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Sustainable recovery of bioactive compounds from *Nelumbo nucifera* using ultrasound-assisted extraction optimized through response surface methodology

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Nelumbo nucifera (lotus flower) is a promising natural source of phenolic compounds, flavonoids, and alkaloids, recognized for their potent bioactive and antioxidant properties. This study optimized a green extraction approach, ultrasound-assisted extraction (UAE) to enhance the yield of these functional compounds while minimizing environmental impact. A response surface methodology (RSM) using a Box–Behnken design (BBD) was employed to investigate the effects of extraction time (10, 25, and 40 min), temperature (50, 60, and 70 °C), and ultrasonic full power rate at 20.5 kHz (40, 65, and 90% power rate). Optimal conditions, 10 min at 57.45 °C and 90% (18.45 kHz), achieved a high total phenolic content (TPC) of 114.52 mg GAE per g, total flavonoid content (TFC) of 0.057 mg QE per g, and strong antioxidant activity (DPPH, 90.91%, ABTS, 91.61%, and ferric reducing antioxidant power, FRAP, 0.072 mg TE per g). The process demonstrated excellent energy efficiency, with reduced energy consumption (617.97 kJ) compared to conventional thermal extraction methods. Thermodynamic analysis confirmed spontaneous extraction of phenolic and antioxidant compounds (negative ΔG), while entropy changes (ΔS) indicated process irreversibility and thermal sensitivity. Overall, UAE operation reduced solvent, saved energy, and effectively preserved heat-sensitive bioactive compounds, highlighting the environmental advantages of UAE. This study underscores UAE as a sustainable and scalable technique for extracting functional compounds, offering considerable potential for applications in food, nutraceutical, and pharmaceutical industries committed to green processing technologies.

Sustainability spotlight

This study highlights ultrasound-assisted extraction (UAE) as a sustainable alternative to conventional thermal methods for recovering bioactive compounds from *Nelumbo nucifera*. By optimizing low-temperature, energy-efficient conditions, the process minimizes environmental impact while preserving heat-sensitive phytochemicals. The reduced energy consumption and solvent use position UAE as a green, scalable solution for the food, nutraceutical, and pharmaceutical industries, aligning with global goals for sustainable and eco-friendly processing technologies.

Introduction

The lotus flower (*Nelumbo nucifera*) holds cultural significance in certain religions, leading to significant waste accommodation during religious festivals. These wasted materials were estimated to range between 10 and 20 tons per event.[1,2] Despite this, only 10–15% of the waste is recycled, while improper disposal poses significant environmental risks, including water pollution and eutrophication.[3,4] Community-led initiatives in locations such as Varanasi have improved waste management, achieving recycling rates of up to 30%.[5] However, more robust policies and educational efforts are required to implement effective waste disposal strategies at religious sites. Moreover, improved management of lotus flower waste presents considerable economic potential, with estimates suggesting it could be worth millions.[3]

In addition to its cultural and environmental relevance, the lotus flower has gained attention for its rich content of bioactive compounds, particularly phenolics, flavonoids, and alkaloids, which exhibit antibacterial, antidiabetic, and antioxidant properties.[1,2] Recent studies have highlighted the diverse applications of lotus flower extract in the food industry,

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emphasizing its nutritional value, antioxidant activity, and sensory benefits.[1,2] As a plant-based ingredient, lotus flower extract aligns with the rising demand for vegan products and has been successfully incorporated into vegan pastries.[3] Furthermore, consumer preference studies indicate that its vibrant coloration enhances the visual appeal of beverages, positively influencing consumer perception. The extract's natural preservative properties extend the shelf life of food products while preventing microbial contamination. Additionally, its phenolic content offers potential health benefits, including anti-inflammatory and anti-obesity effects, supporting its classification as a functional food ingredient.[6] Studies have demonstrated that the incorporation of lotus extract into products such as bread, dairy, and beverages enhances both nutritional value and sensory attributes.[7]

Conventional methods for obtaining extracts from food materials often involve maceration or Soxhlet extraction, both of which require specific sample preparation steps, varying temperatures, and extended extraction times. These techniques typically rely on large quantities of organic solvents, which can lead to the breakdown or loss of bioactive compounds.[8] Additionally, the potential toxicity and safety risks associated with residual solvents raise concerns about the suitability of these extracts for practical applications.

As a result, there is a growing emphasis on developing sustainable and eco-friendly extraction techniques that minimize environmental impact while ensuring safer product outcomes. Many of these methods require less energy and help preserve the stability of bioactive compounds.[9] By adopting green extraction approaches, it is possible to enhance the yield of valuable phytochemicals from plant materials while simultaneously reducing health risks and lowering energy consumption.[10,11]

As interest in sustainable and eco-friendly extraction techniques grows, the need for innovative methods that promote extraction efficiency while lowering energy usage has become increasingly critical.

Ultrasound-assisted extraction (UAE) is an environmentally friendly technique that utilizes high-frequency sound waves above 20 kHz to enhance the extraction process.[12] This method is recognized for its efficiency, requiring less time and fewer materials while preserving the nutritional quality of extracts. Additionally, UAE improves mass transfer and reduces energy consumption, allowing for a more effective extraction process compared to traditional methods.[13–15] When applied to food materials immersed in liquid or osmotic solutions, ultrasound induces alternating cycles of expansion and compression within the food structure, creating microscopic channels that facilitate better extraction.[16] Moreover, the cavitation effect generated by ultrasound leads to the formation and collapse of tiny bubbles, which can facilitate solvent penetration, disrupt cellular structures and cause physical modifications through shockwave generation.[17]

UAE demonstrated effectiveness in extracting phytochemicals from various food materials such as longan seeds,[18,19] lotus leaves,[20] hog plum,[21] apple peel,[22] lotus seed[23] and hemp seeds.[24]

To maximize the effectiveness of ultrasound-assisted extraction (UAE), optimizing extraction conditions and analyzing thermodynamic parameters are essential. Several models, including general regression models, have been developed to assess how operational factors influence the extraction of bioactive compounds.[25–27] Examining thermodynamic parameters provides valuable insights into the spontaneity of the extraction process. Additionally, studying the kinetics and thermodynamics of UAE is crucial for understanding its fundamental mechanisms and evaluating its potential for large-scale industrial applications.[28,29]

To the best of the authors' knowledge, no previous study has specifically examined the optimal extraction conditions for lotus flower extract (LFE) using water as a green solvent, considering energy consumption, extraction yield, phytochemical content, and thermodynamic properties of the ultrasound-assisted extraction (UAE) process. Therefore, this study aimed to evaluate the effects of exposure time, temperature, and ultrasonic power rate on energy consumption, extraction yield, total phenolic content, total flavonoid content, and antioxidant activity using different assays during UAE. Additionally, the research sought to identify the optimal UAE conditions for LFE and to analyze the thermodynamic properties governing the extraction process.

