

Article

Microwave-Assisted Extraction of Garlic Polyphenols: Optimization, Profiling, and In Vitro Digestion

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Abstract

Objective: This study aimed to develop and optimize a rapid, eco-friendly microwave-assisted extraction (MAE) process for recovering total phenolic content (TPC) and total flavonoid content (TFC) from garlic (*Allium sativum* L.), while evaluating the predictive performance of response surface methodology (RSM) versus artificial neural networks (ANNs) and assessing the in vitro gastrointestinal stability of key polyphenols. **Methodology:** A four-factor, three-level central composite design (CCD) was implemented to evaluate the effects of extraction time, temperature, ethanol concentration, and solvent-to-solid ratio. A second-order polynomial RSM model was benchmarked against a 4-10-2 multilayer perceptron ANN trained by backpropagation. Optimal conditions were derived using the Derringer–Suich desirability function and confirmed experimentally. Individual polyphenols were profiled via LC-MS/MS and monitored across simulated oral, gastric, and intestinal digestion phases. **Principal Results:** The ANN model demonstrated superior predictive performance ($R^2 = 0.9999$ training, 0.9974 validation, 0.9939 testing) compared to the RSM model ($R^2 = 0.9721$ for TPC and 0.9925 for TFC). Experimental validation under optimal conditions—1.50 min, 55 °C, 75% ethanol, and a 29 mL/g ratio—yielded a TPC of 2.487 mg GAE/g FW and a TFC of 21.356 mg QUE/g FW. During simulated gastrointestinal digestion, significant degradation occurred during the intestinal phase, resulting in low final recoveries for gallic acid (16.9%), caffeic acid (19.9%), and luteolin (22.6%). **Conclusions:** MAE coupled with ANN modeling provides a highly accurate, rapid, and green extraction strategy for garlic polyphenols. However, the marked degradation of target compounds during intestinal digestion highlights the necessity of encapsulation or protective delivery systems to preserve their biological functionality in food applications.



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Keywords: response surface methodology; artificial neural network; LC-MS/MS; bioaccessibility and bioavailability

1. Introduction

Garlic (*Allium sativum* L.) is among the most widely studied food plants, with both culinary and medicinal uses dating back centuries. Although sulfur compounds typically dominate its bioactivity, the non-sulfur fraction, particularly phenolic acids and flavonoids, makes a significant and synergistic contribution to its antioxidant, antibacterial, anti-inflammatory, and cardioprotective properties [1]. Phenolic content in garlic varies widely

among cultivars and growing regions, reflecting genetic, agronomic, and environmental influences [2–4].

The efficiency of phenolic recovery strongly depends on extraction conditions. Conventional solvent extraction, maceration, and distillation are limited by low yields, long processing times, and excessive solvent consumption [5]. Conventional extraction technologies offer several advantages, including well-established scalability, cost-effectiveness, and reliable performance across a wide range of applications. Their long history of use has led to optimized protocols and infrastructure, making them accessible and practical for large-scale operations. However, these methods often involve toxic solvents, generate significant waste, and consume considerable energy, raising environmental and safety concerns.

In contrast, green extraction technologies prioritize sustainability by minimizing environmental impact through reduced solvent use, lower energy consumption, and safer operational conditions. These approaches align with increasing regulatory and consumer demands for eco-friendly processes. Nonetheless, green extraction methods currently face challenges such as higher initial investment costs, potential difficulties in scaling up from laboratory to industrial levels, and technical limitations depending on the target compounds and matrices.

Green extraction technologies, particularly ultrasound-assisted extraction, microwave-assisted extraction, and pressurized liquid extraction, address these limitations by reducing energy and solvent consumption, thereby providing a shorter extraction time while preserving labile analytes [6–8].

By critically comparing both approaches, it becomes clear that while conventional methods remain advantageous for their maturity and economic feasibility, green technologies present a promising alternative that requires further development to overcome existing constraints. This balanced perspective enables a comprehensive understanding of the trade-offs involved in selecting extraction technologies.

Two modeling frameworks dominate the optimization of such processes. Response surface methodology is a multivariate regression technique that captures linear, interaction, and quadratic effects with a limited number of experimental points and was applied to optimize the extraction of chromones [9], polysaccharides [10], secoisolariciresinol diglucoside [11], and natural pigments [12]. Artificial neural networks, particularly multilayer perceptrons, were trained using backpropagation, where nonlinear input–output relationships are modeled without the need for a predefined equation [13–15]. ANNs have been used to refine the extraction of essential oils of *Diplotaenia cachrydifolia* [16], *Orthosiphon stamineus* oil [8], Passiflora seed oil [17], and Garcinia hull phenolics [18]. A comparative RSM vs. ANN strategy typically exploits the predictive interpretability of RSM and the flexibility of ANN, with the best model selected on the basis of their coefficient of determination (R^2), mean absolute deviation, and root-mean-square error.

Food matrices function as gatekeepers that regulate whether phenolics are released, protected, or sequestered during digestion [19,20]. Their interactions with proteins, fiber, lipids, and minerals can be noncovalent or covalent, both of which can affect release and absorption processes [19,20]. This matrix dependence explains why the same nominal phenolic content can produce different biological outcomes across foods, processing states, and digestive conditions. For these reasons, phenolic-rich foods cannot be treated as interchangeable with isolated compounds. Phenolics frequently bind to dietary fiber and proteins, and these interactions can reduce immediate release while preserving compounds for later microbial transformation in the colon [20,21]. Lipids and carbohydrates may also modulate bioavailability, indicating that some phenolics can behave differently across matrices [19,22]. Such interactions help explain why the functional value of anthocyanins, flavonoids, and bound phenolics is often lower or more variable than predicted from

composites alone [21,23]. Processing can modify the accessibility of phenolics by disrupting cell walls, changing solubility, or releasing bound forms [23,24]. Particle size reduction in wheat bran increased phenolic release and bioaccessible content, illustrating how physical structure affects exposure [24]. Fermentation and related biotechnological approaches may likewise increase bioavailability by liberating bound phenolics or altering matrix architecture [23,25].

