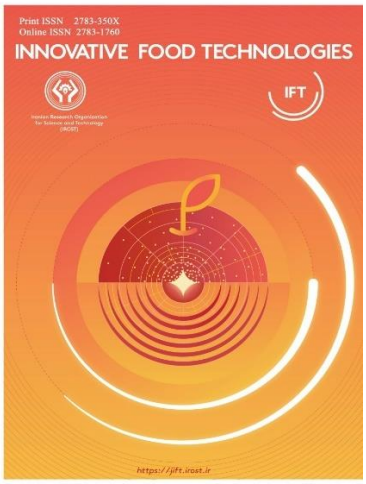
Journal Pre-proofs



Optimized Extraction of Phenolic Compounds from Pistachio (Pistacia vera L.) Green Hull Using Ultrasound-Assisted Extraction: A Response Surface Methodology Analysis

Nooshin Noshirvani, Amir Daraei Garmakhany, Mehran Banibayat

DOI: https://doi.org/ 10.22104/ift.2026.8443.2317

To appear in: Innovative Food Technologies (IFT)

Received Date: 1 August 2026
Revised Date: 21 October 2026
Accepted Date: 8 November 2026

Please cite this article as Nooshin Noshirvani, Amir Daraei Garmakhany, Mehran Banibayat, Optimized Extraction of Phenolic Compounds from Pistachio (Pistacia vera L.) Green Hull Using Ultrasound-Assisted Extraction: A Response Surface Methodology Analysis, *Innovative Food Technologies (2026)*, doi: https://doi.org/ 10.22104/ift.2026.8443.2317

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2026 The Author(s). Published by irost.org.

Journal Pre-proof

Optimized Extraction of Phenolic Compounds from Pistachio (*Pistacia vera L.)* Green Hull Using Ultrasound-Assisted Extraction: A Response Surface Methodology Analysis

Nooshin Noshirvani[1*], Amir Daraei Garmakhany[1*], Mehran Banibayat[1]

1- Department of Food Science and Technology, Faculty of Engineering and Natural Resources, Bu-Ali Sina University, Hamedan, Iran.

Corresponding Authors:
n.noshirvani@basu.ac.ir
amirdaraey@basu.ac.ir

ABSTRACT

This study investigates the optimization of polyphenol extraction from pistachio green hulls (PGHs), a by-product rich in health-promoting phenolic compounds, utilizing Ultrasound-Assisted Extraction (UAE) and Response Surface Methodology (RSM). The effects of extraction time (60, 210, 360 minutes), temperature (40, 50, 60 ℃), and ethanol concentration (0%, 50%, 100% v/v) on the yields of total phenolic compounds (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity were evaluated. The correlation coefficients (R^2) for the models were 0.9666 (TPC) and 0.9386 (TFC), indicating high predictive ability. The DPPH radical-scavenging activity model, however, was not statistically significant ($R^2 = 0.5934$; $p = 0.6312$) and was therefore not used as a standalone predictive response for optimization; instead, DPPH radical-scavenging activity was included as a supplementary experimental response, while the optimization was based primarily on TPC and TFC. Therefore, the Central Composite Design (CCD) enabled the identification of optimal conditions based primarily on TPC and TFC: 60 min, 54.07 °C, and 43.33% ethanol (v/v), with predicted values of 7.175 mg GAE/g (TPC) and 4.003 mg CATCHIN/g (TFC). The corresponding DPPH radical-scavenging activity value (78.560%) was recorded as a supplementary experimental observation rather than a model-based prediction. For practical verification, the condition was rounded to 60 min, 54 °C, and 43.33% ethanol, under which experimental TPC, TFC, and DPPH radical-scavenging activity values reached 7.9 mg/g, 4.4 mg/g, and 80.5%, respectively, exceeding yields obtained through single-variable extraction trials. These findings highlight PGHs as a promising source of bioactive compounds and support the application of UAE-RSM as an efficient approach for valorizing agricultural by-products. However, the weak DPPH radical-scavenging activity model indicates that complementary antioxidant assays are needed in future studies.

Keywords: Sustainable Processing, Food Waste, Green Extraction, Bioactive Compounds, Agro-industrial by-products

Journal Pre-proofs

1. Introduction

Pistachio green hull (PGH), the outer green layer of pistachio fruit (*Pistacia vera* L.), constitutes 40% to 60% of the fresh fruit weight and represents one of the major by-products generated during pistachio processing (Ordoñez-Cano et al., 2025). Although PGH is commonly regarded as an agricultural residue, it contains considerable amounts of phenolic acids, flavonoids, and other polyphenolic constituents (Noshirvani et al., 2015; Noshirvani et al., 2017; Noshirvani et al., 2024). These compounds are characterized by phenolic rings and can be classified into phenolic acids, flavonoids, lignans, and stilbenes (Noshirvani et al., 2019; Abbas et al., 2017). Their antioxidant activity is mainly attributed to the ability to donate electrons and form relatively stable phenoxyl radicals, which enables them to interrupt oxidative chain reactions (Noshirvani & Abolghasemi Fakhri, 2025). Accordingly, PGH represents a promising source of natural antioxidants and value-added functional ingredients. The recovery of phenolic compounds from PGH can therefore provide an effective strategy for valorizing pistachio-processing residues and generating value-added products. However, the efficiency of phenolic recovery is strongly influenced by extraction conditions, including solvent composition, temperature, extraction time, and the physical characteristics of the plant matrix (Lee et al., 2020; Rajaei et al., 2010).

The recovery of phenolic compounds from plant materials is closely related to the extraction method employed. Conventional extraction techniques, such as maceration and Soxhlet extraction, are often associated with long extraction times and relatively high consumption of solvents and energy, which can limit their efficiency and sustainability (Khurramov, et al., 2024). Consequently, several alternative extraction technologies have been developed, including accelerated solvent extraction (ASE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) (Brglez Mojzer et al., 2016). Among these techniques, UAE has emerged as an effective approach for recovering phenolic and other bioactive compounds from plant materials (Gouda et al., 2021). The effectiveness of UAE is primarily associated with acoustic cavitation, acoustic streaming, and disruption of plant cell structures. High-frequency ultrasonic waves promote the formation, growth, and collapse of microscopic bubbles within the extraction medium, generating localized shear forces, microturbulence, and energy that can disrupt plant tissues and improve solvent penetration (Abi-Khattar et al., 2022; Rombaut et al., 2021). Through these mechanisms, UAE can improve extraction efficiency and promote the recovery of polyphenols while reducing extraction time and solvent consumption compared with conventional extraction approaches (Chemat et al., 2017; Azmir et al., 2013). Moreover, the ability of UAE to improve extraction efficiency with relatively lower resource requirements makes it compatible with the principles of sustainable and green extraction (Dimitrov et al., 2019).

Several studies have investigated the phytochemical composition and biological potential of PGH. Goli et al. (2005) were among the first to report that aqueous extracts of PGH possess high phenolic content and notable antioxidant activity comparable to recognized phenolic sources (Nateghi and Hosseini., 2023). Rajaei et al. (2010) subsequently applied response surface methodology (RSM) to optimize UAE of PGH, achieving a high coefficient of determination ($R^2 \approx 0.95$) for total phenolic yield across temperatures of 25–65 °C and sonication times up to 45 min. More recent studies have further characterized the phenolic profile of PGH. Seifzadeh et al. (2019) identified 34 polyphenolic compounds in PGH extract using UHPLC/MSn, including galloylshikimic acids, gallic acid, theogallin, galloyl-O-hexoside, quercetin-O-hexoside, and pyrogallol, with eight of these compounds reported for the first time in pistachio

hulls. Noorolahi et al. (2020) demonstrated that the tannin-enriched fraction of PGH exhibited higher antioxidant activity than the crude extract and also showed pancreatic lipase inhibitory effects. Additionally, Ordoñez-Cano et al. (2025) reported that solid-state fermentation of PGH with *Aspergillus niger* GH1 increased total phenolic content and antioxidant capacity by 129% and 1039%, respectively.

RSM, initially introduced by Box and Wilson (1992), is a mathematical and statistical approach that can be used for process development, optimization, and evaluation of the effects and interactions of experimental variables (Myers & Montgomery, 2002). RSM has been successfully applied to the optimization of biochemical and food-related processes, including the extraction of bioactive compounds from plant materials (Azarifar et al., 2019). However, previous optimization studies on PGH have primarily focused on total phenolic yield or individual compound characterization, while simultaneous optimization of multiple quality responses—specifically total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity—remains comparatively limited (Imtiaz et al., 2025; Bannour et al., 2026) Furthermore, the effects of extraction temperature and sonication time on these multiple responses have not been systematically evaluated for PGH within a unified optimization framework. Increasing temperature and sonication time does not necessarily result in greater recovery of bioactive compounds, as excessive thermal exposure or prolonged sonication may adversely affect thermolabile phenolic constituents. Therefore, this study aimed to optimize UAE conditions for the recovery of bioactive compounds from PGH using RSM. Extraction temperature and sonication time were evaluated within practical ranges. TPC and TFC were considered as the primary response variables for optimization, while DPPH radical-scavenging activity was included as a supplementary response to provide additional information on the antioxidant potential of the extracts. It should be noted that the DPPH radical-scavenging activity response was not used as a standalone predictive response for optimization due to the complexity of antioxidant reactions in multi-component phenolic extracts. Previous studies, including Rajaei et al. (2010), have applied RSM to optimize phenolic extraction from PGH. The present study builds on this work by extending the optimization to multiple responses (TPC and TFC) within a unified RSM framework, while reporting DPPH radical-scavenging activity as a supplementary experimental response. Rather than claiming methodological novelty, this study aims to provide a systematic characterization of UAE behavior for this specific waste stream and to identify a practical, shorter extraction condition. This integrated optimization provides a practical basis for the efficient valorization of PGH into phenolic-rich extracts with potential food and other value-added applications. The extraction time range of 60–360 min was included in the experimental design to characterize the time-dependent and nonlinear behavior of phenolic and flavonoid recovery across a broad design space. Importantly, the 360-min treatment was considered only an extended experimental condition for evaluating the response surface and was not intended to represent a practically preferred UAE condition. Since prolonged sonication may increase energy consumption and the risk of thermal degradation, shorter extraction times are generally more desirable for practical and scale-up applications. In the present study, the multi-response optimization ultimately identified 60 min as the optimal extraction time, demonstrating that effective recovery of bioactive compounds could be achieved without prolonged sonication. Importantly, the extended extraction time of 360 min was included only as the upper experimental boundary of the response surface design to characterize the time-dependent and potentially nonlinear extraction behavior, rather than as a practically advantageous UAE condition. The main objective was to identify a shorter and practically relevant extraction time

that could maximize the recovery of phenolic compounds and antioxidant activity while minimizing prolonged sonication, energy consumption, and potential thermal degradation.

Material and methods

2.1 Plant materials

Fresh pistachios (*Pistacia vera*) with green hulls were purchased from a local market in Kermanshah Province, Iran.

2.2 Sample preparation

The hulls were manually removed from the pistachio nuts and dried at ambient temperature (20-25 ℃) until a constant weight was achieved. The dried pistachio green hulls were then ground into a fine powder using an electric grinder and passed through a 40-mesh sieve (approximately 425 µm) to obtain a relatively uniform particle size. The prepared powder was stored at 4 °C until extraction.

2.3 Chemical reagents

The compounds used in this study 1,1-diphenyl-2-picrylhydrazyl (DPPH), aluminum chloride, potassium acetate, sodium carbonate and Folin–Ciocalteu phenol reagent were all of analytical grade, obtained from the EMSURE® manufactured by the chemical company Merck & Co (Darmstadt, Germany). Ethanol (analytical grade) was obtained from Merck (Darmstadt, Germany). Ethanol–water mixtures were prepared at the required concentrations on a volume/volume (v/v) basis, with ethanol concentration expressed as the percentage of ethanol volume in the total solvent volume. Thus, 0% (v/v) ethanol corresponded to distilled water, whereas 100% (v/v) ethanol corresponded to pure ethanol. Intermediate concentrations represented the corresponding volume percentage of ethanol in the ethanol–water solvent system.

2.5 UAE system and experimental procedure

UAE of PGH was performed using a rectangular ultrasonic bath (Sonica®, Model 2200 ETH, 40 kHz, Italy) with an open top and internal dimension of 24 × 14 × 10 cm. The bath had a nominal average power of 130 W and a maximum peak power of 260 W and was equipped with two piezoelectric transducers and a 175 W heating element. The stainless-steel tank was filled to a working volume of approximately 2.35 L. The ultrasonic amplitude and cavitation intensity were factory-fixed and could not be adjusted by the operator; therefore, no specific amplitude value was available from the manufacturer. The reported power values represent the nominal specifications of the equipment, as the actual acoustic power delivered to the extraction medium was not independently measured. To ensure reproducibility, the bath loading, working volume, sample volume, vessel position, solvent-to-solid ratio, and extraction configuration were kept constant throughout all experimental runs. For each extraction, 2 g of PGH powder was mixed with 10 mL of ethanol–water solvent at the concentration specified by the experimental design, corresponding to a solvent-to-solid ratio of 5:1 (mL g^{-1}). The extraction was performed at the temperature and for the duration specified by the experimental design. During sonication, the temperature of the water in the ultrasonic bath was monitored using a thermometer positioned directly in the bath water. Because sonication gradually increased the bath temperature, ice-cold water was added when necessary to maintain the bath temperature close to the target value. This procedure was applied consistently throughout the experiments to minimize temperature deviations among treatments. After extraction, the mixtures were centrifuged at 5000 rpm for 25 min using a centrifuge (Selecta Lab, Model TDZ5-WS). The supernatants were subsequently filtered through Whatman No. 1 filter paper and stored at 4 °C until biochemical analyses.

Journal Pre-proofs

2.6 Determination of TPC in PGH extracts

The TPC was measured using the method described by Shabanian et al. (2021), based on the Folin-Ciocalteau procedure. The procedure involved combining 0.5 mL of extract samples with 2.5 mL of distilled water and adding 250 microliters of Folin-Ciocalteau reagent. After keeping the mixture in a dark environment for 5 min, 2 mL of 7.5% sodium carbonate was added. After a 2-hour incubation period at room temperature, the absorbance of the reaction was evaluated by a UV–Vis spectrophotometer (A&E Lab, Model STM UV-9000, UK) at 760 nm. The findings were expressed in terms of gallic acid equivalents.