2. Materials and methods

2.1 Chemicals and reagents

The experiment employed high-purity chemicals, reagents, and solvents of analytical grade. The utilized materials and substances include Folin&Ciocalteu's phenol reagent, gallic acid, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPT), Trolox, and quercetin from Sigma-Aldrich, Germany, 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Fluka in U.S.A., and 2,2′-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) from AK Scientific, U.S.A.

2.2 Preparation of lotus flower extract (LFE)

Lotus flowers were procured from a lotus flower garden in Saraburi, Thailand. They were cleaned and dried using a tray dryer at 50 °C and 10% humidity until the moisture content reached below 10% ± 1.50. The dried sample was ground using lab grinder 50-mesh sieves (Pertan, 120, Finland). The obtained sample was kept at 4 °C in an aluminum foil-laminated bag for further use. The extraction process was carried out with an ultrasonic homogenizer (JY89-IIIDN, T.Science Trading Limited Partnership, Maladamez, Thailand) with an input power of 1200 W (20.5 kHz). The extraction conditions included ultrasonic power rate levels of 40%, 60%, and 90% with extraction times of 10, 25 and, 40 min, as well as three different temperature level settings: 50 °C, 60 °C, and 70 °C. The extract was filtered with a Büchner funnel using Whatman No.1 filter paper for removal of cell debris and any course particles. The obtained filtrate was evaporated to eliminate the solvent, then kept at 4 °C until use.

2.3 Calculation of the ultrasonic energy consumption (EC) of UAE

The ultrasonic energy consumption, supplied electrically was transformed into mechcanical vibration (E_v) by the ultrasonic probe. This vibration propagated through the solvent acting as a medium leading to microbubble formation (cavitation) and an increase in the medium's temperature. This process can be understood as the translocation of electrical energy into vibration and subsequently into cavitation. The ultrasonication process involved three key energy conversions: electrical to acoustic to cavitation (sonication), which could be quantified using eqn (1)–(3), as discussed by AlYammahi *et al.* (2022).[30]

$$E_e = P \times t \qquad (1)$$

$$E_a = m \times C_p \times \Delta T \qquad (2)$$

$$EC = E_e - E_a \qquad (3)$$

where P is the power supplied (W), t is the extraction time (s), m is the mass of the medium (g), C_p is the specific heat of water (4.2 J g^{-1} °C), and ΔT stands for temperature change in the medium.

2.4 Determination of the extraction yield (EY) of LFE

Based on the method described in ref. 31, around 10 mL of each extract was placed in a Petri dish and dried in a convection oven (Memmert, UN, Schwabach, Germany) at 50 ± 5 °C until a stable weight was obtained. The extraction yield was measured by determining the proportion of the extracted solid mass relative to the initial sample weight.

2.5 Determination of total phenolic content (TPC) of LFE

LFE sample of 0.1 mL was combined with Folin-Ciocalteu reagent in 7% Na_2CO_3, and incubated for 90 min. The absorbance (Biospectrometer basic, 6135CQ201735, Germany) was measured at 630 nm.[32] The total phenolic content was determined using a gallic acid standard curve (absorbance = 0.0179 concentration + 0.112, r^2 = 0.999) and expressed as milligram gallic acid equivalents (mg GAE) per gram of extract.

2.6 Determination of the total flavonoid content (TFC) of LFE

LFE was mixed with 25 μL of 1 mol L^{-1} sodium acetate solution, 25 μL of 10% w/v aluminum chloride solution, and 1940 μL of 50% v/v ethanol. After thorough mixing, the absorbance of the solution was measured at 415 nm.[33] The flavonoid content was determined using a quercetin standard curve (absorbance = 1.3362 concentration + 0.023, r^2 = 0.999) and expressed as milligram quercetin equivalents (mg/QE) per gram of extract.

2.7 Determination of antioxidant activity using DPPH scavenging assay

Two milliliters of LFE were combined with DPPH solution (0.1 mmol L^{-1} DPPH in ethanol) and incubated in the dark for 30 min. The absorbance was measured at 517 nm to determine the percentage of scavenging activity.[33]

2.8 Determination of antioxidant activity using ABTS scavenging assay

One milliliter of LFE was mixed with ABTS solution, prepared by combining 7 mmol L^{-1} ABTS with 2.45 mmol L^{-1} $K_2S_2O_8$ in a 1 : 1 v/v ratio and incubating in the dark for 12 hours. The mixture was incubated in the dark for 6 min before measuring absorbance at 734 nm to determine the percentage of scavenging activity.[33]

$$\% \text{ activity} = 1 - \frac{A_{sample}}{A_{control}} \times 100 \qquad (4)$$

where $A_{control}$ denotes the mixture of methanol and DPPH/ABTS solution, while A_{sample} refers to the solution containing the extract and DPPH/ABTS solution.

2.9 Determination of ferric ion reducing antioxidant power (FRAP)

LFE sample of 0.1 mL was mixed with 2 mL of distilled water, followed by the addition of a working solution composed of TPTZ (10 mmol L^{-1}), sodium acetate (300 mmol L^{-1}), and $FeCl_3$ (20 mmol L^{-1}) in a ratio of 10 : 1 : 1 v/v/v. The resulting mixture was thoroughly mixed and its absorbance was measured at 596 nm.[33] The FRAP value was calculated using a Trolox standard curve (absorbance = 11.05concentration − 0.6784, r^2 = 0.996) and expressed as milligram Trolox equivalents (mg TE) per gram of extract.