In vitro digestion models are indispensable for estimating bioaccessibility, but their interpretive value depends on standardized definitions and comparable protocols [26,27]. A digestion assay therefore needs to be understood as an exposure model, not as a direct substitute for biological efficacy.

Polyphenol bioavailability depends not only on extraction yield but on stability through the gastrointestinal tract. Recovery during digestion was reported to vary widely among polyphenols depending on the matrix in which they are embedded [28–31]. For garlic, only one prior study explicitly examined the bioaccessibility of its phenolics under simulated digestion [32].

The state of the art in extracting phenolic compounds from garlic involves a range of both conventional and emerging green extraction technologies. Conventional methods such as solvent extraction using ethanol, methanol, or acetone remain widely used due to their established protocols, cost-effectiveness, and scalability. These methods efficiently recover phenolics but often rely on toxic solvents and require longer extraction times, which may lead to the degradation of sensitive compounds.

Emerging green extraction techniques, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and pressurized liquid extraction (PLE), offer significant advantages in terms of sustainability and efficiency. These methods reduce solvent use, energy consumption, and extraction time, aligning with eco-friendly and safety considerations. For example, UAE and MAE enhance mass transfer and cell disruption, improving yield and preserving bioactivity. SFE, particularly with CO₂, provides selective extraction without solvent residues, although it involves higher initial costs and technical complexity.

Despite these advances, green extraction techniques face challenges such as scalability from laboratory to industrial production, higher capital investment, and variability in extraction efficiency depending on garlic matrix and phenolic profiles. Optimizing extraction parameters (temperature, time, solvent type, and power intensity) is critical to maximizing phenolic yield and bioactivity.

Overall, the current trend emphasizes integrating green extraction methods with process optimization to enhance phenolic recovery from garlic while minimizing environmental impact. Combining conventional and green approaches or hybrid techniques may offer practical solutions that balance efficiency, cost, and sustainability.

In the present work, the MAE of garlic polyphenols was optimized using both RSM and ANNs under identical experimental conditions. These two models were statistically compared, the resulting polyphenolic profile was quantified by LC-MS/MS, and the stability of individual phenolics was monitored through a three-step in vitro gastrointestinal digestion process.

2. Materials and Methods

2.1. Garlic Sample

In this investigation, garlic bulbs with long white stems and short green leaves from China, Serbia, and Spain were purchased from a local supermarket and transported to the laboratory. Roots and decaying foliage were removed, while cloves were washed, sliced into ~1 cm² pieces, and stored at 4 °C. Prior to extraction, samples were homogenized

with the domestic food processor SM 3734 (Severin, Sundern, Germany). The material was reduced to a coarse particle size (approximately 2 mm) using a food processor prior to extraction. All analyses were completed within 10 days of purchase.

2.2. Chemicals and Reagents

All analytical grade solvents and reagents were bought from Merck KGaA (Darmstadt, Germany). Sigma-Aldrich (St. Louis, MO, USA) provided phenolic acids and flavonoids standards, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) ascorbic acid, Folin–Ciocalteu reagent, sodium-carbonate, sodium-nitrite, aluminum-chloride, sodium hydroxide, pepsin from porcine gastric mucosa (≥ 250 units/mg), α -amylase from *Bacillus* sp. (≥ 1500 Units/mg), pancreatin from porcine pancreas ($8 \times$ USP specification). The Direct-Q3 water purification system (Millipore, Billerica, MA, USA) was used to prepare solutions and reagents with deionized water ($18.2 \text{ M}\Omega/\text{cm}$ at 25°C).

2.3. MAE Procedure

MAE was performed in an ETHOS 1 Advanced Microwave Labstation (Milestone Srl, Sorisole, Italy) without stirring, and 50 mL vessels were used in accordance with an adapted protocol created by Maeng et al. [33]. Fresh garlic (1 g) was combined in 50 mL extraction vessels and extracted under the time, temperature, ethanol concentration, and solvent-to-solid ratio combinations dictated by the central composite design (Supplementary Material Table S1). After cooling to room temperature, extracts were vacuum-filtered through Whatman paper (GE Healthcare, Buckinghamshire, UK) into 50 mL Falcon tubes and stored at 4°C until analysis.

MAE is often regarded as a more energy-efficient extraction technique than conventional solvent extraction because microwave heating directly couples electromagnetic energy with the sample–solvent system, resulting in rapid volumetric heating, shorter extraction times, and lower overall energy consumption. However, the extent of the energy savings depends on the extraction conditions, equipment configuration, sample matrix, and process scale, and should therefore be evaluated quantitatively using metrics such as specific energy consumption.

2.4. Response Surface Methodology and Artificial Neural Networks

Based on the literature, adequate ranges of independent process variables, namely extraction time (X_1), extraction temperature (X_2), ethanol concentration (X_3), and solvent-to-solid ratio (X_4), were determined for a preliminary investigation. X_1 varied between 1 and 5 min, while X_2 , X_3 , and X_4 were fixed at 60°C , 60%, and 20 mL/g, respectively. Then, X_2 varied between 40 and 80°C , while X_1 , X_3 , and X_4 were held constant at 3 min, 60%, and 20 mL/min, respectively. Then, X_3 changed between 20 and 100%, while the other three factors stayed constant. Similarly, X_4 varied between 10 and 30 mL/g, while other variables remained constant. Initial experimental results demonstrated an increase in response variables with corresponding increases in extraction time (X_1 : 1–5 min), extraction temperature (X_2 : 40 to 80°C), ethanol concentration (X_3 : 20–100%), and solvent-to-solid ratio (X_4 : 10–30 mL/g). These variables were consequently chosen as the most significant independent variables.