2.7 Determination of TFC in PGH extracts

The quantification of TFC was conducted using the methodology described by Woisky & Salatino (1998). The procedure involved combining 0.5 mL of the sample with an equal volume of 2% $AlCl_3$ in ethanol. Following a one-hour incubation period at ambient temperature, the absorbance was determined at 420 nm.

Journal Pre-proofs

2.8 Free radical scavenging activity

The radical scavenging ability of the PGH extracts was assessed using the 1,1-diphenyl-2-picrylhydrazyl method, as described in the work of Shabanian et al. (2021). The DPPH radical-scavenging activity was quantified using the formula:

$$\text{DPPH Scavenging Activity (\%)} = \left[\frac{\mathbf{A}_{blank}-\mathbf{A}_{sample}}{\mathbf{A}_{blank}}\right] \times 100 \qquad \text{Eq (1)}$$

where $\mathbf{A}_{blank}$ is the absorbance of the blank sample known as control and $\mathbf{A}_{sample}$ is the absorbance of the samples at 515 nm.

2.9 Experimental design

RSM was employed to determine the effects of extraction temperature, extraction time, and ethanol concentration on the recovery of phenolic compounds from PGH. The experimental design was generated using Design-Expert software (version 12) based on a central composite design (CCD) comprising 15 experimental runs. To minimize potential bias associated with the order of experiments, the experimental runs were randomized. All non-central design points were performed once, whereas five replicate experiments were conducted at the central point (210 min, 50 °C, and 50% ethanol) to estimate the experimental error and assess the reproducibility of the extraction procedure. The replicate central-point observations were included in the analysis of variance (ANOVA) and in the fitting of the response surface models. The extraction-time levels were selected to provide a broad experimental range for evaluating the time-dependent and potentially nonlinear behavior of phenolic and flavonoid recovery during UAE. Accordingly, 60 and 360 min were selected as the lower and upper boundaries of the experimental range, respectively, while 210 min was used as the central level of the response surface design. This range was informed by previous studies reporting effective UAE durations of approximately 60–120 min, as well as studies using longer extraction periods to characterize extraction kinetics and extended recovery. Rajaei et al. (2010), for example, investigated UAE of pistachio green hulls using sonication times of up to 45 min. Therefore, the broader range used in the present study was intended to evaluate whether extending sonication beyond commonly reported short-duration UAE conditions would improve extraction responses and to adequately capture potential curvature and interaction effects in the RSM model. The 360-min level was used only as the upper boundary of the design space (see Introduction). The following independent variables were evaluated using an ethanol–water solvent system (Table 1): extraction time (60–360 min), extraction temperature (40–60 °C), and ethanol concentration (0–100%, v/v). Ethanol concentration was expressed as the volume percentage of ethanol in the total ethanol–water solvent volume. Thus, 0% (v/v) ethanol corresponded to distilled water, 50% (v/v) corresponded to a 1:1 (v/v) ethanol–water mixture, and 100% (v/v) corresponded to pure ethanol. Numerical optimization using RSM was performed based on TPC and TFC. These responses were selected because their regression models were statistically significant and showed satisfactory coefficients of determination. DPPH radical-scavenging activity was measured as a supplementary experimental response; however, it was excluded from the numerical optimization because its regression model was not statistically significant ($p = 0.6312$; $R^2 = 0.5934$). Accordingly, DPPH measurements were used to describe the experimentally observed antioxidant potential of the extracts, rather than to predict or optimize antioxidant activity. As described in the Results section, the DPPH radical-scavenging activity model did not reach statistical significance and was therefore not used as a standalone predictive response for optimization. In the optimization process, these independent variables were designated as X_1, X_2, and X_3, respectively. The experimental data were fitted to a second-order polynomial model, and the corresponding

regression coefficients were estimated. A generalized second-order polynomial model (Eq. 2) was used in the response surface analysis:

$$y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} b_{ij} X_i X_j \qquad \text{Eq (2)}$$

In the Eq (2), b_0, b_i, b_{ii}, and b_{ij} represent regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively, while X_i and X_j denote independent variables.

Results and discussions

3.1 Analysis of antioxidant characteristics and phenolic compounds in PGH extracts

3.1.1 Interpretation of TPC distribution via 3-D surface plots

Extending the extraction time to 360 min allowed the time-dependent extraction response of phenolic and flavonoid compounds to be evaluated over a broad experimental range. Under the conditions investigated, prolonged treatment was associated with changes in phenolic and flavonoid recovery; however, these changes cannot be attributed exclusively to the ultrasonic effect because extended sonication may also involve cumulative thermal effects and possible changes in solvent composition due to evaporation. Ultrasound-induced cavitation and associated micro-jet effects may facilitate solvent penetration and disruption of plant cell structures, thereby promoting mass transfer. Nevertheless, at prolonged extraction times, the contribution of these effects may be accompanied by thermal and solvent-related effects, and excessive heating may also promote degradation of thermolabile phenolic compounds (Kumar et al., 2021). Therefore, the 360-min condition was considered an extended experimental condition for characterizing the overall extraction response rather than as evidence of a purely ultrasonic contribution. **Figure 1.** depicts the changing trend in total phenolic compounds. **Figure 1-A** illustrates the response surface and corresponding contour plot for TPC in relation to extraction time (A, 60–360 min) and temperature (B, 40–60 °C). At mid-range conditions (≈ 200 minutes, ≈50 °C), the blue trough, TPC reaches the lowest value (~6.3 mg GAE/g), while the highest yields (~7.4 mg GAE/g, red plateaus) are observed at the two design-space extremes: short-cool extractions (60 minutes/40 °C) or long-hot extractions (360 minutes/60 °C). Degradation of labile phenolics, re-adsorption of released compounds onto matrix components, or decreased solubility of specific subclasses at mid-range solvent compositions can all contribute to the decrease in TPC under some intermediate conditions. These non-linear effects, which reflect intricate kinetics between release, solubilization, and degradation, have been noted in UAE studies (Jurinjak Tusek et al., 2018; Tan Quoc & Nguyen, 2022). The interaction diagram in **Figure 1-A** further illustrates the combined effect of extraction time and temperature on TPC. The non-parallel and crossing trends of the response lines indicate that the effect of extraction time depends on the extraction temperature, confirming an interaction between these two factors. At lower temperature, increasing the extraction time produces a different change in TPC compared with that observed at higher temperature. This interaction is consistent with the response-surface and contour plots, which show that the effect of extraction time is not uniform across the investigated temperature range. The significant time × temperature interaction obtained from ANOVA ($p < 0.01$) statistically supports this observed dependence between the two factors.

Fig 1-A

Figure 1-B depicts the response surface, corresponding contour plot, and interaction diagram for TPC as a function of extraction time and solvent concentration, closely resembling the saddle-shaped topology depicted in Figure 1-A. Importantly, the surface features a saddle point under mid-range circumstances (~180–240 min, 40–60% solvent), where TPC decreases to approximately 6.4 mg GAE/g; this is not a true minimum, but rather a point of lower yield

relative to the adjoining ridges, which correspond to maximal phenolic yields (~7.2–7.4 mg GAE/g) under two design-space extremes: short time/low solvent (60 min, 20–30%) and long time/high solvent (360 min, 80–100%). The curvature of the isoclines confirms a strong interaction effect: adding solvent boosts yield greatly in short periods of time, but decreases returns over longer periods unless time is extended correspondingly. This can be related to the decreased polarity of pure ethanol compared to a combination of water-ethanol, as polar solvents such as water have a stronger capacity to remove phenolic compounds from the cellular structure of plants (Gharekhani et al., 2010). Chan et al (2016), reported that the presence of small amounts of water in the extraction solvent enhances its ability to diffuse into plant tissues. This diffusion allows for more effective heating of the plant materials, thereby improving the efficiency of active component transfer into the solvent. The interaction diagram further clarifies this relationship by showing the effect of solvent concentration at different extraction times. At short extraction times (e.g., 60 min), increasing solvent concentration from 0% to approximately 40–60% markedly improves TPC; however, at longer extraction times (e.g., 360 min), the TPC response becomes less dependent on solvent concentration, and the curves tend to converge. This indicates that the positive effect of solvent concentration is more pronounced at shorter extraction times, whereas prolonged sonication partially compensates for lower solvent concentrations. The crossing pattern of the interaction lines confirms that the two variables do not act independently, supporting the significant time × solvent interaction detected in the ANOVA ($p < 0.01$).

Fig 1-B

Figure 1-C demonstrates the response surface and corresponding contour plot for TPC plotted against extraction temperature (B: 40-60 °C) and ethanol concentration (C: 0-100 % v/v). It closely echoes the saddle-shaped topology in **Figures 1-A** and **1-B**. The mid-range region (≈50 °C, 40-60 % solvent) has a saddle point where TPC drops into the trough (~6.4 mg GAE/g). This is not a local minimum, but rather a point with decreased yield relative to the ridge-based extremes. Both extraction regimes produce maximal TPC (~7.2–7.6 mg GAE/g), with the opposing ridges appearing under either hotter condition with high solvent concentration or shorter, cooler extractions with moderate solvent. Furthermore, the contour projection displays curved isoclines, demonstrating that while increasing temperature improves extraction at low solvent concentrations, it diminishes improvements at higher solvent levels unless concentration is also increased. This temperature-dependent behavior suggests that the beneficial effect of increasing extraction temperature on TPC is strongly influenced by solvent composition, with the combined use of moderate temperatures and intermediate ethanol concentrations providing more favorable conditions for phenolic recovery than increasing temperature alone (Espín et al., 2000). The interaction diagram further clarifies the combined effects of extraction temperature and ethanol concentration on TPC. The non-parallel and intersecting trends of the lines indicate that the effect of extraction temperature depends on the solvent concentration. At lower ethanol concentrations, increasing the extraction temperature generally enhances TPC, whereas at higher ethanol concentrations, the response to increasing temperature becomes less pronounced and tends to decline at the upper temperature range. The convergence and crossing of the interaction lines at approximately 50–55 °C indicate that temperature and solvent concentration do not act independently. This observation is consistent with the significant temperature × solvent concentration interaction identified by the ANOVA ($p < 0.01$), confirming that the effect of temperature on phenolic recovery is dependent on solvent composition. Subsequently, water-based extracts typically exhibit the lowest levels of TPC, primarily due to the high polarity of

water, which limits its ability to effectively dissolve non-polar or moderately polar phenolic compounds. The strong hydrogen bonding network of water further restricts its interaction with certain hydrophobic phenolic molecules, resulting in reduced extraction efficiency. Despite this limitation, the presence of water significantly impacts the ultrasonic extraction process. Water's lowered vapor pressure and viscosity intensify ultrasonic cavitation, enhancing the formation and subsequent collapse of cavitation bubbles. This creates localized high-pressure and high-temperature conditions that facilitate the breakdown of plant cell walls. Consequently, while water alone may not be an optimal solvent for maximizing the extraction of TPC, its role in modulating the cavitation process contributes to the overall efficiency of UAE techniques when used in combination with other solvents. Table 2 presents the ANOVA results for the fitted models, whereas Table 3 summarizes the critical statistical diagnostics used to evaluate their adequacy and predictive performance for TPC, TFC, and DPPH scavenging activity of PGH throughout the design space. The fitted model explained 96.7% of the response variability for TPC, according to the overall model, which was significant ($p = 0.0035$) and had a high coefficient of determination ($R2 = 0.9666$). TPC was significantly influenced by the following main effects: solvent concentration (X3, $p = 0.0194$), extraction temperature (X2, $p = 0.0014$), and extraction time (X1, $p = 0.0330$). The quadratic effect of solvent concentration ($X3^2$, $p = 0.0007$) and significant two-way interactions (X1×X2, X1×X3, X2×X3; all $p < 0.01$) further supported the finding that TPC varied significantly across the factor levels. The non-significant lack of fit ($p = 0.9643$) supports the adequacy of the model.

As a result, the 3D surface plots and contour plots (**Figures 1A-C**) show statistically significant variations in TPC throughout the design space. Further research is needed to explore the synergistic effects of water and other solvents in optimizing the extraction of a broad range of phenolic compounds from various plant sources (Esmaeilzadeh Kenari et al., 2014). In addition, water-based extraction contains large amounts of impurities such as organic acids, proteins and soluble sugars, which are detectable and interfere with the determination of the TPC (Chirinos et al., 2007).

In fact, our findings are consistent with the results reported in the literature Sirijeerachai et al. (2021), Martínez-Ramos et al. (2020), Teo et al. (2010), Thoo et al. (2010), which suggest that the use of binary ethanol-water systems is more effective for extracting phenolic compounds than using either solvent alone.

Equation 3. represents the quadratic equation of the statistical model used to calculate the quantity of total phenolic compounds in PGH extracts. The variables A, B, and C in this equation represent the extraction time (minute), temperature (°C), and solvent concentration (v/v%).

$$Y1 = 7.10 + 0.0258\times A + 0.1195 \times B + 0.0520 \times C + (-0.3465) \times AB + -0.3285 \times AC + (-0.2402) \times BC + (-0.0411) \times A2 + (-0.0691) \times B2 + (-0.1336) \times C2 \quad \text{Eq (3)}$$

Fig 1-C

3.1.2 Interpretation of TFC distribution via 3-D surface plots

Soluble phenolic compounds are primarily found in the vacuoles in the cell, whereas the majority of lignin, flavonoids, and insoluble polyphenols accumulate in the cell wall to form hydrogen and hydrophobic bonds with proteins and polysaccharides (Dixon, & Paiva., 1995). Consequently, flavonoids are close to insoluble or poorly soluble in water, which is in line with the data we acquired through this research, where we regarded the increase in the TFC yield in the extractions as the amount of ethanol in the solvent increased. The polarity of the solvent affected the extraction's selectivity: water preferred polar phenolic acids and glycosylated flavonoids, whereas higher ethanol fractions made less polar aglycones and tannins more soluble,

changing the extracts' flavonoid composition (Muhamad et al., 2014). Nonetheless, water and low levels of ethanol can penetrate the cells. Therefore, higher concentration amounts demonstrate protein denaturation, which impedes polyphenol solubility and affects extraction rate.

