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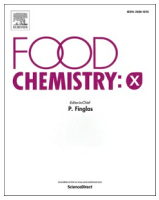

Optimization of ultrasound-assisted extraction of phenols from *Crocus sativus* by-products using sunflower oil as a sustainable solvent alternative

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A R T I C L E I N F O

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A B S T R A C T

In the last decade, there's been a rising emphasis on eco-friendly solvents in industry and academia due to environmental concerns. Vegetable oils are now recognized as a practical, non-toxic option for extracting phytochemicals from herbs. This study presents a novel, green, and user-friendly method for extracting phenolic content from *Crocus sativus* L. waste using ultrasound. It replaces conventional organic solvents with sustainable sunflower oil, making the process eco-friendly and cost-effective. The effects of temperature (18–52 °C), ultrasonic time (5–55 min), and solid-solvent ratio (5–31 g/100 mL) were assessed by applying response surface methodology (RSM) and Central composite design. The combined impact of solid-solvent ratio, temperature, and ultrasonic time led to heightened phenolic content and antioxidant activity in the enriched oil. However, when these variables were at their maximum levels, there was a decline in these attributes. The specific conditions found to be ideal were a solid-to-liquid ratio of 26 g/100 mL, a temperature of 45 °C, and a duration of 45 min. The optimum extraction condition yielded the expected highest phenolic content (317.15 mg/ Kg), and antioxidant activity (89.34%). The enriched oil with flower saffron enabled the utilization of renewable natural ingredients, ensuring the production of a healthy extract or product. Also, enriched oils find diverse applications in areas such as food, aquaculture, and cosmetics.

1. Introduction

Synthetic preservatives are widely used in the food industry to maintain quality and prolong shelf life, but their impact on food composition and health is concerning (Mahato et al., 2019; Singh et al., 2021). Natural antioxidants offer a promising alternative, gaining traction for their ability to stabilize foods without the drawbacks of their synthetic counterparts (Shahid et al., 2022). Scientists are investigating natural alternatives from medicinal and aromatic plants to replace artificial food preservatives. These plants offer antimicrobial and antioxidant properties that can safely preserve food without harmful chemicals (Vijaya Kumar et al., 2009; Yap et al., 2021).

Crocus sativus L., a medicinal plant renowned for its saffron-producing stigmas, has garnered significant attention due to its

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Table 1
Coded and real levels of independent variables.

Variables	Levels				
	-α	-1	0	+1	+α
Temperature X_1 (°C)	18	25	35	45	52
Time X_2 (min)	5	15	30	45	55
Solid/Liquid X_3 (g/100 mL)	5	10	18	26	31

therapeutic properties, culinary applications, and high economic value. Saffron contains 150+ volatile compounds, with crocin, safranal, and picrocrocin being key. Despite their diverse origins, these contents lend saffron its color, flavor, and aroma (Jarukas et al., 2020; Moraga et al., 2009; Rahaiee et al., 2015; Rubio et al., 2008; Slimani et al., 2022). Studies highlight their potent antioxidant effects both *in vitro* and *in vivo* (Cerdá-Bernad et al., 2022; Clinton, 2009; Delam et al., 2023; Kun et al., 2006; Memarzia et al., 2023). Saffron's powerful antioxidants make it a key ingredient for new products in food, medicine, and cosmetics (Abu Safe et al., 2023; Amatto et al., 2024; Colucci Cante et al., 2022; Rahaiee et al., 2015).

In recent years, there has been a growing focus on utilizing advanced technologies like ultrasound assisted extraction alongside environmentally friendly solvents such as sunflower oils to extract phenolic content from vegetable waste and by-products. This approach has proven its advantages, not only in minimizing health risks but also in promoting environmental preservation in a financially viable and sustainable manner for our planet (Jaski et al., 2022; Kyriakoudi et al., 2024; Li et al., 2013; Younis et al., 2022). Recently, it's common to enrich edible fatty acids with antioxidants, enhancing their stability, nutrition, and pharmaceutical value (Bouaziz et al., 2008; Irwandi Jaswir, 2011; Kaderides et al., 2015; Ninčević Grassino et al., 2020). While previous research has explored various extraction methods for *C. sativus* flowers, ultrasonic-assisted extraction with sunflower oil as a solvent remains underexplored.

