 Ash determination

Ash determination was performed only dried chicory root according to AOAC Method No: 940.26 [20].

2.3 Statistical analysis

All analyses were carried out in triplicate, and the data were reported as mean ± standard deviation. Response Surface Methodology (RSM) was applied using Minitab Statistical Software (Version 18). The significance of the model and its terms was assessed by analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$). When necessary, differences among means were determined using Tukey's multiple comparison test.

3 Results and discussion

The moisture content and ash values of the dried chicory root sample were determined as 8.8±0.14% and 1.61±0.29%, respectively. Also, other quality parameters of chicory root extracts based on the Box-Behnken design table were shown in Table 2.

According to the data presented in Table 2, the pH values of the extract samples ranged from 0.86 to 1.00. The lowest pH value was observed in Sample No. 15, which was obtained using 80% ultrasound amplitude, 15% solvent concentration, and a 5-minute extraction time. This indicates that high ultrasonic energy and solvent concentration can render the extraction medium more acidic. The soluble solid content (SSC), reflecting the amount of water-soluble dry matter, showed limited variation across all samples, ranging from 20.5 to 21.3. The highest SSC values were observed in samples extracted with 10% and 15% solvent concentrations. This suggests that the concentration of dissolved compounds (likely polyphenols and sugars) increases with higher solvent concentration. Regarding color parameters, L* values (lightness) ranged between 27.8 and 35, with the highest L* value observed under conditions of 80% amplitude, 5% solvent concentration, and 5 minutes of extraction. This result implies that lower solvent concentrations yield lighter-colored extracts.

Table 2. Quality parameters of chicory root extracts

No	Extraction Condition	pH	SSC	L*	a*	b*
1	A=70%; C=5%; D=7 min	1.0±0.05^{a}	20.5±0.5^{b}	31.8±0.05^{abcd}	-0.26±0.1^{g}	17.9±0.07^{h}
2	A=60%; C=15%; D=5 min	0.92±0.02^{a}	20.8±0.29^{a}	30.4±0.03^{bcde}	3.17±0.05^{b}	24.1±0.1^{a}
3	A=70%; C=10%; D=5 min	0.90±0.01^{a}	21.3±0.14^{a}	34.4±0.03^{ab}	-0.99±0.05^{i}	18.9±0.02^{g}
4	A=80%; C=10%; D=3 min	0.91±0.06^{a}	20.8±0.29^{a}	34±0.06^{ab}	-1.07±0.04^{j}	17.5±0.04^{i}
5	A=70%; C=15%; D=7 min	0.88±0.07^{a}	21.3±0.06^{a}	31±0.02^{abcde}	1.48±0.04^{c}	22.2±0.07^{d}
6	A=60%; C=10%; D=7 min	0.99±0.04^{a}	20.8±0.29^{a}	30.5±0.05^{bcde}	0.92±0.03^{e}	20.2±0.05^{f}
7	A=70%; C=15%; D=3 min	0.89±0.02^{a}	21.1±0.14^{a}	29±0.01^{de}	3.15±0.06^{b}	23.4±0.1^{b}
8	A=60%; C=10%; D=3 min	0.87±0.01^{b}	21.1±0.14^{a}	27.8±0.05^{e}	0.49±0.06^{f}	17.4±0.05^{i}
9	A=80%; C=10%; D=7 min	0.93±0.01^{a}	20.8±0.29^{a}	33.8±0.01^{ab}	-1.25±0.03^{k}	10.1±0.02^{m}
10	A=70%; C=5%; D=3 min	0.95±0.01^{a}	20.8±0.25^{a}	34±0.01^{ab}	-0.93±0.01^{i}	17±0.01^{j}
11	A=70%; C=10%; D=5 min	0.88±0.01^{a}	21±0.25^{ab}	30.5±0.03^{bcde}	1.31±0.04^{d}	22.9±0.01^{c}
12	A=60%; C=5%; D=5 min	0.95±0.01^{a}	20.8±0.29^{a}	32.8±0.05^{abcd}	-0.74±0.03^{h}	17.1±0.01^{j}
13	A=70%; C=10%; D=5 min	0.91±0.01^{a}	21.2±0.29^{a}	30.9±0.33^{abcd}	-0.67±0.04^{h}	15.7±0.2^{k}
14	A=80%; C=5%; D=5 min	0.94±0.01^{a}	21.1±0.14^{a}	35±0.04^{a}	-1.42±0.02^{l}	11.2±0.03^{l}
15	A=80%; C=15%; D=5 min	0.86±0.01^{c}	21.3±0.29^{a}	29.5±5.76^{cde}	4.99±0.03^{a}	21±0.01^{c}