Figure 2-A presents the three-dimensional response surface and corresponding contour plot illustrating the effects of extraction time (60–360 min) and temperature (40–60 °C) on the total flavonoid content (TFC) of pistachio green hull extracts. The fitted quadratic model was statistically significant ($p = 0.0149$; $R^2 = 0.9386$), indicating that it explained a substantial proportion of the variability in TFC. Analysis of variance showed that extraction temperature had a significant effect on TFC ($p = 0.0082$), whereas the linear effect of extraction time was not significant ($p = 0.8996$). Importantly, the time–temperature interaction was also not statistically significant ($p = 0.4918$). Therefore, although the response surface and contour plot illustrate the combined variation of TFC across the experimental ranges of these two factors, the observed curvature should not be interpreted as evidence of a statistically significant interaction between time and temperature. The significant linear and quadratic effects of temperature suggest that temperature-related changes contributed to the observed response pattern. Overall, these findings indicate that extraction temperature was a more influential factor than extraction time for TFC within the investigated experimental range.

Fig 2-A

Figure 2-B illustrates the three-dimensional response surface, contour plot, and interaction diagram for total flavonoid content (TFC) as a function of extraction time (60–360 min) and ethanol concentration (0–100%, v/v). The response surface indicates that TFC varies non-linearly across the investigated combinations of extraction time and ethanol concentration. As indicated by the response pattern, the lowest TFC was observed at the longest extraction time and the highest ethanol concentration, contrary to the previously stated interpretation. The interaction diagram provides a visual representation of the combined effects of these two factors on TFC; however, the analysis of variance showed that the time–ethanol concentration interaction was not statistically significant ($p = 0.3933$). The observed response pattern suggests that the effect of extraction time on TFC should be considered in conjunction with solvent composition, while the individual effects and their statistical significance should be interpreted according to the fitted quadratic model. Overall, these results highlight the importance of evaluating extraction time and ethanol concentration within the experimental design space rather than assuming that increasing extraction time or decreasing ethanol concentration will consistently improve flavonoid recovery.

Fig 2-B

Figure 2-C presents the three-dimensional response surface, contour plot, and interaction diagram illustrating the effects of ethanol concentration (0–100%, v/v) and extraction temperature (40–60 °C) on TFC of PGH extracts. The contour plot indicates that relatively high TFC values occur within the intermediate temperature range and at low-to-moderate ethanol concentrations. In contrast, lower TFC values are observed at higher temperatures combined with high ethanol concentrations. This response pattern suggests that the effects of temperature and solvent composition on TFC depend on their combined levels within the investigated experimental range. The interaction diagram further illustrates differences in the predicted TFC profiles across ethanol concentrations as extraction temperature changes. Consistent with the ANOVA results, the temperature–ethanol concentration interaction was statistically significant ($p = 0.0488$), indicating that the effect of temperature on TFC varied with solvent concentration.

These findings emphasize the importance of considering both factors jointly when interpreting the extraction response. However, the observed pattern should not be attributed solely to solvent polarity or thermal degradation without additional experimental evidence.

The quadratic equation of the statistical model for calculating the amount of total flavonoid contents in the PGH extracts is given in equation 4., The variables A, B, and C in this equation represent the extraction time (minute), temperature (°C), and solvent concentration (v/v%).

$$Y2 = 4.05 + 0.0027 \times A + (-0.1808) \times B + (-0.1290) \times C + (-0.0877) \times AB + (-0.1104) \times AC + (-0.2484) \times BC + 0.0093 \times A2 + (-0.2004) \times B2 + (-0.0532) \times C2 \quad \text{Eq (4)}$$

Fig 2-C

3.1.3 Interpretation of DPPH distribution via 3-D surface plots

It should be emphasized that the DPPH response was included in this study as a supplementary experimental response rather than as a primary predictive response for optimization. As shown in Table 2, the fitted quadratic model for DPPH was not statistically significant ($p = 0.6312$) and showed a relatively low coefficient of determination ($R^2 = 0.5934$). Therefore, the response surface and contour plots presented in Figures 3-A to 3-C should be interpreted as visual representations of the experimental data rather than as reliable predictive models. The DPPH results are discussed below in terms of observed trends and possible mechanisms, but they should not be considered as robust model-based predictions. Therefore, the DPPH regression model was excluded from the numerical optimization. DPPH measurements were retained as experimental data to provide supplementary information on the radical-scavenging activity of the extracts. Any observed variation in DPPH values is discussed descriptively and is not interpreted as evidence of a statistically validated response model. Antioxidant activity can be measured in different ways. Due to the varying phenolic compounds in the extracts, several of them are recognized using different methods. The disparity in antioxidant activity among various methods is primarily determined by the hydrophilic and hydrophobic properties of the existing phenolic compounds and their proportion. The DPPH radical scavenging test basically measures the antioxidant activity of water-soluble phenolic compounds. Therefore, when two extracts have similar results in the DPPH method, it is an indication that they have the same amount of hydrophilic molecules (Erami et al., 2012).

Figure 3-A shows the DPPH radical scavenging response surface (%) and its contour plot as a function of temperature (B: 40–60 °C) and extraction time (A: 60–360 min). The red plateaus and contour projection indicate that antioxidant activity reaches its lowest (~75.5%) at mid-range conditions (≈ 200 min/50 °C), the blue trough, while the highest inhibition (~83–84%) occurs at the two design-space extremes: short-cool (60 min/40 °C) and long-hot (360 min/60 °C).

Fig 3-A

Figure 3-B illustrates the DPPH radical scavenging response surface (%) and its contour plot as a function of ethanol concentration (C: 0–100% v/v) (C: 0–100% v/v) and extraction time (A: 60–360 min). The central trough, or lowest of antioxidant activity, occurs under mid-range conditions (≈200 min/50 % v/v). While it climbs toward the edges of the design-space, it peaks (~79–80 %) at opposite corners (short/high strength: 60 min/100 % v/v; long/low strength: 360 min/0 % v/v). This convex topology is highlighted by the nearly circular contour rings, where activity increase with increasing distance from the center in either time or concentration. Additionally, in **Figure 3-B**, the graph demonstrated a linear increase in DPPH radical scavenging activity as the ethanol concentration in the solvent mixture rose. This observation emphasizes the critical role of solvent polarity in enhancing the extraction of antioxidant compounds (Rauf et al., 2018). The increasing ethanol content likely improved the solubility of

hydrophobic antioxidant molecules, facilitating their transfer into the solvent. Additionally, this finding aligns with the influence of time as a factor in optimizing extraction efficiency, as both solvent polarity and sufficient extraction duration are essential for maximizing the yield of bioactive compounds.

Fig 3-B

Figure 3-C depicts the DPPH radical scavenging response surface (%) and its contour plot as a function of extraction temperature (B: 40–60 °C) and ethanol concentration (C: 0–100% v/v). The surface shows a convex profile with minimum activity (~ 75.5) at mid-range conditions (≈ 50 °C /50 % v/v) and rises to its highest values (~ 79–80 %) at the two diagonal corners: low/low (0 °C /0 % v/v) and high/strong (360 min/100 % v/v). The approximately concentric contour lines emphasize a central trough flanked by maxima at these opposite extremes. In addition, our findings and those of Altemimi et al. (2016), highlight the significance of extraction time and solvent polarity in maximizing extraction efficiency. Increasing the concentration of ethanol increased the solubility of bioactive chemicals, which is consistent with Altemimi et al.'s (2016) discovery that solvent composition plays an important role in antioxidant extraction.

The quadratic equation of the statistical model for calculating the DPPH radical scavenging activity in the PGH extracts is given in equation 5. The variables A, B, and C in this equation represent the extraction time (minute), temperature (°C), and solvent concentration (v/v%).

$$Y3 = 77.77 + -0.2951 \times A + 0.7282 \times B + -0.0286 \times C + 1.15 \times AB + -0.5173 \times AC + 1.79 \times BC + 0.6396 \times A^2 + 1.05 \times B^2 + 0.7253 \times C^2 \quad \text{Eq (5)}$$

Fig 3-C

The non-significant DPPH model may be attributed to several factors. First, the DPPH assay primarily measures the radical scavenging activity of hydrophilic phenolic compounds, while PGH contains a complex mixture of phenolic compounds with varying hydrophobicity. Second, synergistic and antagonistic interactions among phenolic compounds in a multi-component extract can influence the overall antioxidant activity in ways that are not captured by a quadratic polynomial model. Third, experimental variability in the DPPH assay (e.g., reaction kinetics, light sensitivity, and pH dependence) may have contributed to the low R^2. Therefore, the DPPH response was treated as a supplementary experimental observation rather than a standalone predictive response.

3.2 Optimization of the extraction process

Extraction time, temperature, and ethanol concentration are important factors influencing the recovery of phenolic compounds and antioxidant constituents from PGH. In the present study, extraction time was investigated over a range of 60–360 min, while extraction temperature and ethanol concentration varied from 40 to 60 °C and from 0 to 100% (v/v), respectively. These ranges were selected based on preliminary single-factor experiments and were used to characterize the effects of the extraction variables and their interactions on the responses. The objective was to characterize the response patterns over the selected range and identify an effective extraction condition that balanced compound recovery with processing time and energy requirements.

UAE promotes the release of intracellular compounds through acoustic cavitation, which facilitates cell-wall disruption and solvent penetration. However, extending the extraction time does not necessarily result in a proportional increase in phenolic recovery. As readily extractable compounds are released, the process may approach equilibrium, resulting in diminishing returns. Prolonged sonication may also increase energy consumption and expose sensitive compounds to acoustic and thermal stress. Previous studies have demonstrated that effective UAE can be

achieved over substantially shorter periods. For example, Rajaei et al. (2010) investigated UAE of PGH at temperatures of 25–65 °C using extraction times of up to 45 min. Other response surface methodology (RSM) studies have reported optimal extraction times of approximately 60–120 min (Che Sulaiman et al., 2017; Muzolf-Panek et al., 2022; Wu et al., 2021). Kinetic investigations have also described an initial period of rapid extraction followed by slower recovery and a potential plateau after approximately 1–2 h (Sant Anna et al., 2013; Garcia-Montalvo et al., 2022; Suan et al., 2025). Moreover, UAE treatments lasting only 4–10 min have been reported to produce results comparable to conventional stirring for 120–180 min (Dent et al., 2015; Anaya-Esparza et al., 2018).

Longer extraction periods have also been used in studies employing conventional maceration, Soxhlet extraction, enzymatic treatment, or indirect UAE (Osorio-Tobón, 2020; Psarrou et al., 2020; Sridhar et al., 2021; Maaiden et al., 2022; Klausen et al., 2023). However, these approaches differ in their extraction mechanisms and operating conditions and therefore do not directly establish that prolonged sonication is advantageous in UAE. The effects of extraction duration should instead be evaluated in relation to the target compounds, extraction yield, compound stability, and energy requirements (Antony & Farid, 2022; Yao et al., 2022). Accordingly, the inclusion of longer extraction times in the present design enabled the response patterns to be examined across the investigated range, whereas the final operating condition was selected through multi-response optimization.

Table 1

A central composite design (CCD) was employed within the RSM framework to evaluate the effects of extraction time ((X1)), temperature ((X2)), and ethanol concentration ((X3)) on the extraction responses. The independent variables and their experimental levels were established based on preliminary single-factor experiments. The experimental design comprised three factors at three coded levels, with the corresponding experimental runs and factor settings presented in Tables 1 and 2. The center-point experiment was performed in duplicate. The responses included TPC and TFC. The effects of the individual factors, their interactions, and quadratic terms were evaluated using the fitted response surface models. The analysis of variance (ANOVA) for the regression coefficients of the quadratic polynomial models is presented in Table 2.

The response surface analysis indicated that the effects of extraction time, temperature, and ethanol concentration differed among TPC and TFC. These differences may be associated with variations in the composition of PGH phenolics and their extraction kinetics. Therefore, simultaneous consideration of the responses was necessary to identify a balanced operating condition rather than selecting conditions based on a single response alone.

Numerical multi-response optimization, based primarily on TPC and TFC, predicted an optimum extraction condition of 60 min, 54.07 °C, and 43.33% (v/v) ethanol, with predicted values of 7.175 mg GAE/g for TPC and 4.003 mg CATCHIN/g for TFC. The corresponding DPPH value (78.560%) was recorded as a supplementary experimental observation rather than a model-based prediction, since the DPPH model was not statistically significant. The overall desirability of the predicted solution was 0.819, primarily driven by the strong model performance for TPC and TFC. For experimental verification, the conditions were adjusted to 60 min, 54 °C, and 43.33% (v/v) ethanol. Under these conditions, the experimentally determined TPC, TFC, and DPPH values were 7.900 mg GAE/g, 4.400 mg CATCHIN/g, and 80.500%, respectively. These experimental results were reasonably consistent with the model predictions, supporting the suitability of the fitted models for identifying an effective extraction condition. Overall, the results demonstrate that a 60-min UAE treatment under the verified conditions can achieve

substantial phenolic recovery and antioxidant activity from PGH without requiring the longest extraction duration investigated in the experimental design.