This study addresses this gap by developing an ultrasonic-assisted extraction technique to efficiently extract phenolic content from *C. sativus* by-products using sunflower oil. This study also investigates the influence of various parameters, including temperature, time, and solid-to-liquid ratio, on the extraction efficiency of phenolic content and the antioxidant activity of *C. sativus* flower. Thus, the response surface methodology was chosen to optimize the extraction parameters using the Central composite design.

2. Materiel and methods

2.1. Phenolic content extraction

Plant material used in this study was composed of *C. sativus* flower from Taliouine region (province of Taroudant, Morocco). The flowers were obtained from the Fogoug Agricultural Cooperative in October–November 2019. Taliouine region is situated at an elevation of 1586 m, with coordinates of approximately 30.53 latitude and − 7.92 longitude. Phenolic content was extracted from *C. sativus* flower, utilizing ultrasound at a frequency of 35 kHz, employing sunflower oil as alternative solvent. The mixture was then centrifuged at 1411 relative centrifugal force (RCF) for 10 min, and the supernatant was recovered. The oil we used is characterized by its low phenolic content, almost negligible, and a reduced percentage of 2,2-Diphenyl−1-picrylhydrazyl (DPPH). These characteristics allowed us to consider enriching it with saffron bioproducts.

2.2. Experimental design and optimization

Optimization strategies offer a structured approach to identifying the optimal conditions for extracting bioactive content from plants through the analysis of interactions within different mixtures (Fadil et al., 2024, 2022; Garcia et al., 2010). In this study, a central composite design was used to determine the effect of variables; temperature (X_1, expressed as °C), sonication time (X_2, indicated in min) and material/solvent (X_3, measured in g/100 mL). These three factors were thoughtfully selected to guarantee precise adjustment during the extraction procedure (Table 1). The experimental procedure's diagram is illustrated in Fig. 1.

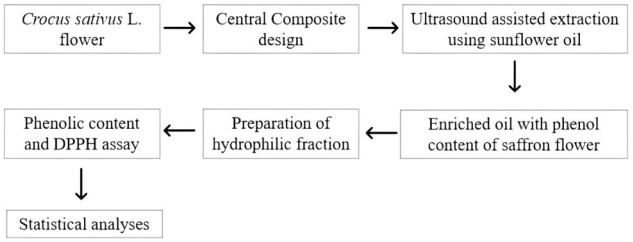

Fig. 1. Diagram of the experimental procedure.

2.3. Preparation of hydrophilic fraction

The hydrophilic fraction of the enriched oil was prepared. Initially, 10 g of each sample were mixed with 20 mL of water-methanol (2:8) at room temperature (24 ± 2 °C) for 20 min (Arranz et al., 2008). Then, the mixture was centrifuged at 1411 RCF, and the supernatant was recovered. The latter is evaluated for its phenolic content as well as its antioxidant capacity.

2.4. Total phenolic content determination

The quantification of total phenolic content (TPC) was conducted through a colorimetric assay that relies on the oxidation of phenols by the Folin-Ciocalteu reagent. The blue product generated, measured at 725 nm, corresponds proportionally to the phenol concentration (Singleton & Rossi, 1965). To convert absorbance values to milligrams of gallic acid equivalents per kilogram of dry matter (mg GAE/ Kg DM), a calibration curve with gallic acid was utilized.

2.5. Radical-scavenging activity

In order to assess the antioxidant capacity of saffron, samples were exposed to a DPPH solution. The degree of DPPH reduction, determined by the reduction in absorbance, indicates the sample's capability to neutralize free radicals (Brand-Williams et al., 1995).

$$\text{Antioxidant activity } (\%) = (\text{Abs DPPH–Abs final})/(\text{Abs DPPH}) \text{ x100} \quad (1)$$

2.6. Mathematical model

The model fit was evaluated by the coefficient of determination (R^2) and by the *p*-values. The following polynomial equation of the Xi function was fitted for each factor evaluated at each experimental point:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \varepsilon \quad (2)$$

Where Y is the predicted response; β_0 represents the mean response value; β_1, β_2, and β_3 are the coefficients of the main terms; β_{11}, β_{22}, and β_{33} are the coefficients of the quadratic terms; β_{12}, β_{13}, and β_{23} are the coefficients of the interaction terms; and ε is the error term.