A: Amplitude, C: Concentration, D: Duration. SSC: Soluble solid content. Results were expressed as mean ± SD. Values of parameters with different superscript letters along the same column are significantly different according to Tukey's test ($p \leq 0.05$)

The a* values (red/green) were generally negative (indicating greenish tones), though notable positive increases were recorded under certain conditions. Notably, Sample No. 15 showed the highest a* value of 4.99, suggesting that higher amplitude and concentration enhance the extraction of colored compounds, such as flavonoids. The b* values (yellowness) ranged from 10.1 to 24.1. The most intense yellow tones were observed in high-concentration conditions (Samples No. 2 and 7), indicating that pigments in the extracts, such as carotenoid derivatives or Maillard reaction products are influenced by solvent concentration. In conclusion, ultrasonic extraction parameters were found to directly affect not only the levels of bioactive compounds but also the physicochemical properties of the extracts (pH, SSC, color). Among these, solvent concentration emerged as one of the most influential parameters on extract characteristics. Ultrasound amplitude and extraction duration, particularly at higher levels, had significant effects on both color parameters and pH value.

Variance data of the results of TPC analysis of dried chicory root extracts and model summary were given in Table 3 and Table 4, respectively.

Table 3. Response surface regression: total phenolic content

Source	DF	Seq SS	C*	Adj SS	Adj MS	F-Value	›-Value
Model	9	10701.0	99.24%	10701.0	1189.0	72.72	0.000
Linear	3	1119.8	10.39%	1119.8	373.27	22.83	0.002
Amplitude	1	638.8	5.92%	638.8	638.82	39.07	0.002
Concentration	1	72.6	0.67%	72.6	72.59	4.44	0.089
Duration	1	408.4	3.79%	408.4	408.39	24.98	0.004
Square	3	4273.6	39.63%	4273.6	1424.54	87.13	0.000
A*A	1	200.0	1.85%	63.7	63.65	3.89	0.106
C*C	1	3469.7	32.18%	3674.8	3674.76	224.76	0.000
D*D	1	604.0	5.60%	604.0	603.96	36.94	0.002
2-Way Interaction	3	5307.6	49.22%	5307.6	1769.19	108.21	0.000
A * C	1	4783.8	44.37%	4783.8	4783.82	292.59	0.000
A * D	1	426.2	3.95%	426.2	426.22	26.07	0.004
C*D	1	97.5	0.90%	97.5	97.53	5.97	0.058
Error	5	81.8	0.76%	81.8	16.35		
Lack-of-Fit	3	79.8	0.74%	79.8	26.61	27.57	0.035
Pure Error	2	1.9	0.02%	1.9	0.97		
Total	14	10782.7	100%				

C*: Contribution, A*A: Amplitude*Amplitude, C*C: Concentration * Concentration, D*D: Duration * Duration, A*C: Amplitude * Concentration, A*D: Amplitude * Duration, C*D: Concentration * Duration

Table 4. Summary of the model

Std	R^2	R^2(adj)	PRESS	R^2(pred)
4.04	99.24%	97.88%	1281.46	88.12%

PRESS: The prediction error sum of squares

The antioxidant activity of plants is primarily attributed to their chemical constituents, most notably natural phenols and polyphenols. These bioactive compounds are particularly abundant in plant parts such as leaves, fruits, seeds, and flowers, which are rich sources of flavonoids and phenolic compounds. Enhancing dietary intake of antioxidants through the consumption of fruits and vegetables with high antioxidant capacity has been shown to contribute positively to overall health and quality of life [21].

The analysis of variance (ANOVA) results for total phenolic content (TPC) is presented in Table 3. The quadratic model was statistically significant ($p < 0.05$), with a coefficient of determination (R^2) of 99.24%, indicating that the model explained a substantial proportion of the variability in the response. The adjusted R^2 (97.88%) and predicted R^2 (88.12%) values were in reasonable agreement, suggesting acceptable predictive capability. However, the lack-of-fit test was statistically significant ($p = 0.035$), indicating that the model does not fully capture all variability within the experimental domain. This may be attributed to the strong nonlinear interaction effects observed between amplitude and concentration, which accounted for 44.37% of total variance. Despite the significant lack-of-fit, the relatively low pure error (0.02%) and high explained variance suggest that the model can still provide useful predictive information within the parameter range studied. Among the linear terms, amplitude ($p = 0.002$) and extraction duration ($p = 0.004$) significantly affected TPC, while

concentration showed a marginal effect ($p = 0.089$). The quadratic effect of concentration (C^2) was highly significant ($p < 0.001$), confirming the presence of curvature in the response surface.