Table 2

3.2.1 Verification of predictive model

While the models for TPC and TFC demonstrated good predictive capability, the DPPH model was not statistically significant ($p = 0.6312$) and showed a relatively low coefficient of determination ($R^2 = 0.5934$). Therefore, the DPPH response was not used as a standalone predictive response for optimization. This may be due to experimental variability or the complexity of interactions among factors such as solvent polarity, thermal degradation, and compound solubility. Nevertheless, the observed experimental values largely followed the model's predicted trends, supporting its overall utility in identifying optimal extraction conditions. Numerical optimization was performed using Design-Expert software with TPC and TFC as the response variables. Both responses were included in the desirability function, whereas DPPH radical-scavenging activity was excluded because its regression model was not statistically significant. The optimum extraction conditions were therefore determined by considering the selected TPC and TFC responses only. DPPH activity was measured experimentally under the selected conditions and is reported as a supplementary result, not as a predicted or optimized response. The optimal conditions and corresponding predicted TPC and TFC values obtained from the revised optimization are presented in this section. Thus, the optimization was based on a multi-response desirability approach rather than maximizing a single response independently. The objective was to identify a combination of extraction time, temperature, and ethanol concentration that provided a balanced and satisfactory performance across all three responses. Based on the numerical optimization, the software identified the following optimal extraction conditions: extraction time = 60 min, extraction temperature = 54.07 °C, and ethanol concentration = 43.33% (v/v). Under these conditions, the predicted values were 7.175 mg GAE/g for TPC, 4.003 mg CATCHIN/g for TFC, and 78.560% for DPPH radical scavenging activity, with an overall desirability of 0.819. **Figure 4** illustrates the response-surface optimization for PGH, showing the individual desirability functions for each independent variable and response, as well as the combined desirability. As shown in Figure 4, the desirability values for extraction time and temperature were both 1.000, while the desirability for solvent concentration was 0.809. Among the responses, TPC showed the highest individual desirability (0.999), followed by TFC (0.722). The DPPH response had a lower individual desirability (0.635), which is consistent with its non-significant model. The combined desirability of 0.819 was therefore primarily determined by the TPC and TFC responses. Among the responses, TPC showed the highest individual desirability (0.999), followed by TFC (0.722) and DPPH (0.635), resulting in a combined desirability of 0.819. This confirms that the selected condition represents a balanced compromise among all three responses rather than maximizing any single response. Although TFC alone showed a higher predicted value under prolonged extraction with 0% ethanol, this condition did not provide the best overall balance among the three responses. Therefore, the multi-response optimization was selected as the basis for the experimental verification presented in Table 4. The verification experiment was conducted under the selected predictead conditions of 60 min, 54 °C, and 43.33% ethanol (v/v). The experimental values were 7.900 mg GAE/g for TPC, 4.400 mg CATECHIN/g for TFC, and 80.500% for DPPH radical scavenging activity. The experimental TPC and TFC values were higher than the corresponding numerical predictions, indicating satisfactory performance of the selected conditions for these primary responses. However, because the DPPH regression model was not

statistically significant ($p = 0.6312$) and showed a relatively low R^2 value (0.5934), the DPPH result should be interpreted solely as an experimental observation and not as validation of the predictive accuracy of the DPPH model. Accordingly, the verification results provide stronger support for the optimized extraction conditions with respect to TPC and TFC than for DPPH.

Table 3

Table 4

Fig 4

3.2.3 Application potential

The optimized PGH extract provides a phenolic-rich material with potential for further investigation as a natural antioxidant in food, cosmetic, and other relevant formulations. However, the present study focused on optimizing the extraction conditions, and the incorporation, stability, and antioxidant performance of the extract in specific food or cosmetic matrices were not experimentally evaluated. Therefore, further formulation and application studies are required to validate its practical potential.

A limitation of the present study is that direct scanning electron microscopy (SEM) analysis was not feasible; therefore, the morphological changes induced by ultrasound could not be experimentally verified in the PGH matrix. Nevertheless, SEM observations reported for UAE of other lignocellulosic plant by-products provide useful supporting evidence for the proposed extraction mechanism (Supplementary Table S1). As summarized in Supplementary Table S1, these studies have reported ultrasound-induced disruption of cell walls, pore formation, and surface roughening, which are consistent with the proposed effects of acoustic cavitation and micro-jet action. It should be emphasized that these observations were obtained from other plant matrices and are cited here only as literature-based mechanistic support; they do not constitute direct morphological evidence for PGH. Future studies should incorporate SEM analysis of PGH before and after UAE to directly characterize ultrasound-induced structural changes and further clarify the relationship between matrix disruption and phenolic compound recovery.

Table S1

Conclusions

In this study, RSM was applied to investigate and optimize the UAE of phenolic and flavonoid compounds from PGH. The fitted quadratic models showed strong explanatory performance for TPC ($R^2 = 0.9666$) and TFC ($R^2 = 0.9386$), and both models were statistically significant. In contrast, the DPPH radical-scavenging model showed limited predictive performance ($R^2 = 0.5934$) and was not statistically significant ($p = 0.6312$); therefore, DPPH radical-scavenging activity was retained only as a supplementary experimental response and was excluded from the numerical optimization, which was based on TPC and TFC. Based on the statistically supported models, multi-response numerical optimization predicted an optimal extraction condition of 60 min, 54.07 °C, and 43.33% (v/v) ethanol, with predicted values of 7.175 mg GAE/g for TPC and 4.003 mg catechin equivalents/g for TFC (overall desirability = 0.819). For practical verification, the condition was rounded to 60 min, 54 °C, and 43.33 % (v/v) ethanol, under which the experimentally determined TPC, TFC, and DPPH radical-scavenging activity reached 7.900 mg GAE/g, 4.400 mg catechin equivalents/g, and 80.500%, respectively. These experimental values were consistent with, and for TPC and TFC exceeded, the model predictions, supporting the reproducibility and practical applicability of the selected condition. Because the DPPH model was not statistically significant, the DPPH value is reported as an experimental observation rather than as validation of a predictive model. Overall, the findings demonstrate that PGH is a promising source of phenolic and flavonoid compounds and that UAE combined with RSM can

be effectively used to characterize and optimize the extraction process. The optimized condition (60 min) achieves substantial recovery of phenolic compounds without requiring prolonged sonication, which is advantageous in terms of energy consumption and reduces the risk of thermal degradation of thermolabile phenolics. Several limitations should be acknowledged. The relatively weak DPPH model indicates that complementary antioxidant assays (e.g., ABTS, FRAP, or ORAC) are needed to provide a more comprehensive assessment of antioxidant capacity. In addition, compound-specific identification was not performed in this study; future work should incorporate HPLC or LC-MS analysis to identify and quantify the individual phenolic compounds in the optimized extracts and to clarify the relationship between phenolic composition and radical-scavenging activity. Direct SEM analysis of PGH before and after UAE was also not feasible; therefore, the morphological changes induced by ultrasound could not be experimentally verified, and the mechanistic interpretation presented here relies on literature-based evidence from other lignocellulosic by-products (Supplementary Table S1). Future studies should incorporate direct SEM analysis, as well as additional process variables such as solvent-to-solid ratio, particle size, pH, and ultrasonic intensity, and should evaluate the stability and performance of the optimized extracts in relevant food or cosmetic matrices.

Abbreviations

PGH Pistachio Green Hull
TPC Total Phenolic Compound
TPY Total Phenolic Yield
TFC Total Flavonoid Content
RSM Response Surface Methodology
UAE Ultrasound-Assisted Extraction

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Nooshin Noshirvani: Conceptualization, Methodology, Formal analysis, Investigation, Review & Editing, Supervision, Project administration, Validation
Amir Daraei Garmakhany: Conceptualization, Methodology, Formal analysis, Investigation, Review & Editing, Supervision, Project administration, Validation
Mehran Banibayat: Writing - Original Draft, Visualization, Methodology, Formal analysis, Investigation

Data availability: Not applicable.

REFERENCES

Abbas, M., Saeed, F., Anjum, F.M., Afzaal, M., Tufail, T., Bashir, M.S., Ishtiaq, A., Hussain, S., Suleria, H.A.R. (2017). Natural polyphenols: An overview. Int. J. Food Prop. 20, 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

Abi-Khattar, A. M., Boussetta, N., Rajha, H.N., Abdel-Massih, R.M., Louka, N., Maroun, R.G., Vorobiev, E., Debs, E. (2022). Mechanical damage and thermal effect induced by ultrasonic treatment in olive leaf tissue. Impact on polyphenols recovery. Ultrason. Sonochem. 82, 105895. https://doi.org/https://doi.org/10.1016/j.ultsonch.2021.105895

Altemimi, A., Watson, D.G., Choudhary, R., Dasari, M.R., Lightfoot, D.A. (2016). Ultrasound Assisted Extraction of Phenolic Compounds from Peaches and Pumpkins. PLoS One 11, e0148758. https://doi.org/10.1371/journal.pone.0148758

Anaya-Esparza, L.M., Ramos-Aguirre, D., Zamora-Gasga, V.M., Yahia, E., Montalvo-González, E. (2018). Optimization of ultrasonic-assisted extraction of phenolic compounds from Justicia spicigera leaves. Food Sci. Biotechnol. 27, 1093–1102. https://doi.org/10.1007/s10068-018-0350-0

Antony, A., Farid, M. (2022). Effect of Temperatures on Polyphenols during Extraction. Appl. Sci. 12. https://doi.org/10.3390/app12042107

Azarifar, M., Ghanbarzadeh, B., Khiabani, M. S., Basti, A. A., Abdulkhani, A., Noshirvani, N., & Hosseini, M. (2019). The optimization of gelatin-CMC based active films containing chitin nanofiber and Trachyspermum ammi essential oil by response surface methodology. *Carbohydrate polymers*, *208*, 457-468. https://doi.org/10.1016/j.carbpol.2019.01.005

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng. 117, 426–436. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2013.01.014

Bannour, Y., Sellami, I. H., Genva, M., Fauconnier, M. L., & Ayadi, M. A. (2026). Phenolic composition of ultrasound-assisted extracts optimized using response surface methodology based on DPPH scavenging activity, total phenolic content, and total flavonoid content from three aromatic and medicinal plants and their mixture. *Journal of Applied Research on Medicinal and Aromatic Plants*, 100732. 10.1016/j.jarmap.2026.100732

Box, G.E.P., Wilson, K.B. (1992). On the experimental attainment of optimum conditions BT - breakthroughs in statistics: methodology and distribution, in: Kotz, S., Johnson, N.L. (Eds.), . Springer New York, New York, NY, pp. 270–310. https://doi.org/10.1007/978-1-4612-4380-9_23

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., Bren, U. (2016). Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* 21. https://doi.org/10.3390/molecules21070901

Ultrason. Sonochem. 26, 176–185. https://doi.org/https://doi.org/10.1016/j.ultsonch.2015.02.008

Chan, C. H., Yeoh, H.K., Yusoff, R., Ngoh, G.C. (2016). A first-principles model for plant cell rupture in microwave-assisted extraction of bioactive compounds. J. Food Eng. 188, 98–107. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2016.05.017

Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A. S., Abert-Vian, M. (2017). Review of green food processing techniques. preservation, transformation, and extraction. Innov. Food Sci. Emerg. Technol. 41, 357–377. https://doi.org/https://doi.org/10.1016/j.ifset.2017.04.016

Che Sulaiman, I.S., Basri, M., Fard Masoumi, H.R., Chee, W.J., Ashari, S.E., Ismail, M. (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. Chem. Cent. J. 11, 54. https://doi.org/10.1186/s13065-017-0285-1

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. Sep. Purif. Technol. 55, 217–225. https://doi.org/https://doi.org/10.1016/j.seppur.2006.12.005

Dent, M., Verica, D.-U., Garofulić, I., Bosiljkov, T., Ježek, D. (2015). Comparison of conventional and ultrasound assisted extraction techniques on mass fraction of phenolic compounds from sage (*Salvia officinalis* L.). Chem. Biochem. Eng. Q. 29. https://doi.org/10.15255/CABEQ.2015.2168

Dimitrov, K., Pradal, D., Vauchel, P., Baouche, B., Nikov, I., Dhulster, P. (2019). Modeling and optimization of extraction and energy consumption during ultrasound-assisted extraction of antioxidant polyphenols from pomegranate peels. Environ. Prog. \& Sustain. Energy 38, 13148. https://doi.org/https://doi.org/10.1002/ep.13148

Dixon, R.A., Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. Plant Cell 7, 1085–1097.

https://doi.org/10.1105/tpc.7.7.1085
Erami, S.R., Jafari, S., Khomeiri, M., Bayat, H. (2012). Antioxidant activity of toyserkani variety of walnut husk and comparison of its antiradical activity with synthetic antioxidants. J. Food Res. (AGRICULTURAL Sci. 22, 39. https://doi.org/https://www.magiran.com/p1003417
Esmaeilzadeh Kenari, R., Mohsenzadeh, F., Amiri, Z.R. (2014). Antioxidant activity and total phenolic compounds of Dezful sesame cake extracts obtained by classical and ultrasound-assisted extraction methods. Food Sci. Nutr. 2, 426–435. https://doi.org/https://doi.org/10.1002/fsn3.118
Espín, J.C., Soler-Rivas, C., Wichers, H.J. (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-Diphenyl-1-picrylhydrazyl Radical. J. Agric. Food Chem. 48, 648–656. https://doi.org/10.1021/jf9908188
Garcia-Montalvo, J., Garcia-Martín, A., Ibañez Bujan, J., Santos Mazorra, V.E., Yustos Cuesta, P., Bolivar, J.M., Ladero, M. (2022). Extraction of Antioxidants from Grape and Apple Pomace: Solvent Selection and Process Kinetics. Appl. Sci. 12. https://doi.org/10.3390/app12104901
Gharekhani, M., Ghorbani, M., Ebrahimzadeh, M.A., Jaafari, S.M., Sadeghi Mahoonak, A.R. (2010). Compare different methods of phenolic and flavonoid compounds extraction from Urtica dioica L. Iran. J. Med. Aromat. Plants Res. 26, 389–405. https://doi.org/10.22092/ijmapr.2010.6800
Goli, A. H., Barzegar, M., & Sahari, M. A. (2005). Antioxidant activity and total phenolic compounds of pistachio (Pistachia vera) hull extracts. *Food chemistry*, *92*(3), 521-525. 10.1016/j.foodchem.2004.08.020
Gouda, M., El-Din Bekhit, A., Tang, Y., Huang, Y., Huang, L., He, Y., Li, X. (2021). Recent innovations of ultrasound green technology in herbal phytochemistry: A review. Ultrason. Sonochem. 73, 105538. https://doi.org/https://doi.org/10.1016/j.ultsonch.2021.105538
Imtiaz, F., Ahmed, D., Mohammed, O. A., Younas, U., & Iqbal, M. (2025). Optimized recovery of phenolic and flavonoid compounds from medicinal plant extracts for enhanced antioxidant activity: A mixture design approach. *Results in Chemistry*, *13*, 101960. 10.1016/j.rechem.2024.101960
Jurinjak Tusek, A., Benković, M., Valinger, D., Jurina, T., Belščak-Cvitanović, A., Gajdoš Kljusurić, J. (2018). Optimizing bioactive compounds extraction from different medicinal plants and prediction through nonlinear and linear models. Ind. Crops Prod. 126, 449–458. https://doi.org/10.1016/j.indcrop.2018.10.040
Khurramov, A., Badraldin, S. Q., Fawzi, M., Hani, M. M., Kadhim, A. J., Saad, T. M., ... & Rakhimova, G. (2024). Optimization of Phenolic Compound Extraction from Pistachio Green Hulls: Influence of Blanching, Harvest Time, Solvent Concentration, and Extraction Method.
Klausen, S.J., Falck-Ytter, A.B., Strætkvern, K.O., Martin, C. (2023). Evaluation of the extraction of bioactive compounds and the saccharification of cellulose as a route for the valorization of spent mushroom substrate. *Molecules* 28. https://doi.org/10.3390/molecules28135140
Kumar, K., Srivastav, S., Sharanagat, V.S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrason. Sonochem. 70, 105325. https://doi.org/https://doi.org/10.1016/j.ultsonch.2020.105325
Lee, W.-J., Suleiman, N., Hadzir, N.H.N., Chong, G. H. (2020). Chapter 12 - Supercritical fluids for the extraction of oleoresins and plant phenolics, in: Inamuddin, Asiri, A.M., Isloor, A.M. (Eds.), Green Sustainable Process for Chemical and Environmental Engineering and Science. Elsevier, pp. 279–328. https://doi.org/https://doi.org/10.1016/B978-0-12-817388-6.00012-X
Maaiden, E., Bouzroud, S., Nasser, B., Moustaid, K., El Mouttaqi, A., Ibourki, M., Boukcim, H., Hirich, A., Kouisni, L., El Kharrassi, Y. (2022). A Comparative Study between Conventional and Advanced Extraction Techniques: Pharmaceutical and Cosmetic Properties of Plant Extracts. *Molecules* 27. https://doi.org/10.3390/molecules27072074
Martínez-Ramos, T., Benedito-Fort, J., Watson, N.J., Ruiz-López, I.I., Che-Galicia, G., Corona-Jiménez, E. (2020). Effect of solvent composition and its interaction with ultrasonic energy on the ultrasound-assisted extraction of phenolic compounds from Mango peels (Mangifera indica L.). Food Bioprod. Process. 122, 41–54. https://doi.org/https://doi.org/10.1016/j.fbp.2020.03.011
Muhamad, N., Muhmed, S., Yusoff, M., Gimbun, J., 2014. Influence of Solvent Polarity and Conditions

on Extraction of Antioxidant, Flavonoids and Phenolic Content from Averrhoa bilimbi. J. Food Sci. Eng. 4, 255–260. https://doi.org/10.17265/2159-5828/2014.05.006