The R-squared coefficient, which indicates the proportion of variation in the mean response explained by the regression and represents the correlation between the observed and predicted responses, highlights the model's lack-of-fit (Fadil et al., 2022).

The model coefficients were considered significant for *p*-values

Table 2
: Experimental matrix of the central composite design based on temperature (X_1), time (X_2), and S/L ratio (X_3).

N	Variables		
	Temperature X_1 (°C)	Time X_2 (min)	S/L Ratio X_3 (g/ 100 mL)
1	18	30	18
2	25	15	10
3	25	15	26
4	25	45	10
5	25	45	26
6	35	5	18
7	35	30	5
8	35	30	18
9	35	30	18
10	35	30	18
11	35	30	18
12	35	30	18
13	35	30	31
14	35	55	18
15	45	15	10
16	45	15	26
17	45	45	10
18	45	45	26
19	52	30	18

<0.05 and they were determined using the *t*-test (only significant coefficients with a *p*-value <0.05 are included).

A contour plot with isoresponse curves pinpointed compromise zones for the desired outcome. Using the "desirability" technique, an optimal setup balancing opposing factors was determined. This approach accounted for trade-offs among parameters to enhance the response. Pareto charts were then used to showcase the importance of various combinations (Fadil et al., 2022).

2.7. Statistical analysis

The experiments of the chosen experimental design were designed and analyzed using the JMP Pro software version 15 (SAS Institute, Cary, NC, USA). A total of 19 combinations were used (Table 2). Analyses were conducted in triplicate to provide degrees of freedom for pure error and lack-of-fit calculations. Results were presented as mean ± SD and analyzed using ANOVA at a 95% confidence level. Mean comparisons were assessed using Tukey's test. Model validation was done *via* ANOVA with a significance level of 95%, comparing predicted responses from different models for statistical significance.

3. Results

3.1. Effect of extraction parameters on oil enriched with C. sativus flowers

Phenolic content in the 19 combinations varies from 80.33 to 378 mg/kg of *C. sativus* flowers. Experiment 18 (26 g/100 mL ratio, 45 °C, and 45 min of extraction time) yielded the greatest amount of phenolic content. On the other hand, experiment 2 (10 g/100 mL ratio, 25 °C, and 15 min as extraction time) provided the lowest concentration (Table 3). The DPPH free radical content ranged from 57.25 to 93.64%. The peak value was documented in experiment 19, conducted with a ratio of 52 g/ 100 mL, at 30 °C, and an extraction duration of 18 min. Conversely, the lowest value was noted in experiment 7, where a ratio of 5 g/ 100 mL was used, along with a temperature of 35 °C, and an extraction time of 30 min.

3.2. Central composite design

The model's high coefficients of determination (R^2), standing at 0.8

Table 3
: Experimental results of the Central Composite design.

N°	Variables			Responses			
	X_1 Temperature (°C)	X_2 Time (min)	X_3 S/L Ratio (g/100 mL)	Phenolic content (mg /kg)		DPPH (%)	
				Observed[a]	Predicted[b]	Observed[a]	predicted[b]
1	18	30	18	270 ± 2	197.21	88.58 ± 0.28	85.51
2	25	15	10	80.33 ± 1.18	144.53	71.48 ± 0.27	69.64
3	25	15	26	95.33 ± 0.33	107.72	71.99 ± 0.23	73.27
4	25	45	10	106.33 ± 0.36	125.87	72.01 ± 0.17	73.14
5	25	45	26	109.67 ± 0.57	144.23	77.00 ± 0.63	81.40
6	35	5	18	81.67 ± 0.63	62.12	65.78 ± 0.06	69.65
7	35	30	5	101.33 ± 0.71	72.32	53.0 ± 0.3	57.25
8	35	30	18	106.0 ± 0.3	122.84	69.90 ± 0.71	73.52
9	35	30	18	127.67 ± 1.49	122.84	74.83 ± 0.15	73.52
10	35	30	18	124.67 ± 1.04	122.84	74.27 ± 0.19	73.52
11	35	30	18	125.67 ± 0.49	122.84	74.21 ± 0.09	73.52
12	35	30	18	127.33 ± 1.34	122.84	74.49 ± 0.23	73.52
13	35	30	31	166.33 ± 0.21	182.59	81.90 ± 0.17	78.20
14	35	55	18	136.6 ± 0.1	144.09	80.12 ± 0.63	76.69
15	45	15	10	114.67 ± 0.74	88.52	73.77 ± 0.52	69.06
16	45	15	26	217.00 ± 1.86	205.88	88.03 ± 0.15	86.59
17	45	45	10	154.33 ± 0.61	150.37	70.98 ± 0.14	69.39
18	45	45	26	378.67 ± 0.18	322.90	90.00 ± 0.73	91.54
19	52	30	18	240.33 ± 0.25	301.47	90.14 ± 0.23	93.64