Based on the single-factor test results and the supposition of a CCD, the extraction factors were refined utilizing response surface methodology. The ranks of the independent variables are shown in Table 1.

Table 1. Independent variables and their levels for central composite design (CCD).

Independent Variable		−1	0	+1
Extraction time (min)	X ₁	1.00	3.00	5.00
Extraction temperature (°C)	X ₂	40.00	60.00	80.00
Ethanol concentration (%)	X ₃	20.00	60.00	100.00
Solvent-to-solid ratio (mL/g)	X ₄	10.00	20.00	30.00

Responses Y₁ (TPC, mg GAE/g) and Y₂ (TFC, mg QUE/g) were fitted to a second-order polynomial:

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

where β_0 is the intercept; β_i , β_{ij} , and β_{ii} are linear, interaction, and quadratic coefficients, respectively; and ε is the residual error.

Backward elimination of non-significant terms ($p > 0.10$) was performed for each model. Adequacy was assessed using R², adjusted R², predicted R², lack-of-fit F-test, and coefficient of variation [34,35], while desirability was optimized using the Derringer–Suich function [36]. All RSM computations were performed in Design-Expert 8.0.

For ANN modeling, a multilayer perceptron with a 4-10-2 topology was implemented in MATLAB 9.5 Neural Networks Toolbox (StatSoft Inc., Tulsa, OK, USA). The four inputs (X₁–X₄) fed one hidden layer of ten neurons, which projected to two outputs (Y₁ and Y₂). Data were partitioned into 70% training (20 points), 15% validation (5 points), and 15% testing (5 points). Weights and biases were initialized randomly between −1 and +1, and hidden neurons were tuned from 1 to 15 by trial and error to minimize validation RMSE. Training terminated when validation errors began to increase (early stopping against overfitting) [15,37].

2.5. Phytochemical Assays

TPC was determined using the Folin–Ciocalteu method [38]: a 0.5 mL extract was reacted with 0.5 mL of reagent and 7.5 mL of water for 10 min; then, 1.5 mL of 20% Na₂CO₃ was added and the mixture was heated at 40 °C for 20 min. Absorbance was read at 755 nm (Cary 300, Palo Alto, CA, USA). Quantification used gallic acid standards (0.5–50 mg GAE/L).

TFC was determined using the aluminum chloride method [38]: a 0.1 g/mL extract was successively reacted with 5 mL of water, 0.3 mL of 5% NaNO₂, 0.6 mL of 10% AlCl₃, and 2 mL of 1 M NaOH; absorbance was read at 510 nm against quercetin standards.

2.6. Evaluation of Antioxidant Capacity

DPPH scavenging was carried out according to the protocol described by Liu et al. [39]: 3 mL of the sample was mixed with 1 mL of 40 µg/mL DPPH and kept in the dark for 30 min, and absorbance was read at 517 nm. The scavenging effect (%) was calculated as $(A_0 - (A_1 - A_2))/A_0 \times 100$, and ascorbic acid and BHT were used as positive standards.

The following equation was used to determine the DPPH radical scavenging effect:

$$\text{DPPH scavenging effect(\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

where A₀ is the absorbance of the control solution without a sample (deionized water instead of the sample), A₁ is the absorbance of the mixture solution with the sample, and

A_2 is the absorbance of the combined solution without DPPH (deionized water instead of DPPH).

2.7. *In Vitro* Digestion

Three-phase simulated digestion was performed according to Ydjedd et al. [40]. The oral phase combined 1 mL of garlic extract obtained under optimal conditions with 5 mL of simulated salivary fluid (SSF) (7.5 mg α -amylase, 25 μ L of 0.3 M $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, pH 7), agitated gently at 37 °C for 2 min. The gastric phase added 5 mL of simulated gastric fluid (SGF) (20 mg pepsin, 2.5 μ L $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, pH 3 with 6 M HCl) and continued at 50 rpm at 37 °C for 2 h. The intestinal phase added 10 mL of simulated intestinal fluid (SIF) (37.5 mg pancreatin, 40 mg bile salts, pH 7 with 1 M NaOH) and continued for 2 h. Aliquots were removed at the end of each phase, centrifuged, and stored at -24 °C.

2.8. LC-MS/MS Quantification of Polyphenolics

Phenolic extraction from digested samples was modified from a published protocol: 0.5 mL of garlic extract was obtained under optimal conditions, vortexed for 2 min, ultrasonicated for 15 min (Sonorex Super RK 100 H Ultrasonic Bath with Heater BANDELIN, Berlin, Germany, power 80 W, frequency 35 kHz), and centrifuged at 4900 rpm for 15 min. The procedure was repeated twice on the residue, and combined supernatants were stored at -80 °C.

Phenolics were analyzed by LC-MS/MS (Shimadzu LC20-AD system coupled to a 3200 QTRAP mass spectrometer, Applied Biosystems, Forester City, CA, USA) using ESI in the negative mode, with a capillary temperature of 450 °C, curtain and auxiliary gases at 0.3 MPa, and a source voltage of -4.5 kV. A Hypersil GOLD aQ column (150 $\times$ 4.6 mm, 5 μ m) was used for separation at 30 °C with 0.1% formic acid (A) and acetonitrile (B) at 1 mL/min under the following gradient: 5% B (0–3 min) $\rightarrow$ 70% B (3–26 min) $\rightarrow$ 100% B (31–36 min). The sample returned to initial conditions in 5 min. Quantification was achieved using selected reaction monitoring with calibration curves ($R^2 > 0.998$, $n = 6$), and limits of detection and quantification were within the 0.0005–0.008 $\mu\text{g/mL}$ range.