In a previous study, TPC values of three chicory root preparations were determined between 816.7 and 906.4 mg/100g d.b. using three different extraction methods, while the highest TPC amount was obtained by the enzyme + pressure-assisted extraction method [22]. The earlier study indicated that solvent type, impregnation duration, number of sonication cycles, and ultrasonic power significantly influenced the UAE of chicory (*Cichorium intybus*) root. Using an orthogonal experimental design, the TPC values of the extracts ranged between 22.34 ± 0.00 and 27.87 ± 0.14 mg GAE/100 g dry extract. The current study further demonstrates that solvent composition is a key factor in enhancing both total phenolic content and antioxidant capacity [23]. Previous studies on chicory root extract showed that the TPC content of the samples ranged from 0.68 to 10.13 mg GAE/g root powder because of the Soxhlet extraction method using three different solvents. The highest amount of TPC was found in methanol extract, and the lowest in hexane extract [24]. It was reported in previous studies that the TPC content was determined as 28.16±1.60 mg GAE/g extract by concentrating the extract obtained by maceration of chicory roots in a rotary evaporator [25]. This value is approximately 20 times higher than the maximum amount of TPC determined in our study. Although there are many reasons for this difference, it is thought that the main factors are differences in chicory species, growing conditions, and extraction parameters applied.

Although the TPC values obtained in the present study were lower than some values reported in the literature, these differences may be attributed to several factors. Variations in chicory cultivar, geographical origin, climatic conditions, harvest stage, drying process, and particle size can strongly affect the phenolic composition of the raw material. In addition, the extraction technique, solvent polarity, extraction temperature, and expression basis of the results may significantly influence the reported TPC values. For example, studies using methanol as the extraction solvent often report higher TPC values due to its greater polarity and extraction efficiency for phenolic compounds. Similarly, Soxhlet extraction and rotary evaporator concentration processes may produce more concentrated extracts, resulting in higher phenolic values compared to direct ultrasound-assisted extraction. Furthermore, some studies express TPC on an extract basis (mg GAE/g extract), whereas the present study reported results on a dry sample basis (mg GAE/100 g dry matter), which may also contribute to apparent differences. Previous studies reported TPC values ranging from 0.68 to 10.13 mg GAE/g root powder for chicory root extracts obtained by Soxhlet extraction with different solvents, with methanol producing the highest phenolic yield. Likewise, macerated chicory root extracts concentrated by rotary evaporation were reported to contain 28.16 ± 1.60 mg GAE/g extract. Therefore, the relatively lower TPC values obtained in the present study should not necessarily be interpreted as lower extraction efficiency, but rather as a consequence of methodological and compositional differences among studies.

The results of the analysis of variance and model summary of the antioxidant activity values of chicory root extracts are shown in Tables 5 and 6, respectively.

For antioxidant activity, the model was also statistically significant ($p < 0.05$), with $R^2 = 99.22\%$, adjusted $R^2 = 97.82\%$, and predicted $R^2 = 87.56\%$, indicating strong explanatory power. Nevertheless, the lack-of-fit test was significant ($p = 0.004$), suggesting that the quadratic model may not entirely represent the complexity of the response behavior. This could be related to the dominant influence of solid-to-liquid concentration (71.58% contribution), which may introduce nonlinear extraction kinetics not fully modeled by a second-order polynomial equation. Despite the significant lack-of-fit, the small residual error (0.78%) and consistent agreement between predicted and experimental validation results (Table 7) indicate that the model remains practically applicable within the investigated factor space.

Table 5. Response surface regression: antioxidant activity

Source	DF	Seq SS	C*	Adj SS	Adj MS	F-Value	P-Value
Model	9	3117.82	99.22%	3117.82	346.42	70.76	0.000
Linear	3	2910.06	92.61%	2910.06	970.02	198.14	0.000
Amplitude	1	441.07	14.04%	441.07	441.07	90.10	0.000
Concentration	1	2249.36	71.58%	2249.36	2249.36	459.46	0.000
Duration	1	219.63	6.99%	219.63	219.63	44.86	0.001
Square	3	118.95	3.79%	118.95	39.65	8.10	0.023
A*A	1	5.44	0.17%	8.70	8.70	1.78	0.240
C*C	1	5.62	0.18%	2.45	2.45	0.50	0.511
D*D	1	107.89	3.43%	107.89	107.89	22.04	0.005
2-Way Interaction	3	88.81	2.83%	88.81	29.60	6.05	0.041
A * C	1	42.59	1.36%	42.59	42.59	8.70	0.032
A * D	1	17.10	0.54%	17.10	17.10	3.49	0.121
C*D	1	29.12	0.93%	29.12	29.12	5.95	0.059
Error	5	24.48	0.78%	24.48	4.90		
Lack-of-Fit	3	24.42	0.78%	24.42	8.14	266.56	0.004
Pure Error	2	0.06	0.00%	0.06	0.03		
Total	14	3142.3	100%				