2.10 Thermodynamic analysis of UAE

The relationship between temperature and power consumption was analyzed by calculating specific enthalpy, entropy, and Gibbs free energy. According to thermodynamic principles, these parameters were determined using the Van't Hoff equation (eqn (5)), Krishnan and Rajan equation (eqn (6)), and Gibbs free energy equation (eqn (7)) (Abishli, Albarri, & Şahin, 2021).[34]

$$K_e = \frac{Y_s}{(Y_{max} - Y_s)} \qquad (5)$$

$$\ln K_e = \left(\frac{\Delta H}{R}\right) \times \frac{1}{T} + \frac{\Delta S}{R} \qquad (6)$$

$$\Delta G = \Delta H - T(\Delta S) \qquad (7)$$

where K_e is the equilibrium constant rate, Y_s is the bioactive compound yield using distilled water, Y_{max} is the maximum bioactive compound yield using methanol, ΔG is the Gibbs free energy (kJ mol^{-1}), ΔH is the enthalpy change (kJ mol^{-1}), ΔS is the entropy change (kJ mol^{-1} K^{-1}), T is the absolute temperature (K), and R is the universal gas constant (8.314 J mol^{-1} K).

2.11 Experimental design

All experiments were conducted in triplicate and data are presented as mean ± standard deviation. The experimental setup

Table 1 Actual and coded value of independent variables that will be assumed in the RSM

Independent variables	Code symbols	Level		
		−1	0	1
Time (min)	X_1	10	25	40
Temperature (°C)	X_2	50	60	70
Power rate (%)	X_3	40	65	90

followed a complete three-level, three-factor (3^3) factorial design, as outlined in Table 1. In this study, response surface methodology (RSM) was employed to analyze and optimize the extraction parameters. A Box–Behnken Design (BBD) (Table 1) was utilized to systematically investigate the effects of key extraction factors, including extraction time, temperature, and ultrasonic power on the overall yield of the bioactive compounds and antioxidant activities (DPPH, ABTS, and FRAP assays). The independent variables investigated were: three extraction durations (x_1: 10, 25, and 40 min), ultrasonic temperatures (x_2: 50 °C, 60 °C, and 70 °C), and ultrasonic power ratio (x_3: 40%, 65%, and 90%). Experiments at the center point (10 min, 60 °C, and 65%) were conducted in quintuplicate. The dependent responses evaluated included energy consumption (EC), extraction yield (EY), total phenolic content (TPC), total flavonoid content (TFC), DPPH and ABTS radical scavenging activity, and ferric ion reducing antioxidant power (FRAP) of LFE.

To predict the responses under various conditions, the second-order polynomial model, as shown in eqn (8) and proposed by Firky *et al.*, 2024,[19] was utilized. Non-linear regression analysis and analysis of variance (ANOVA) were applied to determine the effects of the independent variables on the dependent variables. Significance was evaluated based on *p*-values, where values below 0.05 were considered significant. Additionally, an R^2 value of at least 0.80 was used as a criterion to confirm that the regression model adequately described the variability and variance in the different properties.[35] Minitab18,

Origin Pro 2016 software, and Design – Expert® software, version 13, from Stat-Ease, Inc., Minneapolis, MN, USA were used for experimental design and data analysis.

$$y_n = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i=1}^{k} a_{ii} x_i x_i + \sum_{i=1}^{k-1} \sum_{i=1}^{k} a_{ij} x_i x_i \tag{8}$$

In the equation, y_n represents the predicted response, while x_i and x_j are the input predictors. The term a_0 refers to the intercept of the model, and a_0, a_i, a_{ii} and a_{ij} represent the linear, quadratic coefficients, and cross-product terms, respectively. The variable k indicates the number of input predictors which in this case is three ($k = 3$ variables). These components together define the relationship between the input factors and the predicted outcomes in the experimental model (Table 2).

3. Results and discussion

3.1 Effect of UAE conditions on energy consumption

The energy consumption of the system was calculated using eqn (1)–(3), which describe the transformation between electrical energy (E_e) and seismic energy generated by the ultrasonic probe (E_a). These equations were employed to assess the overall energy consumption (EC) of the system. The obtained results are presented in Table 1. The maximum energy consumption recorded was 2559.66 kJ, observed under conditions of 40 min extraction time, 60 °C temperature, and a power rate of 90% (18.45 kHz). In contrast, the minimum energy consumption was 426.42 kJ, occurring at 10 min extraction time, 70 °C temperature, and a power rate of 65% (13.325 kHz). The results suggest that temperature does not have a direct effect on energy consumption in the UAE process when comparing the maximum and minimum energy values. This finding is consistent with previous studies on the extraction of flavonoids from rowanberry fruit.[36] Furthermore, the results were validated using a linear regression model (Table 4), which demonstrated a strong statistical significance ($P < 0.001$) for the relationship between extraction time and power rate. However, temperature did not exhibit a significant effect. Additionally, the interaction between time and power rate, analyzed using a cross-product effect, showed high statistical significance ($P < 0.001$).

These findings provide empirical evidence that extraction time and power rate are the primary determinants of energy consumption in the UAE process. Both factors exhibit a direct proportional relationship with yield and extraction efficiency. Extended exposure time enhances the ability of seismic waves to effectively disrupt cell structures, aligning with the findings of Yogesh *et al.*,[37] who suggested an optimal exposure time of approximately 5–15 min. Although an increase in temperature can enhance cell disruption and compound diffusion, exceeding a certain threshold may reduce extraction efficiency. This aligns with the research of Wang *et al.*,[38] which indicates that phenolic compounds and anthocyanins begin to degrade at temperatures exceeding 45 °C due to their thermolabile nature.