2.9. Statistical Analysis

All experiments were performed in triplicate. Means $\pm$ SDs were compared using a one-way ANOVA and Tukey's post hoc test using SPSS 20.0; significance was set at $p < 0.05$.

3. Results and Discussion

Thirty CCD runs (six central replicates) were randomized and carried out in triplicate (Supplementary Material Table S1). Backward elimination reduced quadratic models for TPC and TFC, retaining only the statistically significant ($p < 0.10$) linear, interaction, and quadratic terms.

The analysis of variance (ANOVA) results approximated the model's quality and suitability (Table 2). The significance of the predicted extraction yield was substantially governed by linear, interaction, and quadratic effects and can therefore be predicted using a quadratic equation. To obtain a simple and logical model, the backward rejection process was used to eliminate irrelevant terms. In Table 2, the statistical information derived from ANOVA was shown for reduced models.

For TPC (Y_1), the reduced model yielded $R^2 = 0.9721$, adjusted $R^2 = 0.9648$, predicted $R^2 = 0.9517$, and C.V. = 4.50%, values that indicate a strong fit and reliable reproducibility [35]. Insignificant lack of fit ($p = 0.12$) confirmed that the model's residual variance could not be attributed to noise. The model F-value (133.60, $p < 0.00001$) confirmed statistical significance of the overall regression, with significant terms being X_1 , X_2 , X_4 , $X_1 \times X_4$, X_1^2 , and X_2^2 . Adequate precision was 37.71 (where >4 is desirable).

Table 2. ANOVA results for the fitted quadratic polynomial model of extraction of phenolics and flavonoids.

Source	Y ₁ : TPC (mg GAE/g)	Y ₂ : TFC (mg QUE/g)
Model	Significant	Significant
Intercept (β_0)	2.01	19.57
Linear terms		
X_1 (β_1)	−0.14	−3.08
X_2 (β_2)	−0.087	−1.51
X_3 (β_3)		2.82
X_4 (β_4)	0.23	0.34
Quadratic terms		
X_1^2 (β_{11})	0.30	−1.96
X_2^2 (β_{22})	−0.84	−3.84
X_3^2 (β_{33})		−3.53
X_4^2 (β_{44})		−0.068
Interaction terms		
X_1X_2 (β_{12})		−0.25
X_1X_3 (β_{13})		−0.014
X_1X_4 (β_{14})	−0.051	0.98
X_2X_3 (β_{23})		−0.19
X_2X_4 (β_{24})		0.25
X_3X_4 (β_{34})		−1.26
Lack of fit	0.12	7.10×10^{-3}
Pure error	7.96×10^{-3}	3.41×10^{-3}
C.V.%	4.50	0.19
F-value probability	4.32	1.04
R ²	0.9721	0.9999
Adj. R ²	0.9648	0.9991
Predicted R ²	0.9517	0.9925
Adequate precision	37.710	977.282

TPC—total phenolic content; GAE—gallic acid equivalent; TFC—total flavonoid content; QUE—quercetin equivalent; C.V.—coefficient of variation. X_1 —extraction time; X_2 —extraction temperature; X_3 —ethanol concentration; X_4 —solvent-to-solid ratio.

For TFC (Y_2), the fit was more precise ($R^2 = 0.9999$, adjusted $R^2 = 0.9991$, predicted $R^2 = 0.9925$, C.V. = 0.19%), and lack of fit was also insignificant ($p = 7.10 \times 10^{-3}$). Significant terms included linear (X_1 , X_2 , X_3), quadratic (X_1^2 , X_2^2 , X_3^2 , X_4^2), and interaction terms (X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_2X_4 , X_3X_4), where adequate precision was 977.28.

Two-dimensional quadratic profiles can be used to recognize the interaction. 2D response surface diagrams, with contours plotted in Figure 1, are a graphical representation of the correlation between independent and dependent variables.

TPC recovery rose with solvent-to-solid ratios up to ~20 mL/g and with extraction time in a hyperbolic manner, peaking between 2.5 and 4.5 min. TFC similarly increased with time from 1 to 3 min and with temperature from 45 to 70 °C and then declined at higher values, which is consistent with the thermal degradation of flavonoids above ~80 °C [39]. TFC showed a sigmoid increase with ethanol concentration (peak near 80%), reflecting the favorable solubility of flavonoids in aqueous ethanol, and was significantly affected ($p < 0.01$) by interactions between extraction time, temperature, ethanol concentration, and solvent-to-solid ratio.

The Derringer–Suich desirability function [36], calculated using the following equation, was applied to maximize both responses simultaneously:

$$D = (d_1 \times d_2 \times \dots \times d_n)^{1/n}$$

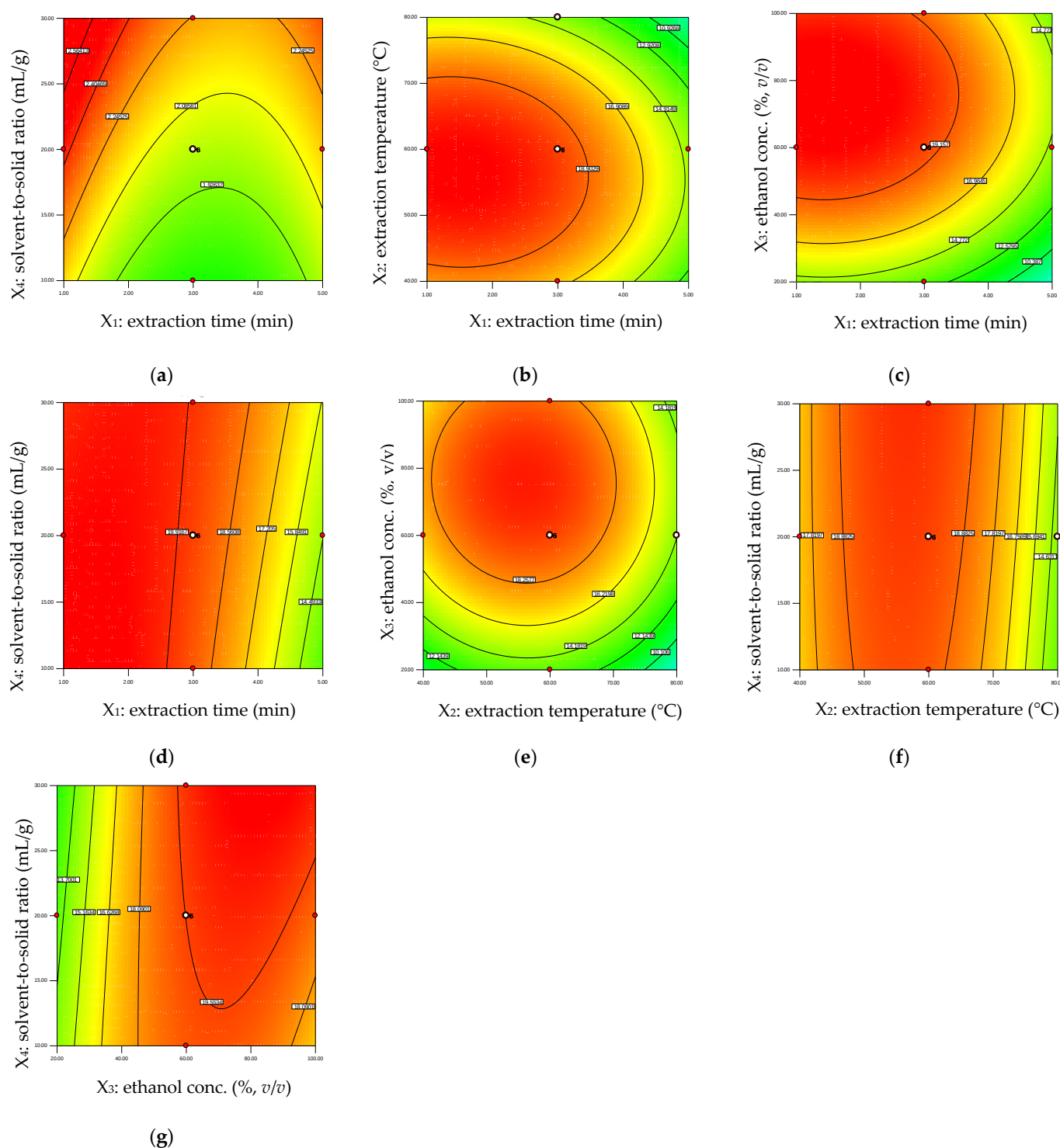


Figure 1. Contour plots showing the effect of independent variables under optimal conditions of MAE on total phenolic content (a) and total flavonoid content (b–g).

A D value greater than zero indicates that all responses are within the desirable range simultaneously, while a D value nearly equal to 1 indicates that the arrangement of the various standards is globally optimal, resulting in response values that are close to the target values presented in Figure 2.

Under optimal conditions, the higher extraction yield of phenolic compounds may be attributed to optimized extraction parameters, which improve solvent penetration, mass transfer, and the solubilization of phenolic compounds while minimizing their degradation. Garlic, at full maturity, is a composite plant of cellulosic microcomponents composed of

lignin and guarded by pectin. The majority of phenolic compounds are covalently linked to polysaccharides in the cell wall. Table 3 displays the outcomes of experiments conducted under optimal extraction conditions using a microwave.

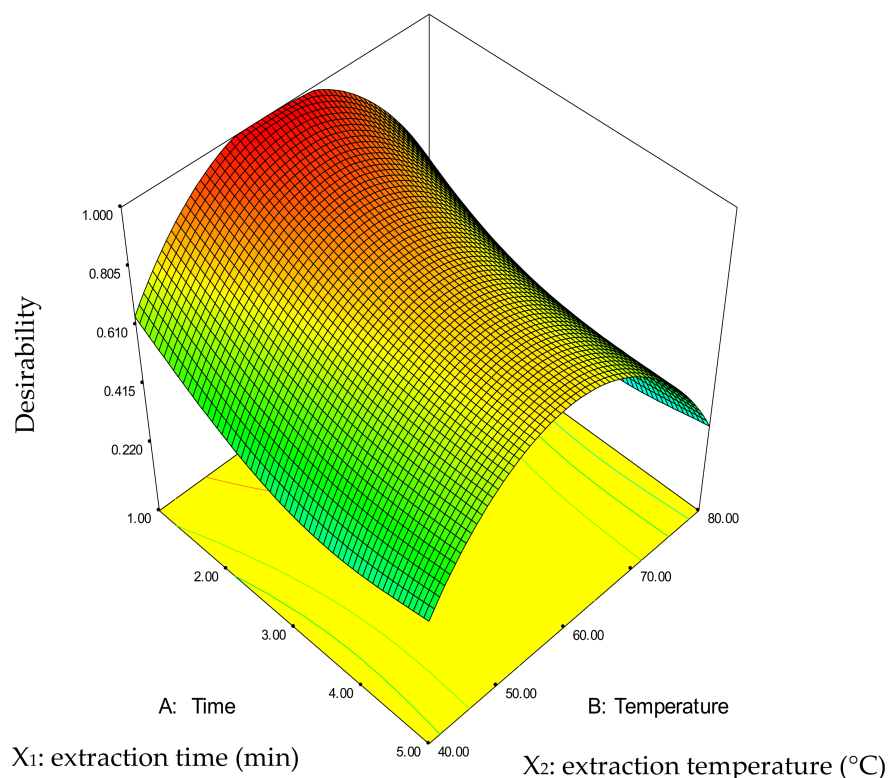


Figure 2. 3D response surface plots showing desirability.