Muzolf-Panek, M., Gliszczyńska-Świgło, A., 2022. Extraction optimization for the antioxidants from Nigella sativa seeds using response surface methodology. J. Food Meas. Charact. 16, 4741–4753. https://doi.org/10.1007/s11694-022-01575-z

Myers, R. H and Montgomery, D. C. (2002). Response Surface Methodology (RSM). Response Surf. Methodol. Process Prod. Optim. using Des. Exp. (2nd ed.) 489–492. https://doi.org/https://www.researchgate.net/publication/307534448_Response_Surface_Methodology_Process_and_Product_Optimization_Using_Designed_Experiments

Nateghi, L., & Hosseini, E. (2023). Investigating the oxidative stability of grape seed oil using aqueous extract of pistachio green hull. *Journal of Food Measurement and Characterization*, *17*(5), 4434-4447. https://doi.org/10.1007/s11694-023-01961-1.

Noorolahi, Z., Sahari, M. A., Barzegar, M., & Ahmadi Gavlighi, H. (2020). Tannin fraction of pistachio green hull extract with pancreatic lipase inhibitory and antioxidant activity. *Journal of Food Biochemistry*, *44*(6), e13208. https://doi.org/10.1111/jfbc.13208.

Noshirvani, N., Fasihi, H., Nourmohammadi, E., Moradipayam, A. (2017). Study on the antioxidant and antifungal activities of extract and pulp of date (*Phoenix dactylifera* L.) by-products. Iran. J. Nutr. Sci. Food Technol. 12(3), 77–88.

Noshirvani, N., Fasihi, H., & Moradipayam, A. (2015). Study on the antioxidant effects of extract and powder of green walnut hulls on the oxidation of sunflower oil. Iran. J. Nutr. Sci. Food Technol. 10(3), 79–90

Noshirvani, N., Bathaeian, N. S., Fasihi, H., & Taheri Ghods, M. (2024). Evaluation and prediction of synergistic antioxidant effects of green walnut hulls, potato peel, and date pulp extracts on the stability of sunflower oil by deep neural networks. *Journal of Food Measurement and Characterization*, *18*(12), 9721-9735. https://doi.org/10.1007/s11694-024-02903-1

Noshirvani, N., 2024. Essential oils as natural food preservatives: special emphasis on antimicrobial and antioxidant activities. J. Food Qual. 2024, 5807281. https://doi.org/https://doi.org/10.1155/jfq/5807281

Noshirvani, N., & Fasihi, H. (2018). Control of Aspergilus niger in vitro and in vivo by three Iranian essential oils. *International Food Research Journal*, *25*(4), 1745-1752. http://www.ifrj.upm.edu.my/25%20(04)%202018/(56).pdf

Noshirvani, N., Fasihi, H., & Nourmohammadi, E. (2019). Evaluation of Antifungal and Antioxidant Properties of Fennel Extract. J. Food Technol. Nutr. 16(2), 67–78.

Noshirvani, N., & Abolghasemi Fakhri, L. (2025). Advances in extending the microbial shelf-life of bread and bakery products using different technologies: A Review. *Food Reviews International*, *41*(1), 87-112. https://doi.org/10.1080/87559129.2024.2386029

Osorio-Tobón, J.F., 2020. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. J. Food Sci. Technol. 57, 4299–4315. https://doi.org/10.1007/s13197-020-04433-2

Ordoñez-Cano, A. J., Ramírez-Esparza, U., Alvarado-González, M., Baeza-Jiménez, R., Espinoza-Hicks, J. C., Prado-Barragán, L. A., & Buenrostro-Figueroa, J. J. (2025). Valorization of Pistachio Green Hull: Advances in Extraction and Characterization of Phenolic Compounds. *Processes*, *13*(12), 3761. https://doi.org/10.3390/pr13123761.

Psarrou, I., Oreopoulou, A., Tsimogiannis, D., Oreopoulou, V., 2020. Extraction Kinetics of Phenolic Antioxidants from the Hydro Distillation Residues of Rosemary and Effect of Pretreatment and Extraction Parameters. Molecules 25. https://doi.org/10.3390/molecules25194520

Rajaei, A., Barzegar, M., Hamidi Esfahani, Z., Sahari, M.A., 2010. Optimization of Extraction Conditions of Phenolic Compounds from Pistachio (Pistachia vera) Green Hull through Response Surface Method. J Agr Sci Tech 12. https://doi.org/https://www.researchgate.net/publication/267425926_Optimization_of_Extraction_Conditions_of_Phenolic_Compounds_from_Pistachio_Pistachia_vera_Green_Hull_through_Respons

e_Surface_Method

Rauf, A., Nawaz, H., Shad, M.A., 2018. Effect of solvent polarity and extraction time on in vitro antioxidant properties of Brassica oleracea Convar Capitata Var L. seeds. Pak. J. Pharm. Sci. 31, 1889–1897. https://doi.org/https://pubmed.ncbi.nlm.nih.gov/30150185/

Rombaut, N., Chave, T., Nikitenko, S.I., Maâtaoui, M.E.I., Fabiano-Tixier, A.S., Chemat, F., 2021. Modification of Olive Leaves' Surface by Ultrasound Cavitation. Correlation with Polyphenol Extraction Enhancement. Appl. Sci. 11. https://doi.org/10.3390/app11010232

Sant Anna, V., Marczak, L., Tessaro, I., 2013. Kinetic Modeling of Anthocyanin Extraction from Grape Marc. Food Bioprocess Technol. 6. https://doi.org/10.1007/s11947-012-1016-1

Seifzadeh, N., Sahari, M. A., Barzegar, M., Gavlighi, H. A., Calani, L., Del Rio, D., & Galaverna, G. (2019). Evaluation of polyphenolic compounds in membrane concentrated pistachio hull extract. *Food chemistry*, *277*, 398-406. 10.1016/j.foodchem.2018.10.001

Sirijeerachai, Guntima, Khunasinkun, Suphattra, Boonleau, Piyapassara, Oonsivilai, Ratchadaporn, Nuchitprasitchai, Aroonsri, 2021. Optimization of Ultrasonic-assisted Extraction of Total Phenolic Compound from Leaves of Chromolaena Odorata L. Using Response Surface Methodology. MATEC Web Conf. 333, 6005. https://doi.org/10.1051/matecconf/202133306005

Shabanian, M., Sari, A.A., Daraei Garmakhany, A., 2021. Optimization of ethanolic extracts of walnut green peel extracted by Microwave method and investigation of their antioxidant properties. J. food Sci. Technol. 18. https://doi.org/10.52547/fsct.18.115.23

Sridhar, A., Ponnuchamy, M., Kumar, P., Kapoor, A., Vo, D.-V., Prabhakar, S., 2021. Techniques and modeling of polyphenol extraction from food: a review. Environ. Chem. Lett. 19, 1–35. https://doi.org/10.1007/s10311-021-01217-8

Suan, T., Zubairi, S., Manuel, R., Mohd-Kasim, Z., Faujan, N.H., 2025. Optimization of ultrasonic-assisted extraction of phenolic compounds from Clinacanthus nutans using ionic liquid (ILs) binary solvent: Application of Peleg's model and response surface methodology. PLoS One 20. https://doi.org/10.1371/journal.pone.0326141

Tan Quoc, L.P., Nguyen, Y., 2022. Optimization of ultrasound-assisted extraction of phenolic compounds from fresh Moringa oleifera leaves with a response surface methodology and comparison with the Soxhlet extraction method. Bull. Chem. Soc. Ethiop. 36, 261–275. https://doi.org/10.4314/bcse.v36i2.2

Teo, C.C., Tan, S.N., Yong, J.W.H., Hew, C.S., Ong, E.S., 2010. Pressurized hot water extraction (PHWE). J. Chromatogr. A 1217, 2484–2494. https://doi.org/https://doi.org/10.1016/j.chroma.2009.12.050

Thoo, Y.Y., Ho, S.K., Liang, J.Y., Ho, C.W., Tan, C.P., 2010. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia). Food Chem. 120, 290–295. https://doi.org/https://doi.org/10.1016/j.foodchem.2009.09.064

Woisky, R., Salatino, A., 1998. Analysis of Propolis: Some Parameters and Procedures for Chemical Quality Control. J. Apic. Res. 37, 99–105. https://doi.org/10.1080/00218839.1998.11100961

Wu, J., Zhang, J., Yu, X., Shu, Y., Zhang, S., Zhang, Y., 2021. Extraction optimization by using response surface methodology and purification of yellow pigment from Gardenia jasminoides var. radicans Makikno. Food Sci. Nutr. 9, 822–832. https://doi.org/10.1002/fsn3.2046

Yao, T., Li, H., Ren, Y., Feng, M., Hu, Y., Yan, H., Peng, L., 2022. Extraction and recovery of phenolic compounds from aqueous solution by thermo-separating magnetic ionic liquid aqueous two-phase system. Sep. Purif. Technol. 282, 120034. https://doi.org/https://doi.org/10.1016/j.seppur.2021.120034

Journal Pre-proofs

Table 1. Independent variables and their amounts used to optimize PGH extracts

Independent variables	Levels and limits of variables		
	-1	0	+1
Extraction time	60	210	360
Extraction temperature	40	50	60
Ethanol concentration (% v/v)	0	50	100

Table 2. The variance of regression coefficients in quadratic polynomial models for PGH extracts

Response	Source	DF[a]	Sum of Squares	Mean of Squares	F-value	p-value	Coefficient	Significance
TPC (R^2 = 0.9666)	Model	9	0.0679	0.0075	16.07	0.0035	7.10	**
	X1 (Time)	1	0.0040	0.0040	8.53	0.0330	0.0258	*
	X2 (Temperature)	1	0.0190	0.0190	40.57	0.0014	0.1195	**
	X3 (Ethanol)	1	0.0054	0.0054	11.52	0.0194	0.0520	*
	X1 × X2	1	0.0208	0.0208	44.28	0.0012	-0.3465	**

	X1 × X3	1	0.0187	0.0187	39.80	0.0015	-0.3285	**
	X2 × X3	1	0.0152	0.0152	32.35	0.0023	0.2402	**
	$X1^2$	1	0.0024	0.0024	5.14	0.0727	0.0411	
	$X2^2$	1	0.0024	0.0024	5.22	0.0712	0.0691	
	$X3^2$	1	0.0255	0.0255	54.33	0.0007	-0.1336	***
	Residual	5	0.0023	0.0005	-	-	-	
	Lack of Fit	1	1.333E-06	1.333E-06	0.00023	0.9643^{ns}	-	ns
	Pure Error	4	0.0023	0.0006	-	-	-	
	Total	14	0.0702	-	-	-	-	
TFC (R^2 = 0.8142)	Model	6	0.1605	0.0268	5.84	0.0130	4.03	*
	X1 (Time)	1	0.0000	0.0000	0.0093	0.9255	0.0027	ns
	X2 (Temperature)	1	0.1482	0.1482	32.38	0.0005	-0.2576	**
	X3 (Ethanol)	1	0.0888	0.0888	19.41	0.0023	-0.1802	**
	X1 × X2	1	0.0420	0.0420	9.17	0.0163	0.1996	*
	X1 × X3	1	0.0330	0.0330	7.20	0.0278	0.1768	*
	X2 × X3	1	0.0018	0.0018	0.3911	0.5492	-0.0252	ns
	Residual	8	0.0366	0.0046	-	-	-	
	Lack of Fit	4	0.0246	0.0061	2.04	0.2539^{ns}	-	ns
	Pure Error	4	0.0121	0.0030	-	-	-	
	Total	14	0.1971	-	-	-		
DPPH (R^2 = 0.5934)	Model	9	21.11	21.11	0.8109	0.6312	77.77	ns
	X1 (Time)	1	0.5224	0.5224	0.1806	0.6885	-0.2451	ns
	X2 (Temperature)	1	0.7069	0.7069	0.2444	0.6420	0.7287	ns
	X3 (Ethanol)	1	0.0016	0.0016	0.0006	0.9820	-0.0286	ns
	X1 × X2	1	0.2301	0.2301	0.0795	0.7892	1.15	ns
	X1 × X3	1	0.0463	0.0463	0.0160	0.9043	-0.5173	ns
	X2 × X3	1	0.8463	0.8463	0.2925	0.6118	1.79	ns
	$X1^2$	1	0.5845	0.5845	0.2020	0.6719	0.6396	ns
	$X2^2$	1	0.5694	0.5694	0.1968	0.6758	1.05	ns
	$X3^2$	1	0.7515	0.7515	0.2598	0.6320	0.7253	ns
	Residual	5	2.89	2.89	-	-	-	
	Lack of Fit	1	1.45	1.45	0.4451	0.5412^{ns}	-	ns
	Pure Error	4	13.02	3.25	-	-	-	
	Total	14	35.58	-	-	-	-	

p < 0.05; ** p < 0.01; *** p < 0.001; ns = not significant. Dashes (—) = not recoverable from the submitted table; complete from Design-Expert output. The DPPH model was not statistically significant (p = 0.6312) and was therefore not used as a standalone predictive response for optimization. DPPH was included as a supplementary experimental response only