The linear term of this regression model (Table 5) demonstrated a strong correlation between extraction time and power

Table 2 Box–Behnken design (BBD) for lotus flower extract using UAE

Conditions

Run	X_1-time (min)	X_2-temperature (°C)	X_3-power rate (%)
1	10	50	65
2	10	70	65
3	40	50	65
4	40	70	65
5	25	50	40
6	25	70	40
7	25	50	90
8	25	70	90
9	10	60	40
10	40	60	40
11	10	60	90
12	40	60	90
13	25	60	65

Table 3 Lotus flower extract values from 13 experiments obtained with BBD for TPC, TFC, antioxidant activities (DPPH, ABTS, and FRAP), EC, and EY

Run	X_1 (time)	X_2 (°C)	X_3 (%)	TPC (mg GAE per g)	TFC (mg QE per g)	DPPH (% scavenging)	ABTS (% scavenging)	FRAP (mg TE per g)	Energy consumption (EC) (kJ)	Extraction yield (EY) (%)
1	10	50	65	94.86 ± 8.05	0.044 ± 0.002	91.82 ± 0.96	71.85 ± 1.30	0.051 ± 0.005	444.90 ± 1.08	2.30 ± 0.48
2	10	70	65	89.11 ± 1.30	0.031 ± 0.003	92.14 ± 1.03	74.99 ± 1.50	0.044 ± 0.004	426.42 ± 1.16	2.52 ± 0.52
3	40	50	65	80.14 ± 1.51	0.036 ± 0.003	92.20 ± 1.09	85.36 ± 1.68	0.048 ± 0.004	1848.90 ± 1.23	3.12 ± 0.54
4	40	70	65	110.31 ± 1.68	0.047 ± 0.003	92.53 ± 1.42	92.86 ± 1.84	0.047 ± 0.003	1830.42 ± 1.28	3.37 ± 0.57
5	25	50	40	104.47 ± 1.84	0.043 ± 0.004	91.35 ± 1.19	84.92 ± 1.99	0.042 ± 0.004	696.90 ± 1.33	2.34 ± 0.59
6	25	70	40	114.89 ± 9.75	0.051 ± 0.003	90.15 ± 1.23	97.59 ± 2.12	0.068 ± 0.004	678.42 ± 1.39	2.82 ± 0.61
7	25	50	90	91.90 ± 1.96	0.039 ± 0.001	91.73 ± 7.78	84.99 ± 2.57	0.072 ± 0.004	1596.90 ± 1.42	2.34 ± 0.62
8	25	70	90	110.98 ± 2.10	0.039 ± 0.002	91.40 ± 1.29	95.24 ± 2.38	0.051 ± 0.003	1578.42 ± 1.46	2.88 ± 0.64
9	10	60	40	100.36 ± 2.17	0.045 ± 0.001	90.77 ± 1.18	94.31 ± 1.90	0.055 ± 0.002	255.66 ± 1.34	2.33 ± 0.59
10	40	60	40	122.51 ± 1.60	0.064 ± 0.003	88.46 ± 1.21	98.01 ± 8.32	0.076 ± 0.002	1119.66 ± 1.36	3.23 ± 0.60
11	10	60	90	123.44 ± 10.47	0.056 ± 0.002	91.61 ± 1.23	97.91 ± 2.09	0.062 ± 0.004	615.66 ± 1.19	2.52 ± 0.61
12	40	60	90	126.54 ± 2.17	0.048 ± 0.002	91.10 ± 1.06	95.68 ± 2.17	0.045 ± 0.005	2559.66 ± 1.24	3.22 ± 0.53
13	25	60	65	104.750 ± 1.80	0.042 ± 0.003	91.56 ± 1.11	96.05 ± 1.59	0.044 ± 0.004	1137.66 ± 1.26	2.43 ± 0.55

rate, with a coefficient of determination ($R^2 = 0.95$) indicating a good fit. Additionally, response surface methodology (RSM) analysis was employed to visualize the relationship between energy consumption, extraction time, and temperature. The resulting 3D surface plot (Fig. 1K and L), generated using a fixed power rate from the predictive model, is presented in Fig. 1. The constants of the predictive model that adequately describes the energy consumption of UAE can be found in Table 4.

3.2 Effect of UAE conditions on extraction yield

The effect of ultrasonic-assisted extraction (UAE) factors on extraction yield (EY) was evaluated to assess the impact of soluble solid compounds in LFE. The extraction yield results, presented in Table 3, ranged from 2.30% to 3.37%. The minimum yield was observed at an exposure time of 10 min, a temperature of 50 °C, and a power rate of 65%, whereas the maximum yield was obtained at 40 min, a temperature of 70 °C, and the same power rate of 65%. As shown in Table 4, extraction time and power rate did not exhibit a direct effect on yield. However, temperature demonstrated a linear relationship with extraction yield ($P < 0.05$). This finding indicates that the interaction between time, temperature, and power rate was

statistically significant, as confirmed by the cross-product regression model ($P < 0.001$). These results highlight the influence of UAE parameters on the physicochemical properties of bioactive compound yield and suggest potential degradation with prolonged exposure to UAE treatment. This phenomenon aligns with the findings of Zhang and Zhu,[39,40] who reported molecular weight reduction in polyphenols due to the breakdown of polyphenolic compounds induced by ultrasonic energy. However, molecular weight loss may be dependent on specific UAE conditions. Furthermore, Xu et al.[41] observed that the loss of soluble solid compounds from blackcurrant during UAE led to an increase in antioxidant activity, enhanced DNA damage protection, and improved enzyme inhibition. These effects were attributed to enhanced hydrolysis and oxidation activity resulting from molecular fragmentation.

The quadratic regression model (Table 5) exhibited a strong fit ($R^2 = 0.943$). Additionally, response surface methodology (RSM) analysis was used to generate a 3D surface plot (Fig. 1M and N), illustrating the relationship between yield, extraction time, and temperature under a constant power rate. The constants of the predictive model that adequately describes the extraction yield of LFE can be found in Table 4.