C*: Contribution, A*A: Amplitude*Amplitude, C*C: Concentration * Concentration, D*D: Duration * Duration, A*C: Amplitude * Concentration, A*D: Amplitude * Duration, C*D: Concentration * Duration

Table 6. Model summary of antioxidant activity result

Std	R^2	R^2(adj)	PRESS	R^2(pred)
2.21	99.22%	97.82%	390.81	87.56%

Std: Standard deviation. PRESS: The prediction error sum of squares

Although the lack-of-fit values for TPC ($p = 0.035$) and DPPH inhibition ($p = 0.004$) were statistically significant, the models were still considered acceptable due to their very high coefficients of determination ($R^2 > 99\%$), high adjusted R^2 values, and strong predictive abilities. In addition, the low pure error values indicated good repeatability among the center points. The significant lack-of-fit may be attributed to the inherent complexity of the extraction system and the presence of nonlinear interactions among the variables. This finding is consistent with previous research indicating that higher solid concentrations in the extraction solvent can lead to greater yields of phenolic compounds due to increased mass transfer gradients and solute availability. The solid-to-liquid ratio is crucial in determining the saturation level and extraction kinetics of polyphenols, as inadequate solvent volume may limit compound solubility, while excessive dilution can reduce mass transfer efficiency. DPPH amounts of the samples were determined to be between 21.56% and 69.89%. The highest antioxidant activity was realized under A=70 %; C=15%; D=3 min conditions.

A previous study evaluated the antioxidant activities of *Cichorium intybus* root extracts using various in vitro models. Analyses conducted with different methanol concentrations reported that the extracts exhibited inhibition rates ranging from 39.75% to 99.98% [24]. Baiano et al. reported that solid/liquid ratio is an important parameter in the extraction of phenolic compounds from chicory and fennel residues and that high phenolic content is obtained under optimum conditions [26]. Moreover, amplitude has generally been reported as a significant factor in literature. For instance, Pingret et al. reported that increasing ultrasound amplitude enhanced the extraction yield of polyphenols from apple pomace [27]. In conclusion, the antioxidant activity achieved in this study is notably efficient, especially considering the short extraction duration and relatively mild processing conditions. This highlights the technological advantage of the UAE as a green and effective method for functional compound recovery.

Table 7. Optimal extraction conditions for TPC and DPPH

	Optimum Extraction Conditions			P	Experimental	CV (%)
Response	A	C	D			
Antioxidant Activity (Inhibition %)	74.85	15	3	68.14	61.06±3.36	6.73
TPC (mg GAE/100 g db)				142.33	133.79±4.43	3.92

A: Amplitude. C: Concentration. D: Duration. P: Predicted. CV (%): Coefficient of variance between predicted and experimental results.

As shown in Table 7, the optimum extraction conditions predicted by the model were 74.85% amplitude, 15% solid-to-liquid concentration, and 3 minutes of extraction time. Under these conditions, the predicted values for TPC and DPPH inhibition were 142.33 mg GAE/100 g and 68.14%, respectively. The experimental values were 133.79±4.43 mg GAE/100 g for TPC and 61.06±3.36% for antioxidant activity. A lower coefficient of variation (CV%) indicates that the data points are more closely clustered around the mean, reflecting lower relative variability within the dataset. These results closely matched the model's predictions, further supporting the robustness and applicability of the model in practical extraction scenarios.

4 Conclusion

This study successfully demonstrated the effectiveness of UAE in enhancing the recovery of phenolic compounds and antioxidant activity from dried chicory (*Cichorium intybus* L.) roots. Through a Box-Behnken experimental design, the effects of amplitude, extraction duration, and solid-to-liquid concentration on bioactive compounds were systematically evaluated. The results showed that solvent concentration had the most pronounced effect on antioxidant activity, while amplitude and duration also played significant roles. In contrast, TPC was influenced primarily by interaction and quadratic terms, particularly the amplitude × concentration interaction, which highlighted the importance of parameter synergy in optimizing extraction efficiency. The optimized extraction conditions 74.85% amplitude, 15% concentration, and 3 minutes duration resulted in high experimental yields of both TPC (133.79±4.43 mg GAE/100 g) and antioxidant activity (61.06±3.36%). The results demonstrate the robustness of the proposed model and indicate that UAE represents an effective, sustainable, and rapid approach for recovering bioactive compounds from plant materials. In general, these findings strengthen the expanding evidence that UAE is an eco-conscious and industrially adaptable technique for producing functional ingredients, with promising uses in the food, nutraceutical, and pharmaceutical sectors.

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Competing interest

The authors declare no competing interests.

Similarity (iThenticate): 19%

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