Journal Pre-proofs

Table 3. Critical statistical diagnostics of quadratic polynomial models for PGH extracts

Critical statistical diagnostics	TPC	TFC	DPPH
Std. Dev	0.0217	0.0677	1.70
C.V. %	0.3057	1.68	2.17
R^2	0.9666	0.8142	0.5934
Adjusted R	0.9064	0.6749	-0.1384
Adeq Precision	14.5483	9.4731	3.4955

Table 4. The result of experimental verification

Term	Extraction time (min)	Extraction temperature (℃)	Solvent concentration (%, v/v)	Total phenolic compounds (mgGA/g hull)	Total flavonoid contents (mg CATECHIN/g hull)	DPPH (%)
Predict	60.000	54.074	43.333	7.175	4.003	78.560
Experiment	60.000	54	43.33	7.900	4.400	80.500

Journal Pre-proofs

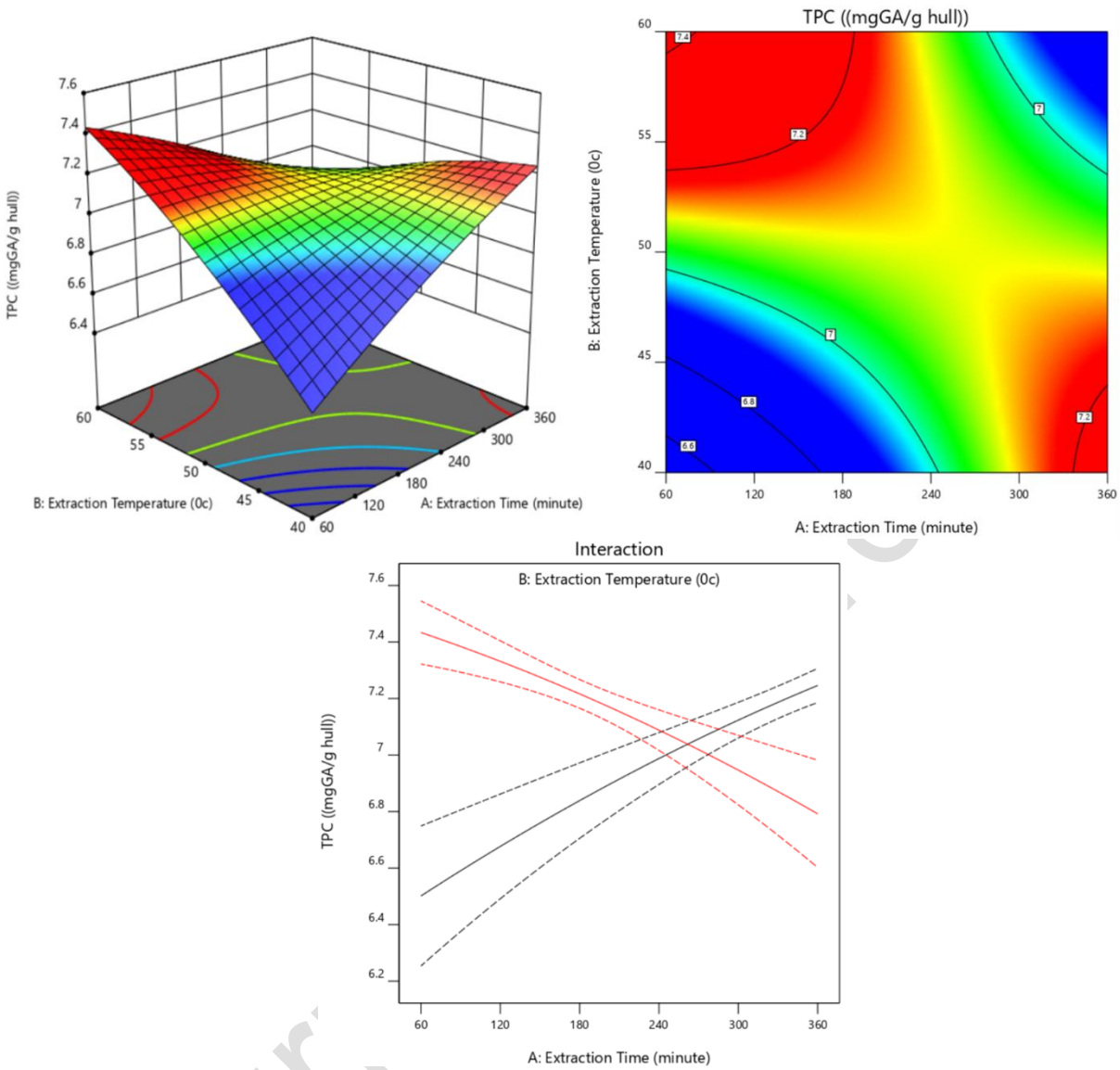


Figure 1-A. Response surface (3D), contour, and interaction plots for total phenolic content (TPC, mg gallic acid equivalents/g PGH) as a function of extraction time (60–360 min) and extraction temperature (40–60 °C), at a fixed ethanol concentration of 50% (v/v).

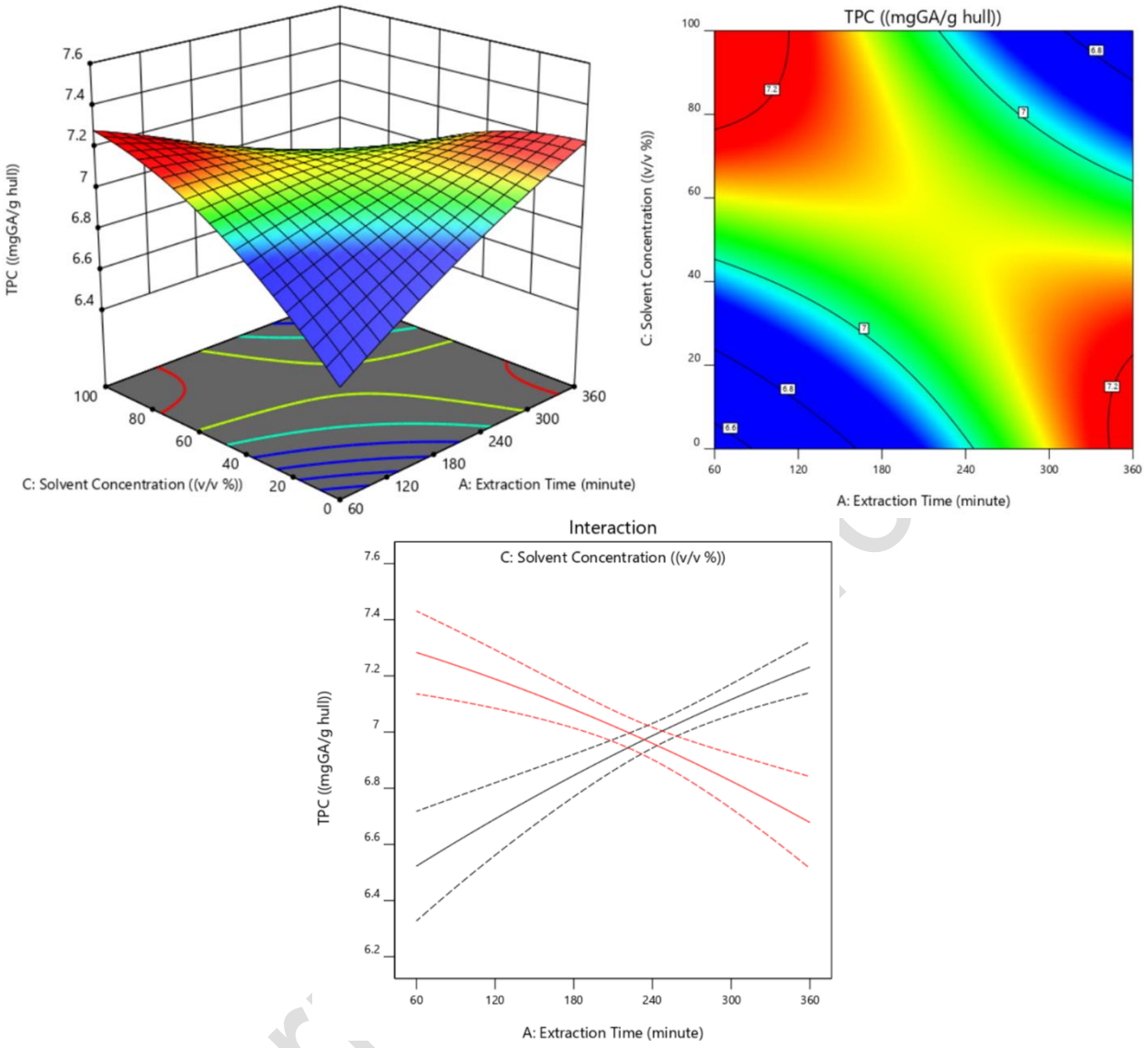


Figure 1-B. Response surface (3D), contour, and interaction plots for TPC (mg gallic acid equivalents/g PGH) as a function of ethanol concentration (0–100% v/v) and extraction time (60–360 min), at a fixed extraction temperature of 50 °C.

Jour

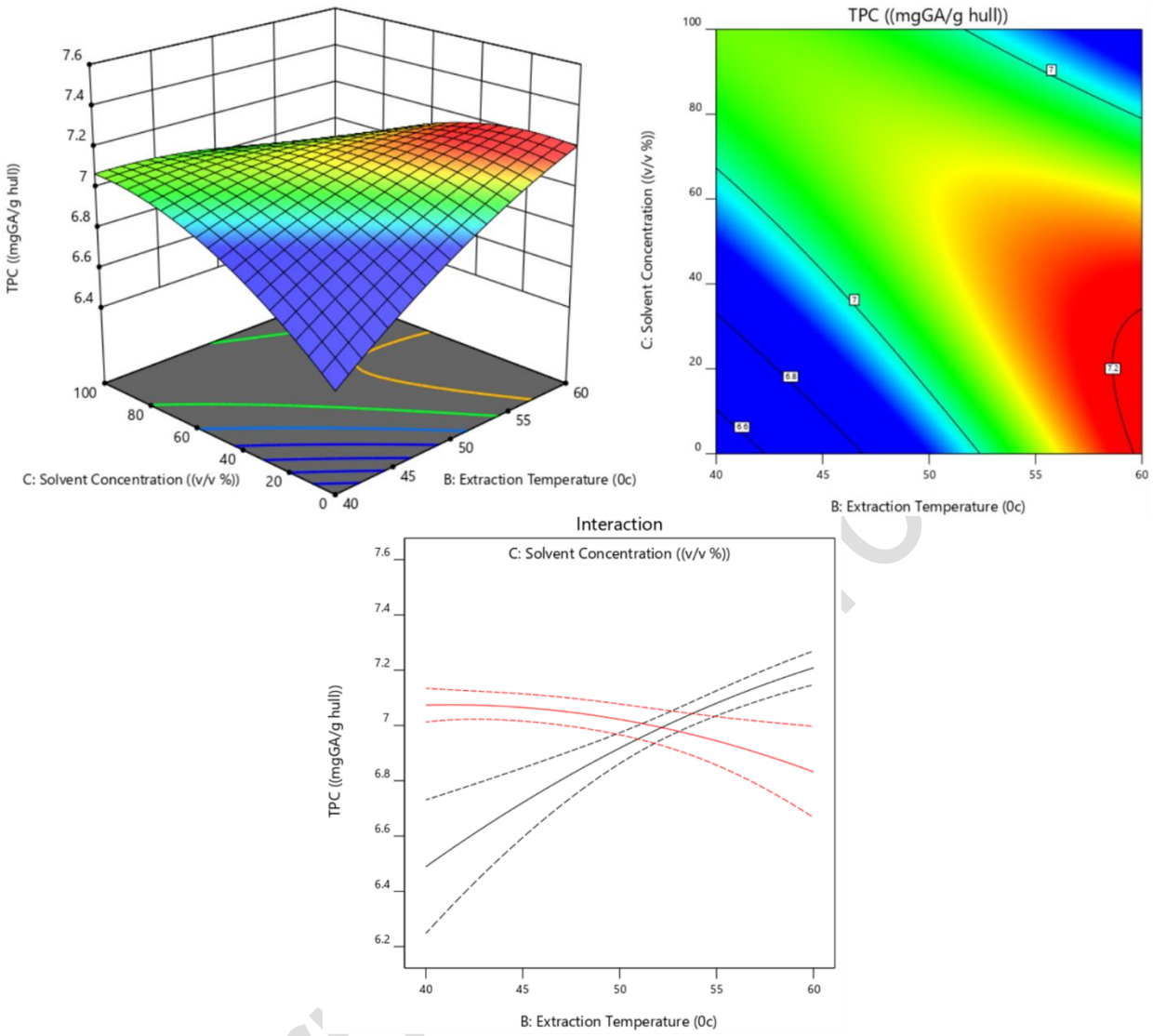


Figure 1-C. Response surface (3D), contour, and interaction plots for TPC (mg gallic acid equivalents/g PGH) as a function of ethanol concentration (0–100% v/v) and extraction temperature (40–60 °C), at a fixed extraction time of 210 min.