Table 4 Analysis of variance for response surface regression model

Model	Variable	EC F-test	EC P-value	EY F-test	EY P-value	TPC F-test	TPC P-value	TFC F-test	TFC P-value	DPPH F-test	DPPH P-value	ABTS F-test	ABTS P-value	FRAP F-test	FRAP P-value
Linear	x_1	0.031	0.864	2.980	0.108	2.440	0.142	0.091	0.767	0.014	0.906	2.370	0.147	0.036	0.966
	x_2	175.760	<0.001[b]	14.250	0.002[a]	0.847	0.374	0.620	0.445	0.889	0.363	2.270	0.156	0.002	0.915
	x_3	72.220	<0.001[b]	0.076	0.788	0.095	0.763	0.833	0.378	2.940	0.110	0.002	0.964	0.012	0.766
Quadratic	x_1	0.470	0.523	0.060	0.313	5.880	0.020[a]	1.480	0.263	0.433	0.531	7.450	0.029[a]	0.010	0.925
	x_2	0.100	0.761	0.281	0.535	8.930	0.122	10.040	0.016[a]	0.096	0.764	7.140	0.032[a]	0.060	0.813
	x_3	0.060	0.822	−0.097	0.156	3.100	0.575	13.510	0.008[a]	7.06	0.032[a]	0.007	0.971	0.472	0.514
Cross-product	x_1	0.010	0.991	44.730	<0.001[b]	4.950	0.040[a]	1.440	0.292	2.650	0.131	14.440	0.002[a]	0.010	0.986
	x_2	54.080	<0.001[b]	180.140	<0.001[b]	0.710	0.518	1.060	0.390	0.830	0.472	5.690	0.029[a]	0.090	0.917
	x_3	22.220	0.001[a]	23.770	<0.001[b]	3.410	0.085	2.860	0.115	6.510	0.021[a]	2.440	0.149	2.400	0.153

[a] P-value < 0.05. [b] P-value < 0.001.

Table 5 Regression coefficients and their significance in regression equation

Bioactive compound	Regression coefficient model of the quadratic equation										
	Intercept	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2	R^2
TPC	105.75	6.74[a]	3.97	1.33	8.98[a]	2.16	−4.76	−11.9[a]	0.754	12.71[a]	0.8830
TFC	0.041	0.0009	0.0023[a]	−0.003[a]	0.006[b]	−0.002	−0.007[b]	−0.006[b]	0.0041[a]	0.008[b]	0.9700
DPPH	91.57	−0.0777	0.0368	0.3137[a]	−0.0630	0.0295	−0.0105	0.0952[a]	0.5757	−0.7542[a]	0.8524
ABTS	96.05	4.2[a]	4.11[a]	−0.126	1.09	−0.607	−1.48	−10.29[a]	−4.5[a]	4.92[a]	0.8740
FRAP	0.044	−0.0002	0.0005	−0.001	0.002	−0.012[b]	−0.009[b]	0.001	0.003	0.013[a]	0.8951
@EC	1137.66	702.00	−9.24	450	—	—	—	—	—	—	0.9502
EY	2.25[a]	0.186[b]	0.408	0.030	0.008	0.015	−0.050	0.175[a]	0.406[a]	0.177[a]	0.9430

[a] P-value < 0.05. [b] P-value < 0.001, @EC, linear regression.

3.3 Effect of UAE conditions on TPC

Phenolic compounds are essential bioactive constituents in plant extracts, associated with various biological activities, including antioxidant and anti-inflammatory properties.[20,32,42] The total phenolic content (TPC) results are presented in Table 3. The maximum TPC recorded was 126.54 mg GAE per g at an exposure time of 40 min, a temperature of 60 °C, and a power rate of 90% (18.45 kHz). In contrast, the minimum TPC was 80.14 mg GAE per g under the same exposure time but at a lower temperature of 50 °C and a power rate of 65% (13.325 kHz). The variation in TPC at the same exposure time suggests that extraction time does not have a direct influence on TPC, a finding further supported by the statistical analysis in Table 4. Time did not show a statistically significant effect on TPC (P > 0.05), whereas temperature exhibited a direct influence, with a quadratic effect (X_1, X_2, P < 0.05) and a significant interaction with time in the cross-product analysis (P < 0.05). These results confirm that both temperature and time influence TPC. The thermal effect in UAE facilitates solvent-induced conversion of mechanical waves into accelerated particle motion, generating minuscule bubbles that subsequently collapse, a process known as cavitation. This phenomenon enhances the release of cellular contents.[32,43] Although TPC remains relatively stable up to approximately 60 °C, it gradually declines beyond this point (Fig. 1A and B). An appropriate temperature can positively impact TPC by enhancing extraction efficiency (Fig. 1A). For instance, an increase of 20 °C resulted in approximately a 10% increase in TPC (Table 3). However, prolonged ultrasonic exposure can lead to complete cellular rupture, improving solvent solubility but also contributing to the thermal degradation of phenolic compounds, which may ultimately affect extraction efficiency.[44,45] Additionally, extended exposure at high temperatures accelerates the degradation of thermally sensitive phenolic compounds.[46] HPLC-DAD analysis of lotus flower extract identified various phenolic compounds with chlorogenic acid, ferulic acid, and coumarin[47] being predominant. Specifically, compounds like coumarin are noted for their potential antioxidant effect by directly scavenging reactive oxygen and nitrogen species.[48] Thermal processes, particularly floral thermogenesis, significantly influence phenolic accumulation.[49] These findings emphasize that extraction conditions directly affect phenolic compounds in lotus flower.

The multiple regression analysis using a second-order polynomial model (Table 5) demonstrated a moderate fit (R^2 = 0.8830). Similarly, response surface methodology (RSM) analysis, represented in a 3D surface plot (Fig. 1A and B), illustrates the relationship between TPC, extraction time, and temperature at a constant power rate. The constants of the predictive model that adequately describes TPC of LFE can be found in Table 4.