a: The predicted value.
b: The observed value is given with the standard deviation of the response in our experimentation.

Table 4
: Regression equation and model fitting for total phenolic content and free radical scavenging activity (DPPH) of enriched oils.

Responses	Equations	*p*-value	LF	R^2
Polyphenols	$Y_1 = 913.08 - 40.27\,X_1 - 3.23\,X_2 - 17.05\,X_3 + 0.44\,X_1^2 - 0.03\,X_2^2 + 0.04\,X_3^2 + 0.13\,X_1X_2 + 0.48\,X_1X_3 + 0.11\,X_2X_3$ (3)	0.0254	0.0591	0.80
DPPH %	$Y_2 = 129.87 - 4.27\,X_1 + 0.19\,X_2 + 0.23\,X_3 + 0.07\,X_1^2 - 0.01\,X_2^2 - 0.03\,X_3^2 - 0.01\,X_1X_2 + 0.43\,X_1X_3 + 0.01\,X_2X_3$ (4)	0.0016	0.0583	0.90

LF: lack of fit; R: regression.

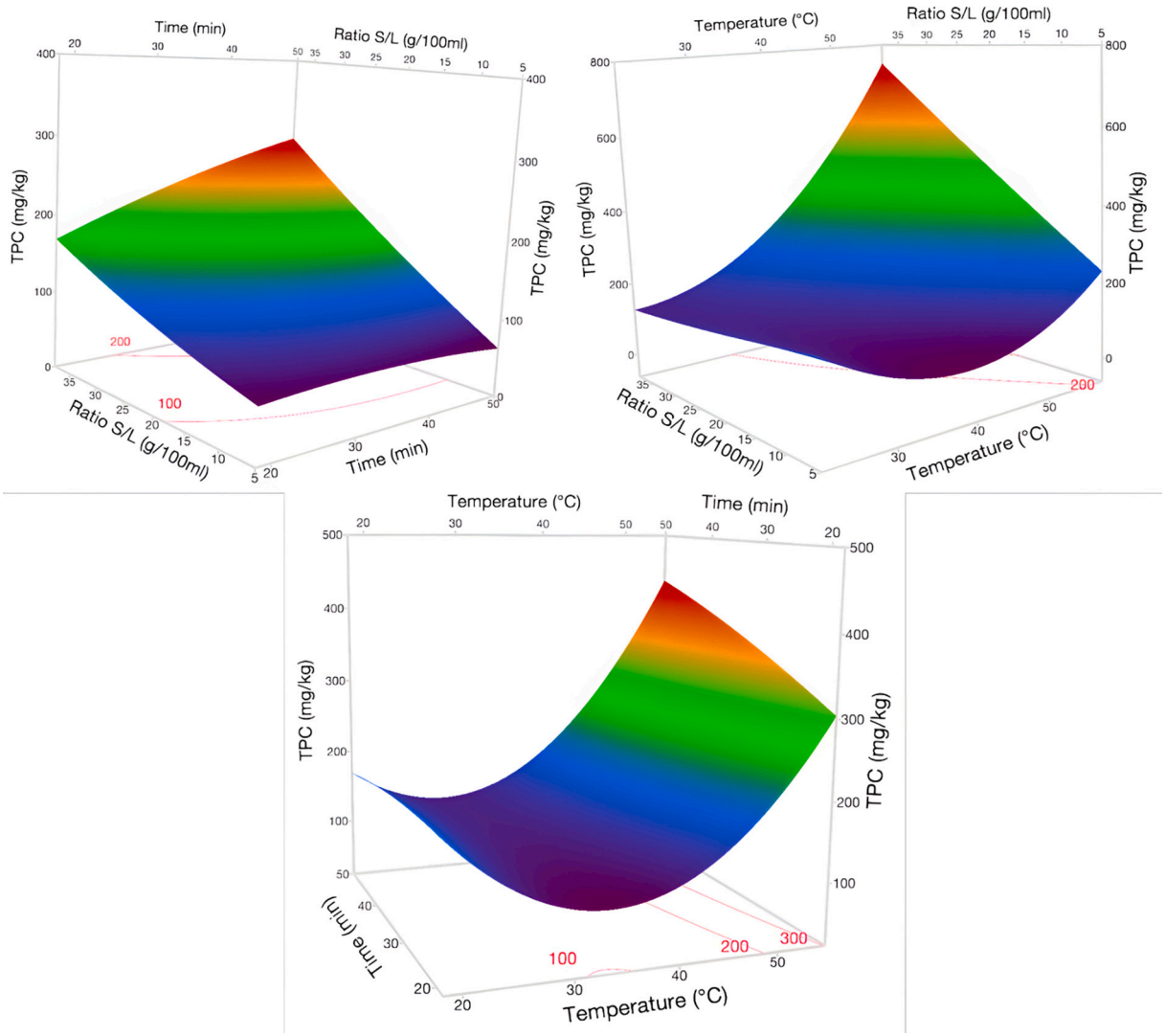

Fig. 2. : Response surfaces showing the combined effect of parameters (S/L ratio, temperature, and extraction time) on the response in phenolic compounds (TPC). (A) estimated S/L ratio and extraction time response surface; (B) estimated S/L ratio and temperature response surface; (C) estimated extraction time and temperature response surface.

for phenolic content and 0.9 for DPPH, highlight its capability to effectively elucidate a substantial proportion of the variation within the dataset. The model demonstrated significant *p-values* for both phenolic content (0.059), and DPPH (0.058), indicating its suitability for the data. The significant lack-of-fit suggests that the pure error of the model serves as an independent measure of the dispersion within the experimental design. Essentially, the lack-of-fit test confirms that the variability in the data arises primarily from the model rather than other factors like experimental error (Myers et al., 2009) (Table 4).

3.3. Effect of extraction parameters