Table 3. The predicted (RSM and ANN) and experimental values of responses at optimum conditions.

	Predicted Values (RSM)	Predicted Values (ANN)	Experimental Values	RSD (%) (RSM)	RSD (%) (ANN)
Time (min)	1.47	1.49	1.50	1.43	0.67
Temperature (°C)	55.38	55.15	55.00	0.49	0.27
Ethanol concentration (%)	74.94	75.02	75.00	0.06	0.03
Solvent-to-solid ratio (mL/g)	29.17	28.96	29.00	0.41	0.14
TPC (mg GAE/g)	2.508	2.501	2.487	0.59	0.56
TFC (mg QUE/g)	21.406	21.387	21.356	0.17	0.14

TPC—total phenolic content; TFC—total flavonoid content; GAE—gallic acid equivalent; QUE—quercetin equivalent; RSM—response surface methodology; ANN—artificial neural network; RSD—relative standard deviation.

The global optimum was predicted at 1.47 min, 55.38 °C, 74.94% ethanol, and 29.17 mL/g, with predicted TPC = 2.508 mg GAE/g FW and TFC = 21.406 mg QUE/g FW. For practical operation, conditions were rounded to 1.50 min, 55 °C, 75% ethanol, and 27 mL/g. Validation experiments under these conditions produced TPC = 2.487 mg GAE/g FW and TFC = 21.356 mg QUE/g FW, with relative standard deviations of 0.59% and 0.17%. Predicted and experimental values did not differ significantly by paired *t*-test ($p > 0.05$), confirming model adequacy. The anticipated response values differed marginally from the observed values. A strong correlation between actual and predicted results indicated that the regression model's response was adequate to replicate the predicted optimization. This investigation revealed cumulative trends that are consistent with the findings of Zhang et al. [41].

3.1. ANN Simulation

The ANN was trained on the same CCD data set as the RSM so that the two models could be directly compared. The 4-10-2 MLP converged to RMSE = 0.0001 (training), 0.6574 (validation), and 0.7810 (testing), and $R = 0.9999$, 0.9974 , and 0.9939 , respectively; these values exceeded the R^2 of the RSM polynomial. The implicit advantage of an ANN is its capacity to encode nonlinear effects without the user-defined ordering of polynomial terms required by RSM.

The global optimum was predicted by the ANN at 1.49 min, 55.15 °C, 75.02% ethanol, and 28.96 mL/g, with predicted TPC = 2.501 mg GAE/g FW and TFC = 21.387 mg QUE/g FW. Predicted and experimental values did not differ significantly according to the paired t -test ($p > 0.05$), confirming model adequacy.

The other key distinction is flexibility toward experimental data: an ANN tolerates informal designs and uses all data points, whereas RSM prunes insignificant terms at a preset threshold. As a result, the ANN modestly outperformed the RSM in the present case but at the cost of reduced interpretability.

Although RSM successfully identified the optimal extraction conditions and quantified the effects of the process variables, the ANN model provided additional value by capturing more complex nonlinear relationships among the input variables. As a result, ANN achieved higher predictive accuracy, as reflected by improved performance metrics, thereby enhancing the reliability of the predicted extraction yield. While RSM offers a statistically interpretable model and facilitates a better understanding of factor interactions, an ANN complements these findings by providing a more robust prediction of the response under both optimal and varying process conditions. Thus, the combined use of RSM and ANNs enables both process interpretation and improved predictive capability.

3.2. Total Phenolic, Flavonoid, and DPPH—Antioxidant Content Across Cultivars

TPC and TFC were highly variable among the garlic origins examined. Serbian “Bosut” garlic had the highest values (TPC 2.501 mg GAE/g FW; TFC 21.32 mg QUE/g FW), followed by garlic with Spanish (1.991; 19.657) and Chinese origins (1.02; 17.452). These cultivar rankings are consistent with previous reports: Korean local cultivars grown near Namhae-gun were reported at 17.86 mg/kg dry matter for total phenolic acids and 29.95 mg/kg for total flavonoids [42]; Spanish Andalusian garlic ranged from 3.4 to 10.8 mg GAE/g [2]; Nagella et al. [4] reported TPC of 33.50–49.89 mg GAE/g across Korean origins, with Hampyeong being the highest and Namdo being the lowest; and aged or differently processed garlic ranged from 5.63 [2,43] to 15.23 mg GAE/g fresh weight [44]. Even within *Allium* species, TPC as low as 0.075–0.080 mg GAE/g has been documented [45], and bulb/bolt TFC was reported at 0.11–0.59 and 0.075–0.67 mg rutin/g FW [3], underscoring the broad range driven by genetics, environment, and processing.

For consistency, only studies reporting results in equivalent units of measurement and on the same basis (e.g., mg GAE g^{−1} dry weight or mg GAE g^{−1} dry extract) were quantitatively compared. Where differences in reporting units or analytical methodologies prevented direct comparison, the results were interpreted qualitatively.

All extracts showed greater antioxidant activity than BHT ($p < 0.01$) but lower than ascorbic acid ($p > 0.05$). Figure 3 shows the effect of phenolic compounds, vitamin C, and BHT on the scavenging of DPPH radicals.