3.4 Effect of UAE conditions on TFC

The total flavonoid content (TFC) was determined and is presented in Table 3. The highest TFC recorded was 0.064 mg QE per g under conditions of 40 min exposure time, 60 °C temperature, and a power rate of 40% (8.2 kHz). In contrast, the lowest TFC was 0.031 mg QE per g at 10 min exposure time, 70 °C temperature, and a power rate of 65% (13.325 kHz). Statistical analysis confirmed a significant quadratic relationship (Table 4) between temperature and power rate (P < 0.05), emphasizing their crucial role in TFC extraction from lotus flowers. Previous studies on the extraction of flavonoids from lotus leaves have reported a decline in TFC beyond 60 °C, with temperature dependency playing a significant role. However, excessive temperatures can lead to a decrease in TFC due to the oxidation of flavonoid compounds.[33] Additionally, prolonged exposure times are not favorable for TFC retention, as extended durations may cause chemical degradation, ultimately limiting the maximum achievable TFC.[50] Although an increase in power rate generally enhances TFC extraction by promoting cellulose breakdown, excessively high frequencies result in a sharp reduction in TFC yield. This effect is likely due to the formation of cell debris, which facilitates flavonoid degradation.[20] Moreover, the flavonoids in lotus flower including quercetin, kaempferol, rutin, and luteolin were identified using UPLC-MS/MS[51] and HPLC.[47] These flavonoids significantly contribute to both antioxidant and anti-inflammatory activities. Their efficacy is also dynamically influenced by floral thermogenesis and other plant chemical regulations as demonstrated by their radical scavenging activity.[49]

The multiple regression analysis using a second-order polynomial model (Table 5) exhibited a strong fit (R^2 = 0.970).
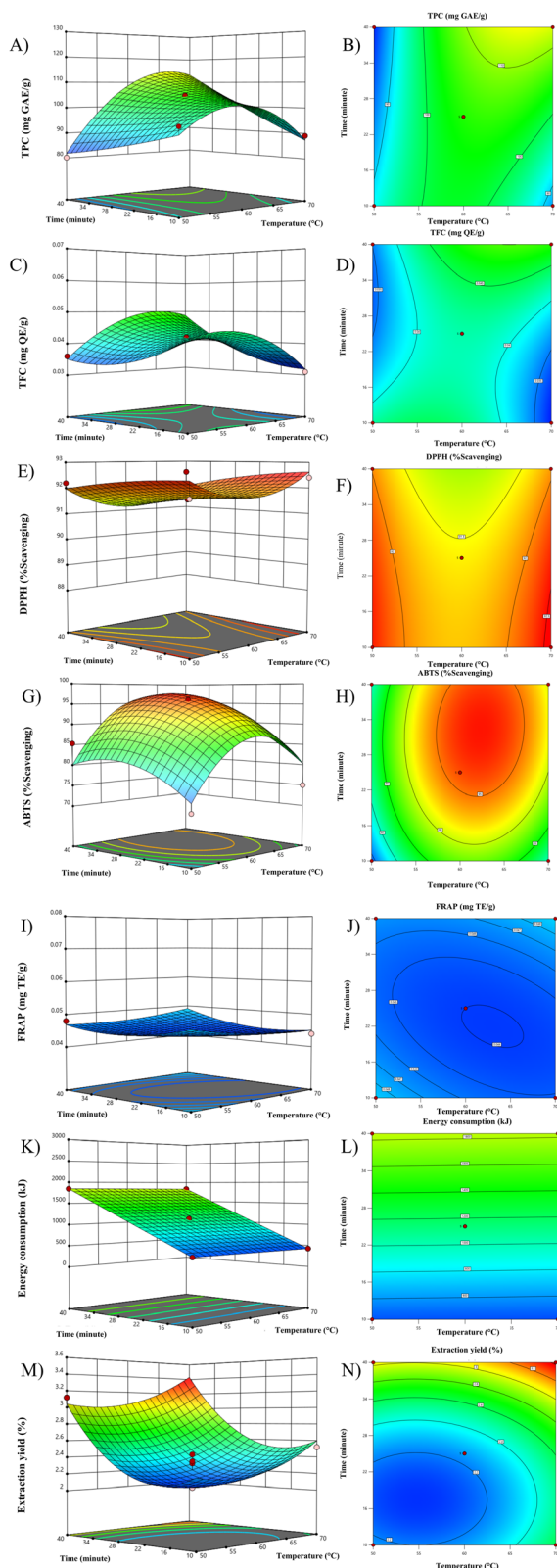
Fig. 1 Response surface methodology (RSM) applied to LFE assessed the relationships between extraction time and temperature, maintaining a constant power rate of 65% across various bioactive compounds. This analysis encompasses antioxidant activities, extraction yield (EY), and energy consumption (EC), as illustrated in (A and B) for total phenolic content (TPC), (C and D) for total flavonoid content (TFC), (E and F) for DPPH radical scavenging activity, (G and H) for FRAP

Similarly, response surface methodology (RSM) analysis, illustrated in a 3D surface plot (Fig. 1C and D), depicts the relationship between TFC, extraction time, and temperature at a constant power rate. The constants of the predictive model that adequately describe TFC of LFE can be found in Table 4.

3.5 Effect of UAE conditions on antioxidant properties

The antioxidant activities of LFE were evaluated using the DPPH, ABTS, and FRAP assays. The free radical scavenging activity was assessed using the DPPH and ABTS assays, while the FRAP assay was used to determine iron-chelating activity by measuring the extract's ability to reduce Fe^{3+} to Fe^{2+}. A higher Fe^{2+} concentration indicates a higher FRAP value. The results of the free radical scavenging activity are presented in Table 3. The minimum antioxidant activities were recorded at 88.46% for the DPPH assay under conditions of 40 min extraction time, 60 °C temperature, and a power rate of 40% (8.2 kHz), while the ABTS assay yielded 84.99% at 25 min extraction time, 50 °C temperature, and a power rate of 90% (18.45 kHz). Conversely, the maximum antioxidant activities were observed at 92.53% for the DPPH assay at 40 min extraction time, 70 °C temperature, and a power rate of 65% (13.325 kHz), while that obtained from the ABTS assay reached 98.01% at 40 min extraction time, 60 °C temperature, and a power rate of 40% (8.2 kHz).