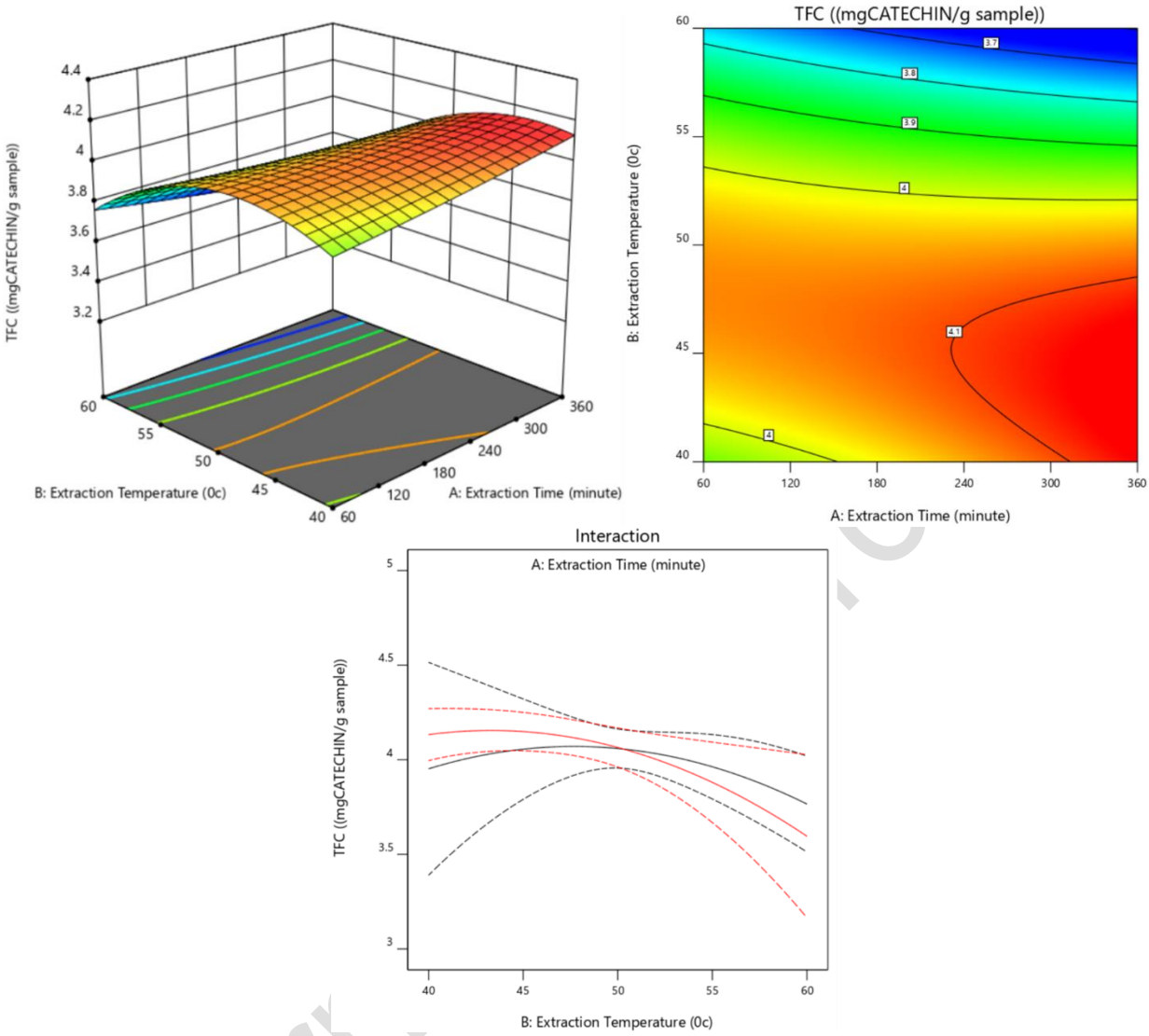


Figure 2-A. Response surface (3D), contour, and interaction plots for total flavonoid content (TFC, mg CATCHIN equivalents/g PGH) as a function of extraction time (60–360 min) and extraction temperature (40–60 °C), at a fixed ethanol concentration of 50% (v/v). Note: the time × temperature interaction was not statistically significant (p = 0.4918); the surface is shown for descriptive purposes.

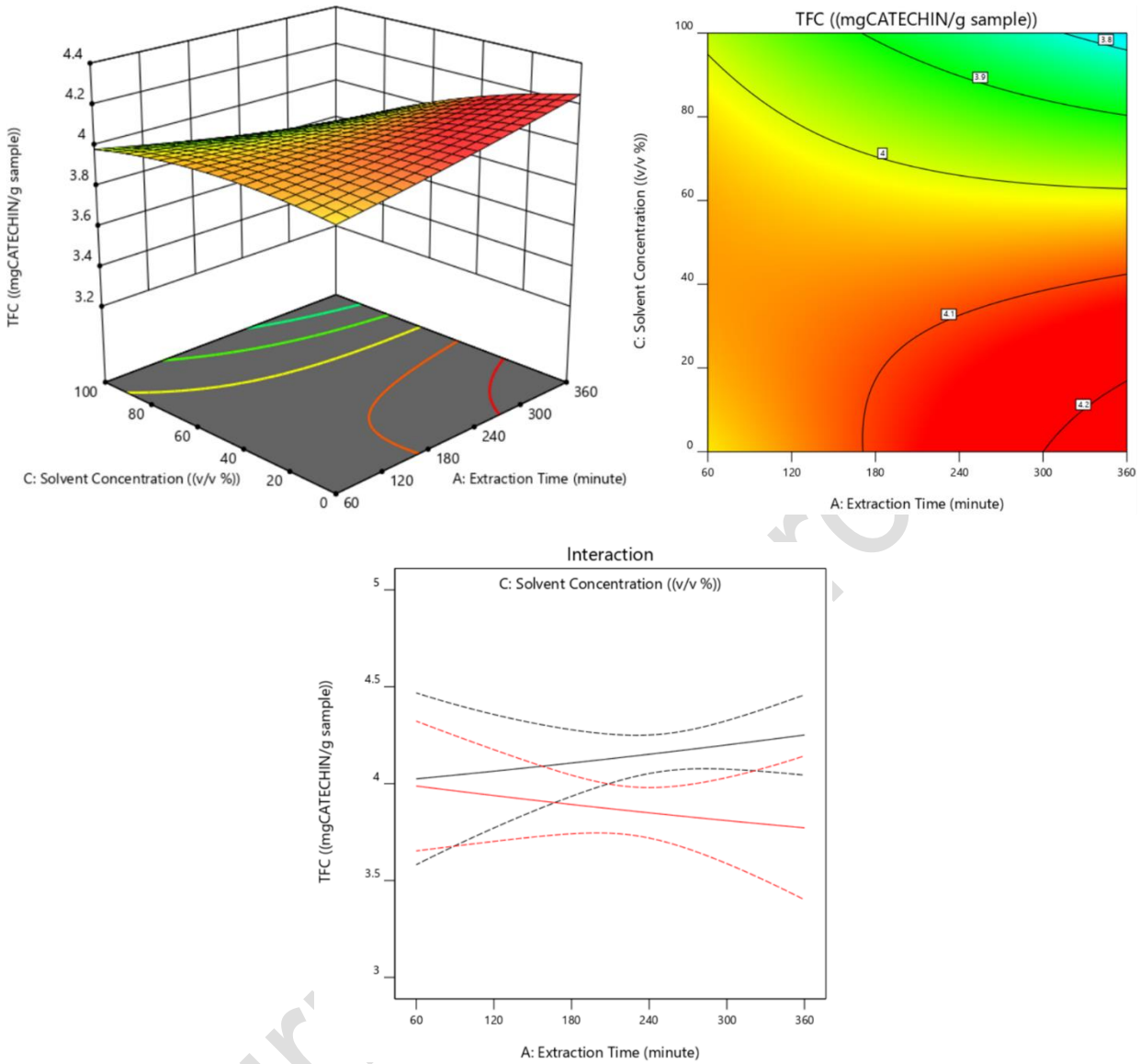


Figure 2-B. Response surface (3D), contour, and interaction plots for TFC (mg CATCHIN equivalents/g PGH) as a function of ethanol concentration (0–100% v/v) and extraction time (60–360 min), at a fixed extraction temperature of 50 °C. Note: the time × ethanol concentration interaction was not statistically significant (p = 0.3933).

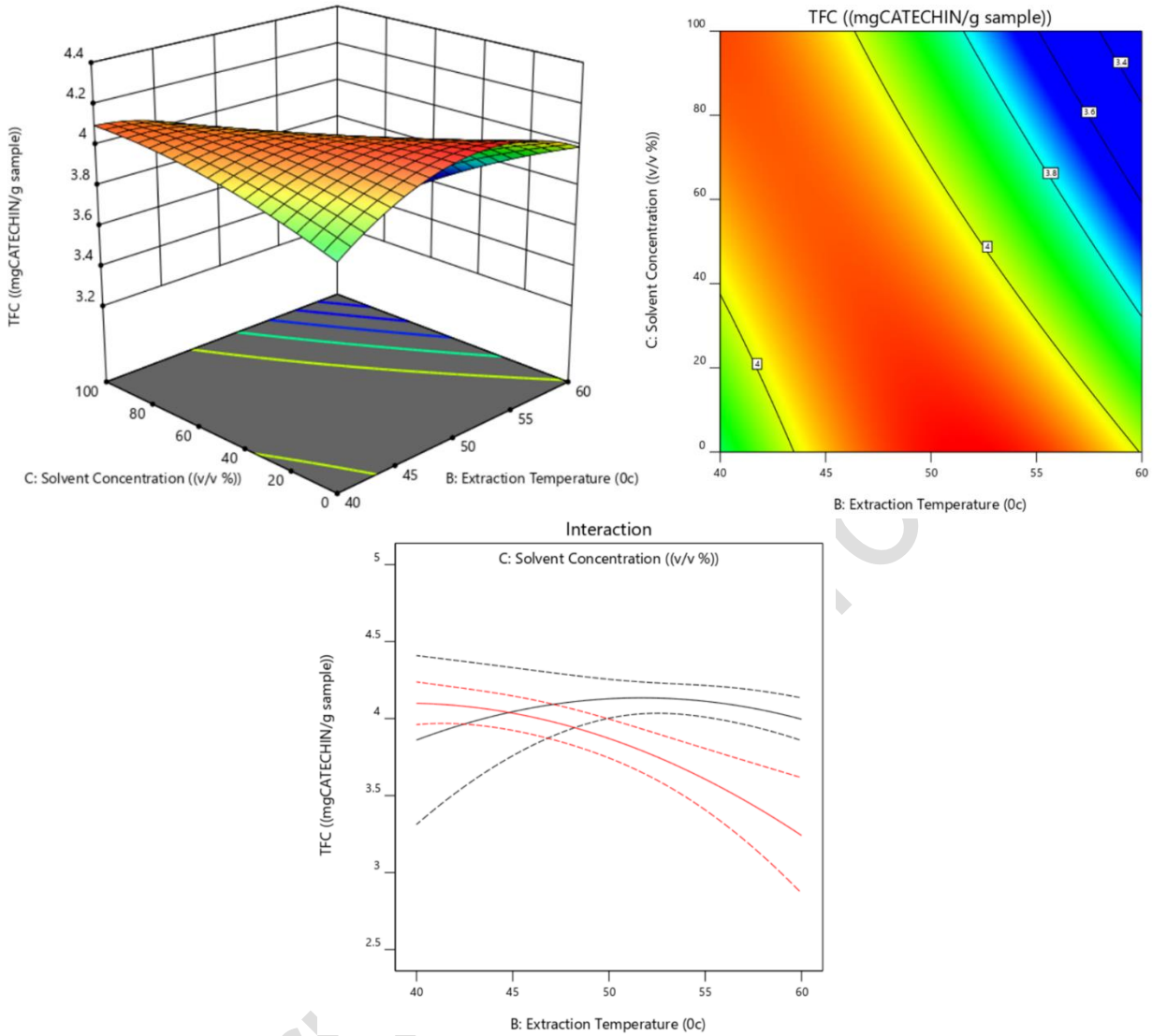


Figure 2-C. Response surface (3D), contour, and interaction plots for TFC (mg CATCHIN equivalents/g PGH) as a function of ethanol concentration (0–100% v/v) and extraction temperature (40–60 °C), at a fixed extraction time of 210 min. The temperature × ethanol concentration interaction was statistically significant (p = 0.0488).

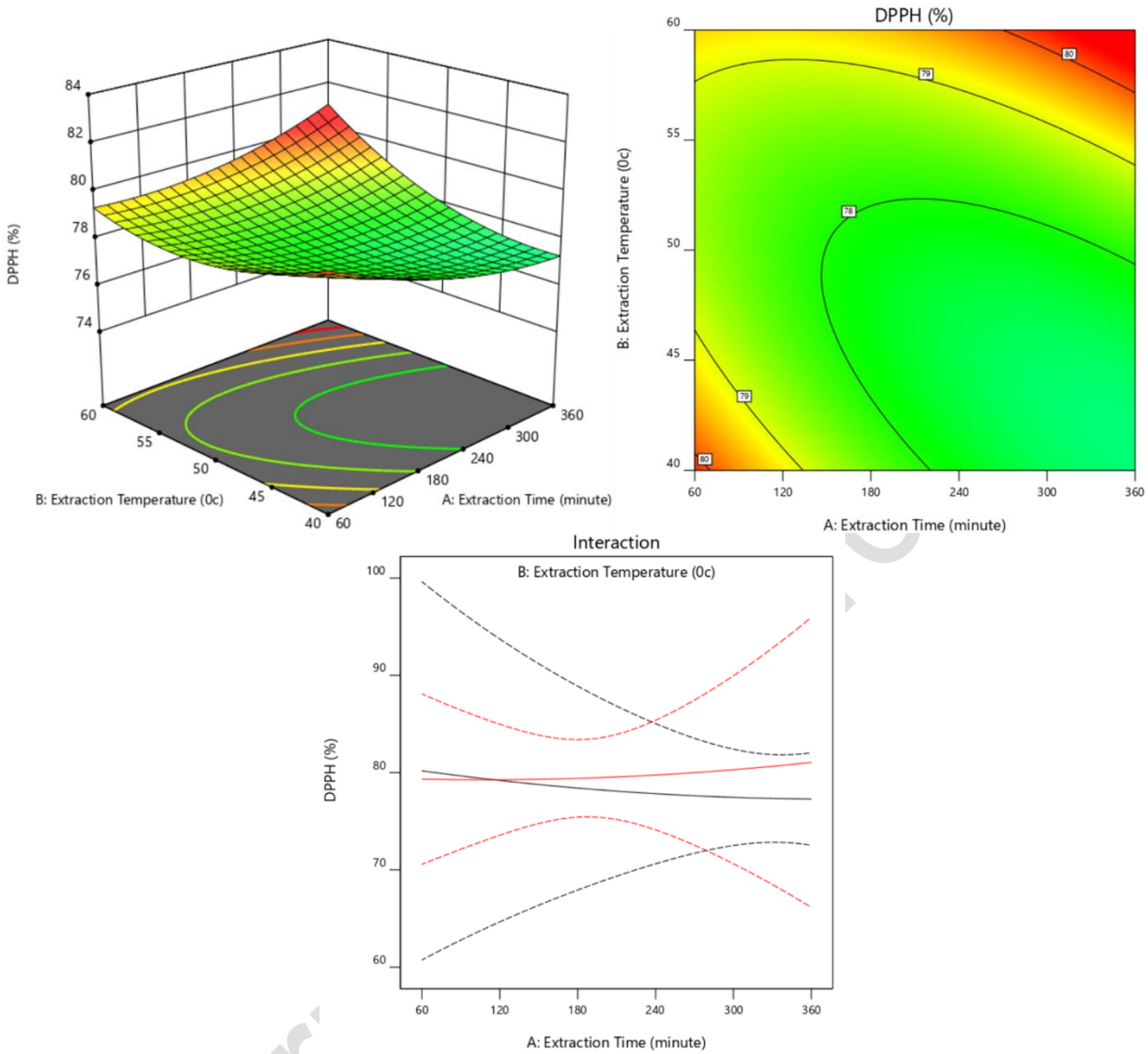


Figure 3-A. Response surface (3D), contour, and interaction plots for DPPH radical-scavenging activity (%) as a function of extraction time (60–360 min) and extraction temperature (40–60 °C), at a fixed ethanol concentration of 50% (v/v). The DPPH regression model was not statistically significant ($p = 0.6312$; $R^2 = 0.5934$); these plots are therefore presented as descriptive visualizations of the experimental data and not as predictive models.