Serbian “Bosut” exhibited an IC₅₀ of 74 µg/mL (not significantly lower than Spanish garlic at 63 µg/mL; $p > 0.05$), and Chinese garlic required 141 µg/mL. Reported IC₅₀ values for Korean cultivars ranged from 95.04 to 166.92 µg/mL [4]; Szychowski et al. [46] reported IC₅₀ values of 4.63 µg/mL and 4.88 µg/mL for Chinese and Portuguese garlic, respectively, with values for Polish “Harnas” (6.52 µg/mL) and Spanish “Castano” (7.59 µg/mL) trailing

closely behind. The ranking in radical scavenging correlates with TPC, supporting the role of phenolics as the principal contributors to antioxidant capacity in garlic.

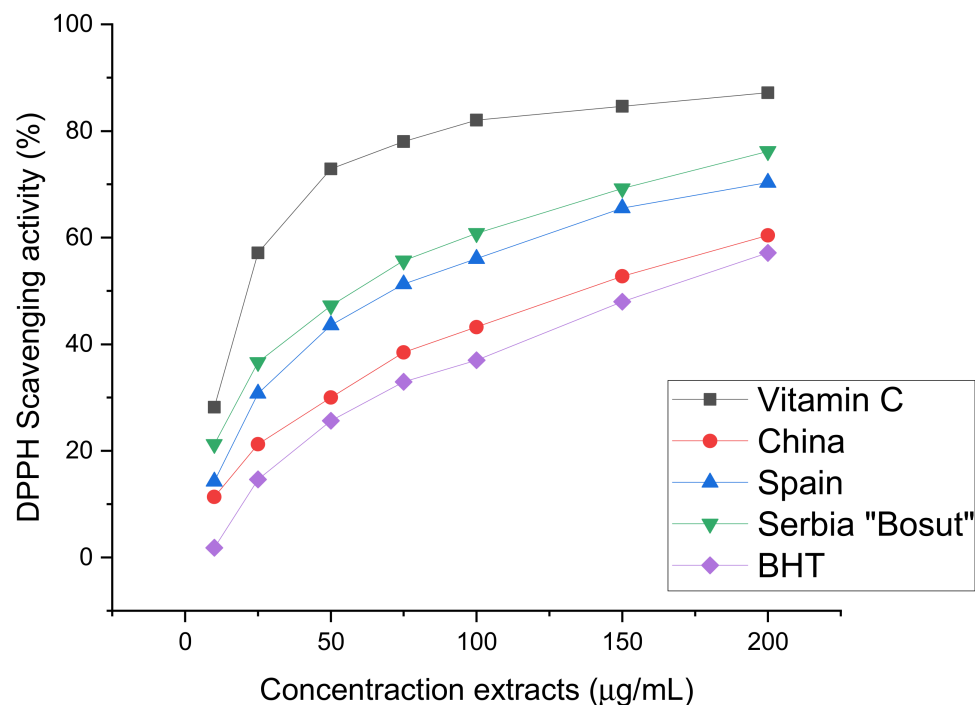


Figure 3. DPPH scavenging activity (%) of garlic with different origins.

Although several studies have reported lower IC_{50} values than those obtained in the present study, direct comparisons should be made with caution. The antioxidant activity of plant extracts is highly dependent on factors such as the extraction technique, solvent composition and polarity, extraction conditions (temperature, time, and solvent-to-solid ratio), plant material characteristics, and the specific conditions of the DPPH assay. Consequently, variations in IC_{50} values among studies in the literature do not necessarily reflect differences in the intrinsic antioxidant potential of the extracts but may instead result from methodological differences. Therefore, the present results should be interpreted within the context of the extraction procedure and analytical conditions employed.

3.3. Polyphenolic Profile by LC-MS/MS

The LC-MS/MS study provided the polyphenolic profile of an extract obtained under ideal conditions (Figure 4). Figure 4 demonstrated the influence of gastrointestinal digestion on the polyphenolic content of fresh garlic extracts. The results revealed a significant reduction in phenolic content during in vitro digestion of the garlic sample.

The extract produced under optimal conditions contained, in total, 308.1 mg/kg of identified phenolic and polyphenolic compounds. Gallic acid was the dominant constituent (92.3 mg/kg), followed by chlorogenic acid (82.12 mg/kg) and naringenin (49.2 mg/kg). Comparable magnitudes of chlorogenic acid (e.g., 52.55 mg/100 g dry matter) have been reported elsewhere [47]. Nagella et al. [4] similarly found gallic acid to be dominant (67.40–196.8 mg/kg) with quercetin at 25.70–56.65 mg/kg and rutin at 51.35–90.45 mg/kg across Korean agroecologies [4].