Statistical analysis (Table 4) revealed that the DPPH assay results significantly decreased with increasing power rate ($P < 0.05$), while extraction time and temperature had no significant effect ($P > 0.05$). In contrast, the ABTS assay demonstrated a significant decrease with increasing temperature ($P < 0.05$), and an interaction effect between temperature and power rate was also observed ($P < 0.05$). These findings confirm that power rate and temperature influence the free radical scavenging ability of LFE. This is consistent with the findings of Yang et al.,[52] who reported a positive correlation between phenolic and flavonoid compounds and DPPH/ABTS scavenging activity in lotus leaf extract, which aligns with the TPC and TFC results in this study.

The FRAP assay results, also shown in Table 3, indicate that the minimum total reducing capacity was 0.042 mg TE per g at 25 min exposure time, 50 °C temperature, and a power rate of 40% (8.2 kHz), whereas the maximum was 0.076 mg TE per g at 40 min exposure time, 60 °C temperature, and a power rate of 60% (12.3 kHz). However, statistical analysis (Table 4) indicated that total reducing capacity was not significantly affected by any of the tested factors. Although LFE exhibited strong free radical scavenging activity, its iron-chelating ability was lower than that of Trolox. This may be attributed to the influence of other compounds, as suggested by Parven et al. and Zahari et al.,[53,54] who confirmed the role of alkaloids in iron-chelating activity.

Additionally, UAE can influence antioxidant activity due to the formation of free radicals, which is associated with increased temperature and prolonged extraction time.[55]

(Ferric Reducing Antioxidant Power), (I and J) for ABTS radical cation scavenging activity, (K and L) for energy consumption (EC), and (M and N) for extraction yield (EY).

However, exposure to high-intensity ultrasound conditions can enhance cavitation, leading to the formation of hydroxyl radicals. This process can have both positive and negative effects, depending on specific extraction conditions. Furthermore, numerous studies have highlighted the role of phenolic compounds and flavonoids in antioxidant activities, including iron chelation, hydrogen atom donation, and electron transfer mechanisms.[33,45,52]

The multiple regression analysis using a second-order polynomial model (Table 5) demonstrated moderate predictive accuracy, with coefficients of determination (R^2 of 0.852, 0.874, and 0.895 for the DPPH, ABTS, and FRAP assays, respectively). Similarly, response surface methodology (RSM) analysis, represented in a 3D surface plot (Fig. 1E–J), illustrates the relationship between DPPH, ABTS, and FRAP with extraction time and temperature at a constant power rate. The constants of the predictive models that adequately describe DPPH, ABTS, and FRAP of LFE can be found in Table 4.

3.6 The optimum conditions of UAE

The optimal extraction conditions were determined using response surface methodology (RSM), employing the Box–Behnken design (BBD) for experimental modeling. The independent variables considered were extraction time (10, 25, and 40 min), temperature (50, 60, and 70 °C), and power rate (40%, 65%, and 90%). The optimization process aimed to maximize the extraction of bioactive compounds and their associated bioactivities, including total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH, ABTS, and FRAP assays). Additionally, energy consumption (EC) and extraction yield (EY) were included as critical parameters for evaluation. The optimal extraction conditions were predicted to be 10 min extraction time, 57.45 °C temperature, and a power rate of 90% (18.45 kHz). Under these conditions, the predicted values for TPC, TFC, DPPH, ABTS, FRAP, EY, and EC were 114.52 mg GAE per g, 0.057 mg QE per g, 90.91%, 91.61%, 0.072 mg TE per g, 2.46%, and 617.98 kJ, respectively, as presented in Table 6. To validate these predictions, experimental verification was conducted, yielding values of 106.50 mg GAE per g, 0.058 mg QE per g, 89.97%, 92.47%, 0.060 mg TE per g, 2.52%, and 617.97 kJ for TPC, TFC, DPPH, ABTS, FRAP, EY, and EC, respectively. The predicted and experimental values showed strong agreement, with a 95% confidence level, as demonstrated by the ranges presented in Table 6. The optimal extraction conditions identified in this study are consistent with the findings of Bao et al.,[56] who reported that ultrasound-assisted extraction (UAE) of lotus seed pods resulted in high phenolic compound yields. Similarly, these results align with the study by Bhadange et al.,[37] which suggested that UAE durations of 5–15 min are optimal to prevent chemical degradation and loss of bioactive compounds. In addition to extraction time, ultrasonic frequency is a critical factor in controlling energy consumption and optimizing bioactive compound extraction. Yusoff et al., Panda et al., and Dzah et al.[57–59] reviewed the impact of ultrasonic frequency, indicating that frequencies exceeding 20–40 kHz can enhance bioactive compound extraction from natural sources. UAE has demonstrated clear advantages over conventional methods such as hot water extraction, agitation and Soxhlet extraction for isolating flavonoids, phenolic, and other bioactive compounds from various parts of lotus flower. In this case, UAE treatment yielded significantly higher levels of TFC than conventional methods primary due to improved cell wall disruption correlated with enhanced antioxidant activities.[20] Ultrasound microwave assisted extraction (UMAE) also greatly outperformed microwave assisted extraction (MAE) in both yield and time efficiency.[23] For nuciferine extraction UAE showed more than 20% higher yields than agitation assisted extraction and significantly outperformed Soxhlet extraction.[60] The findings of the present study further confirm that a lower frequency of 18.45 kHz (corresponding to a 90% power rate) is effective for extracting bioactive compounds from lotus flowers. The combination of optimized time and temperature settings contributes to reduced energy consumption, making it a viable approach for functional ingredient production.