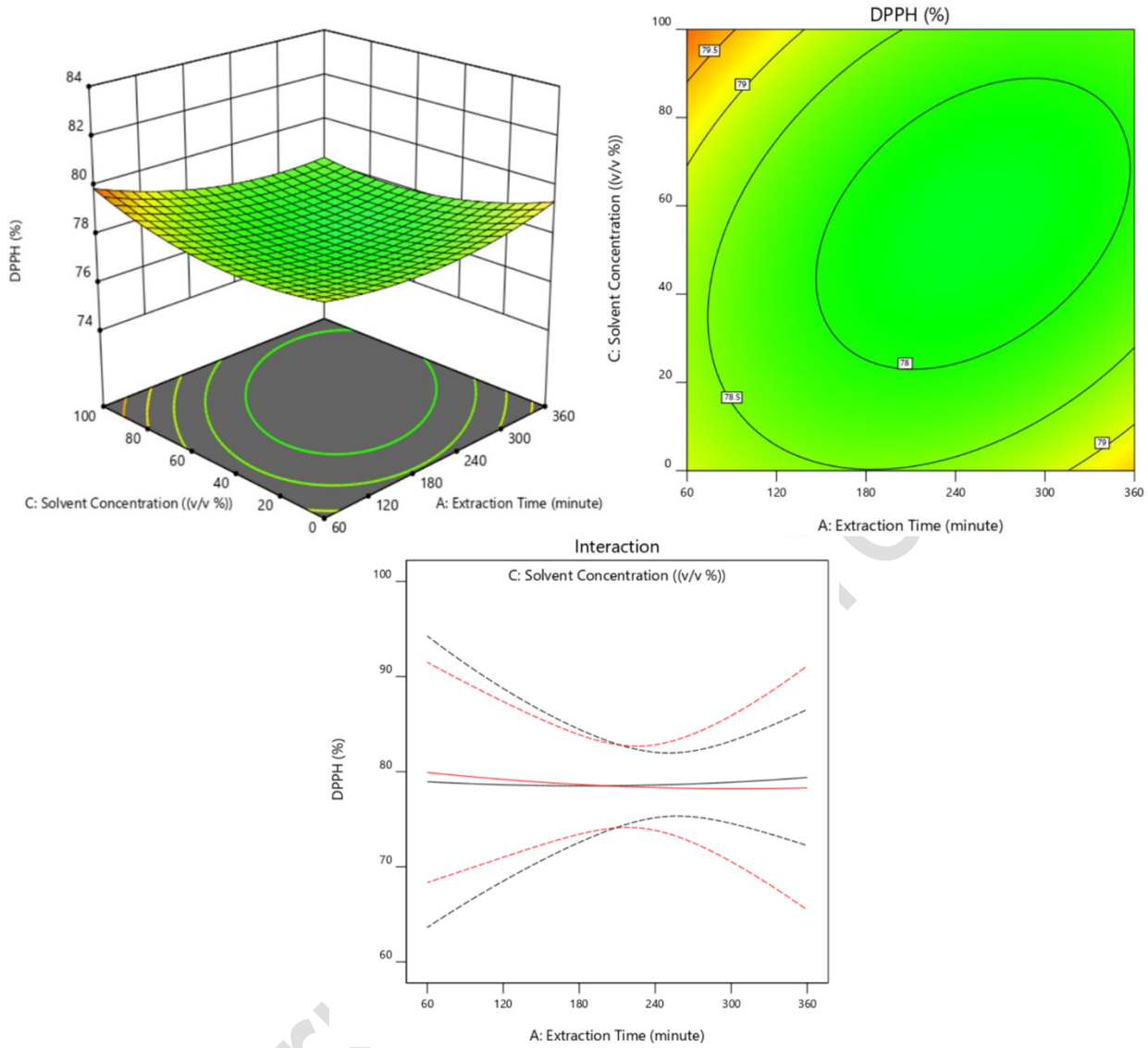


Figure 3-B. Response surface (3D), contour, and interaction plots for DPPH radical-scavenging activity (%) as a function of ethanol concentration (0–100% v/v) and extraction time (60–360 min), at a fixed extraction temperature of 50 °C. The DPPH model was not statistically significant ($p = 0.6312$; $R^2 = 0.5934$); plots are descriptive only.

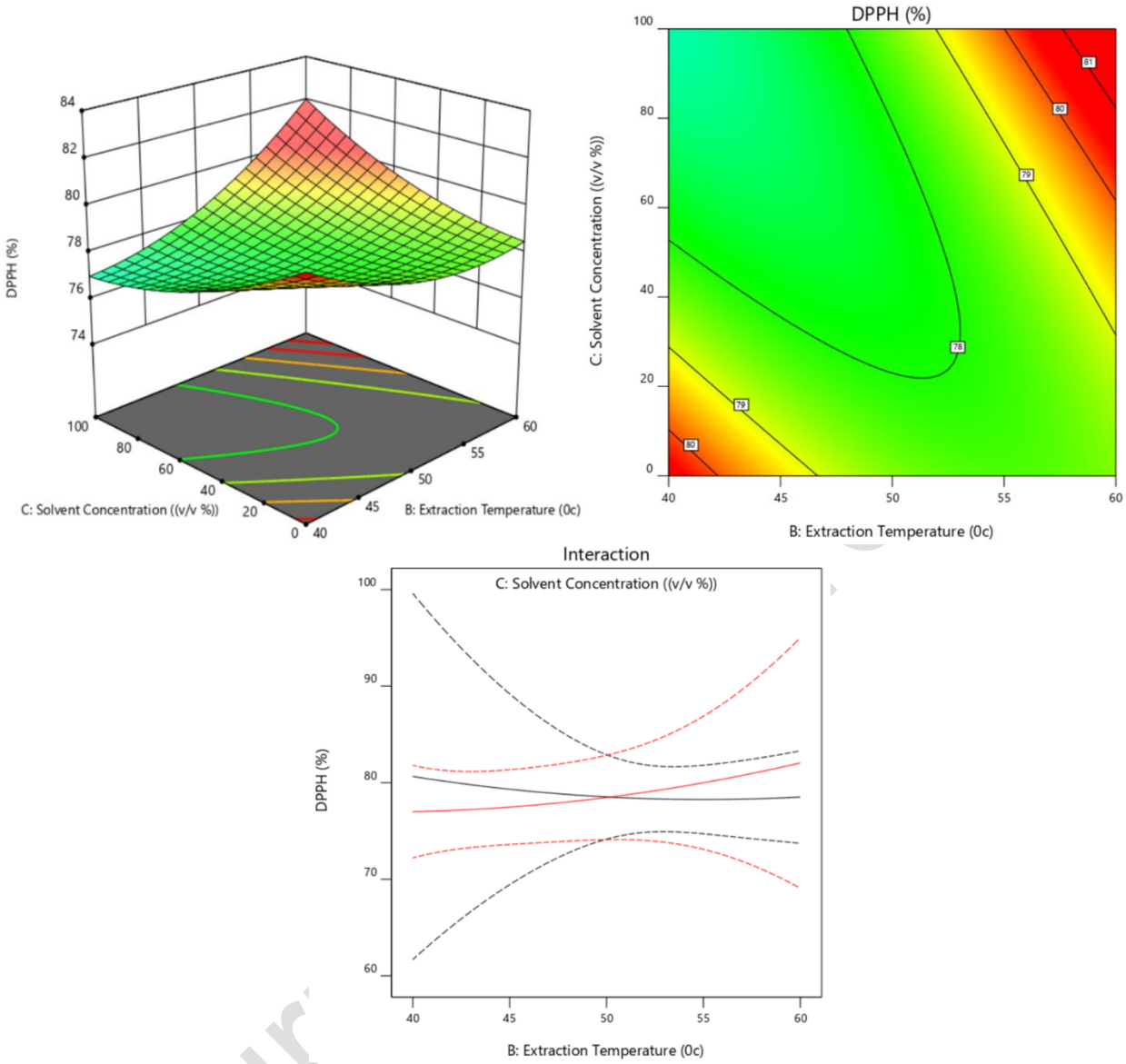


Figure 3-C. Response surface (3D), contour, and interaction plots for DPPH radical-scavenging activity (%) as a function of ethanol concentration (0–100% v/v) and extraction temperature (40–60 °C), at a fixed extraction time of 210 min. The DPPH model was not statistically significant ($p = 0.6312$; $R^2 = 0.5934$); plots are descriptive only.

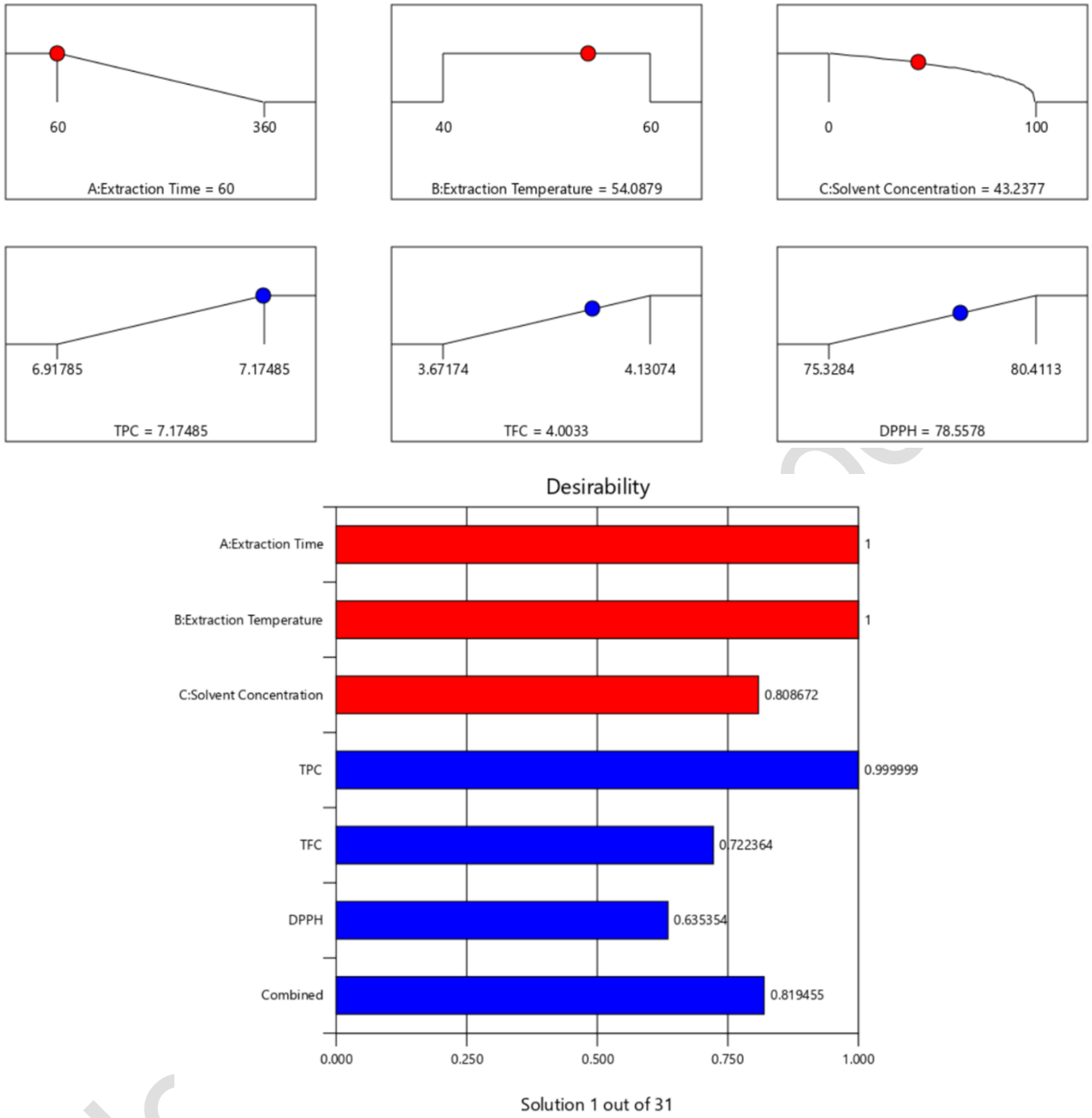


Figure 4. Numerical multi-response optimization of UAE conditions based on TPC and TFC using the desirability function. The predicted optimum was 60 min, 54.07 °C, and 43.33% (v/v) ethanol, with an overall desirability of 0.819 and predicted values of 7.175 mg GAE/g (TPC) and 4.003 mg CATCHIN/g (TFC). DPPH radical-scavenging activity was excluded from the optimization because its regression model was not statistically significant ($p = 0.6312$; $R^2 = 0.5934$).