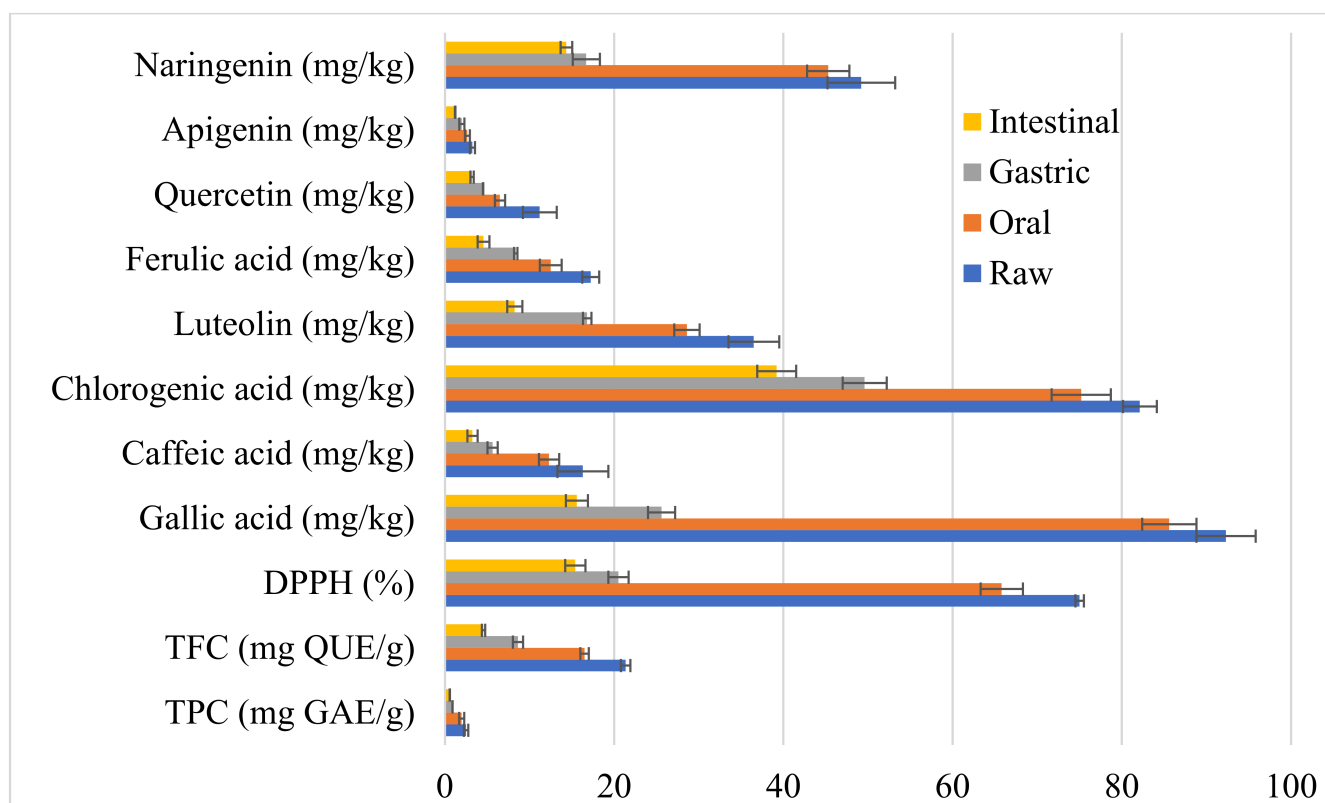


Figure 4. Amounts of total and individual phenolic compounds during in vitro gastrointestinal digestion.

3.4. In Vitro Gastrointestinal Digestion

Simulated digestion affected polyphenols differently at each phase. TPC declined significantly ($p < 0.05$) across digestion, with the lowest values in the intestinal phase and the highest in the gastric phase. Similarly, TFC bioavailability peaked after the gastric phase, with a pronounced drop during intestinal digestion; these observations are in line with results for carob flour [48], pomegranate peel [30], wild blueberry [29], and strawberry grape [49].

Limited solubility in saliva and the short oral phase account for the restricted TPC/TFC release observed initially. Subsequent losses have been attributed to physicochemical transformations (oxidation, complexation with polysaccharides, and protein–phenolic binding) [48,50] and to pH-driven instability of phenolic acids in the basic intestinal environment [28,51]. Gallic acid, chlorogenic acid, and naringenin remained relatively available after oral digestion (85.6, 75.2, and 45.3 mg/kg, respectively), whereas caffeic acid was hardly affected by the oral phase; this is likely due to a matrix effect, as garlic proteins, carbohydrates, and fiber modulate bioaccessibility [28,31].

After gastric digestion, gallic acid and naringenin showed the largest absolute decreases (to 25.6 and 16.7 mg/kg), and caffeic acid declined slightly (5.63 mg/kg). Intestinal digestion produced the largest losses overall: recovery rates during the intestinal phase were 16.9% (gallic acid), 19.9% (caffeic acid), and 22.6% (luteolin).

These findings confirm that the polyphenol content of fresh garlic, dominated by phenolic acids, is poorly stable under intestinal conditions, and that any industrial nutraceutical use of garlic extracts must account for matrix or formulation strategies to protect labile phenolics through gastrointestinal transit.

4. Conclusions

A four-factor, three-level CCD combined with desirability function optimization provided an MAE method that maximized TPC and TFC from garlic. The ANN 4-10-2 MLP achieved even higher correlations and lower RMSE, demonstrating its complementary value for nonlinear process modeling.

Beyond extraction yield, this work quantified for the first time the polyphenolic profile under optimized MAE and tracked individual polyphenols through simulated digestion. The results show that high extraction efficiency does not translate directly into high bioaccessibility: total and individual polyphenols, particularly gallic acid, caffeic acid, and luteolin, suffered substantial losses during simulated intestinal digestion (recoveries < 25%).

The MAE method is fast, energy-efficient, solvent-economical, and environmentally benign, qualifying it as a scalable green alternative to conventional extraction. However, the gap between extraction yield and intestinal bioavailability underscores the need for follow-on encapsulation or co-formulation strategies (e.g., encapsulation, microemulsions, or co-delivery with protective matrices) before garlic polyphenol extracts can be effectively deployed in nutritional or pharmaceutical applications.

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Abbreviations

The following abbreviations are used in this manuscript:

TPC	Total phenolic content
TFC	Total flavonoid content
GAE	Gallic acid equivalent
QUE	Quercetin equivalent
RSD	Relative standard deviation
ANN	Artificial neural network
RSM	Response surface methodology
FW	Fresh weight
LC-MS/MS	Liquid chromatography coupled with mass spectrometry
DPPH	1,1-diphenyl-2-picrylhydrazyl
BHT	Butylated hydroxytoluene
CCD	Central Composite Design

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