3.7 Thermodynamic analysis of UAE

The thermodynamic parameters of UAE, including enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG), were analyzed to assess the thermal effects on the LFE extraction process. The results are summarized in Table 7. The enthalpy (ΔH) values obtained were -10.457 kJ mol^{-1} for total phenolic content (TPC), 12.265 kJ mol^{-1} for total flavonoid content (TFC), 103.620 kJ mol^{-1} for DPPH radical scavenging activity, -373.983 kJ mol^{-1} for ABTS radical scavenging activity, and 12.386 kJ mol^{-1} for ferric reducing antioxidant power (FRAP). The positive ΔH values for TFC, DPPH, and FRAP indicate that these processes are endothermic, meaning that heat input facilitates extraction. In contrast, the negative ΔH values for TPC and ABTS suggest exothermic processes, indicating that

Table 6 Experimental and predicted responses of LFE under optimal conditions

Phytochemical screening	Predicted values	Experimental values	95% Cl
TPC (mg GAE per g extract)	114.52	106.50 ± 2.58	(106.31, 132.86)
TFC (mg QE per g extract)	0.0570	0.0582 ± 0.002	(0.0528, 0.0613)
DPPH (% scavenging activity)	90.91	89.97 ± 5.34	(89.60, 92.12)
ABTS (% scavenging activity)	91.61	92.47 ± 4.20	(83.38, 101.48)
FRAP (mg TE per g)	0.072	0.060 ± 0.003	(0.060, 0.082)
EY (%)	2.46	2.52 ± 1.29	(2.15, 2.78)
EC (kJ)	617.97	617.97 ± 1.52	(705.86, 1070.16)

Table 7 Thermodynamic parameters of LFE for TPC, TFC, and antioxidant activities (DPPH, ABTS, and FRAP assays) using optimal exposure time and power rate at different temperatures

Bioactive compound	T (K)	K_e	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1})	Linnear equation	R^2
TPC	323.15	0.415	−37.087	−10.457	0.082	$\ln(K_e) = -86.903(1/T) + 0.684$	0.994
	333.15	0.424	−37.911				
	343.15	0.431	−38.735				
TFC	323.15	0.471	6.145	12.265	0.0189	$\ln(K_e) = 101.93(1/T) + 0.157$	0.920
	333.15	0.466	5.955				
	343.15	0.452	5.766				
DPPH	323.15	3.180	83.259	103.620	0.063	$\ln(K_e) = 861.09(1/T) + 0.523$	0.967
	333.15	3.124	82.629				
	343.15	3.024	81.999				
ABTS	323.15	2.440	−843.620	−373.983	1.453	$\ln(K_e) = -3107.8(1/T) + 12.077$	0.984
	333.15	2.788	−858.153				
	343.15	2.999	−872.686				
FRAP	323.15	0.476	6.261	12.386	0.0189	$\ln(K_e) = 102.93(1/T) + 0.157$	0.992
	333.15	0.465	6.072				
	343.15	0.457	5.882				

these extractions occur more efficiently without additional thermal acceleration.

The entropy change (ΔS) values were positive under all conditions, suggesting an increase in disorder and irreversibility during the extraction process. This aligns with previous studies demonstrating that ultrasound treatment disrupts plant cell structures, increasing the diffusion of bioactive compounds into the solvent.[61,62] Furthermore, Gibbs free energy (ΔG) was used to assess the spontaneity of the process, with negative values indicating spontaneous reactions. Both TPC (−10.457 kJ mol^{-1}) and ABTS (−373.983 kJ mol^{-1}) exhibited negative ΔG values, confirming their spontaneous extraction under UAE conditions. However, ΔG values generally decreased with increasing temperature, implying that higher thermal input can drive certain extractions toward spontaneity. These findings suggest that the extraction of TPC and ABTS is significantly influenced by temperature, as higher temperatures resulted in lower yields, as depicted in Fig. 1A, B, I and J. This trend is consistent with prior studies indicating that excessive thermal exposure can degrade phenolic compounds due to oxidation and polymerization.[63] Moreover, the positive entropy values further support the hypothesis that UAE facilitates molecular dispersion, enhancing the release of bioactive compounds.[64]

The thermodynamics analysis of LFE indicates that TFC, DPPH, and FRAP are endothermic with improved efficiency under heat input. In contrast, the extraction of TPC and ABTS is exothermic and more favorable at lower temperatures. The consistently positive entropy (ΔS) suggests an increase in system disorder and effective disruption of plant cell structures by ultrasound thereby enhancing the diffusion of bioactive compounds. Negative Gibbs free energy (ΔG) for TPC and ABTS confirms the spontaneous reaction of their extraction under UAE conditions. However, higher temperatures may reduce the yields of thermolabile compounds due to the degradation process. These parameters provide valuable insights into the mechanistic aspects of UAE. While controlled heating can improve mass transfer and extraction efficiency, excessive temperature elevations may lead to the degradation of thermosensitive

compounds, thereby reducing extraction yields. Consequently, optimizing UAE parameters is critical for balancing the efficiency and stability of extracted bioactive compounds.

Conclusion

This study optimized the ultrasound-assisted extraction (UAE) conditions for enhancing the yield of bioactive compounds from the lotus flower, focusing on total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH, ABTS, and FRAP assays). Using response surface methodology (RSM), the optimal extraction conditions were determined as 10 min, 57.45 °C, and 90% power (18.45 kHz), which maximized bioactive compound yields while maintaining extraction efficiency. Thermodynamic analysis revealed a mix of endothermic and exothermic processes, with negative Gibbs free energy (ΔG) values for TPC and ABTS indicating spontaneous extraction and entropy change (ΔS) suggesting an irreversible process influenced by thermal factors. While UAE proved to be an energy-efficient and sustainable method, future studies should focus on process scalability, comparative analyses with conventional techniques, structural stability of extracts, *in vivo* bioavailability assessments, and solvent system optimization to enhance extraction efficiency and practical applications. These findings contribute to the establishment of UAE as a viable technology for producing high-value lotus flower extracts for use in the food, nutraceutical, and pharmaceutical industries.

Data availability

The data supporting the findings of this study are available from the corresponding author upon request.

Author contributions

Thiti Sonphakdi: investigation, formal analysis, data curation, validation and writing – original draft. Mohammad Fikry: validation, data curation and writing – original draft. Saranya Jansamutr: validation and data curation. Kitipong Assatarakul:

conceptualization, data curation, funding acquisition, project administration, supervision, writing – review & editing.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

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