The response TPC is notably influenced by significant terms (*p*-values <0.05), including the constant term, quadratic term of temperature, quadratic term of material/solvent ratio and the interaction terms of X_1X_2, X_1X_3 and X_2X_3 (Eq. 3, Table 4). Similar relationships were observed for IC_{50} of stigmas (Eq. 4, Table 4).

3.4. Parameters optimization and desirability

3.4.1. Appropriacy of the extraction parameters on phenols compounds

Phenolic content surpassing 322.89 mg/kg can be reached by setting the sonication time to its maximum of 45 min and ensuring the highest temperature and material/solvent ratio (Fig. 2). Moreover, according to the desirability function, there is an 85% chance of reaching TPC values of 322.89 mg/Kg by keeping the temperature at 45 °C, maintaining the material/solvent ratio at 26 g/100 mL, and adjusting the sonication time to 45 min (Fig. 4).

3.4.2. Appropriacy of the extraction parameters on DPPH

By adjusting the sonication duration to its maximum of 45 min and optimizing the temperature and the ratio of material to solvent, it is possible to attain antioxidant activity exceeding 91.53%, as depicted in Fig. 3. Furthermore, according to the desirability function, there is an 85% probability of reaching DPPH values of 91.53% by maintaining the temperature at 45 °C, the material/solvent ratio at 26 g/100 mL, and the sonication time at 45 min (Fig. 4).

3.5. Pareto diagrams

The Pareto diagrams provided a visual representation of the significance of various combinations in a hierarchical manner (Fig. 5). These illustrations demonstrated that the level of antioxidants extracted increased with greater solvent concentration and temperature, but

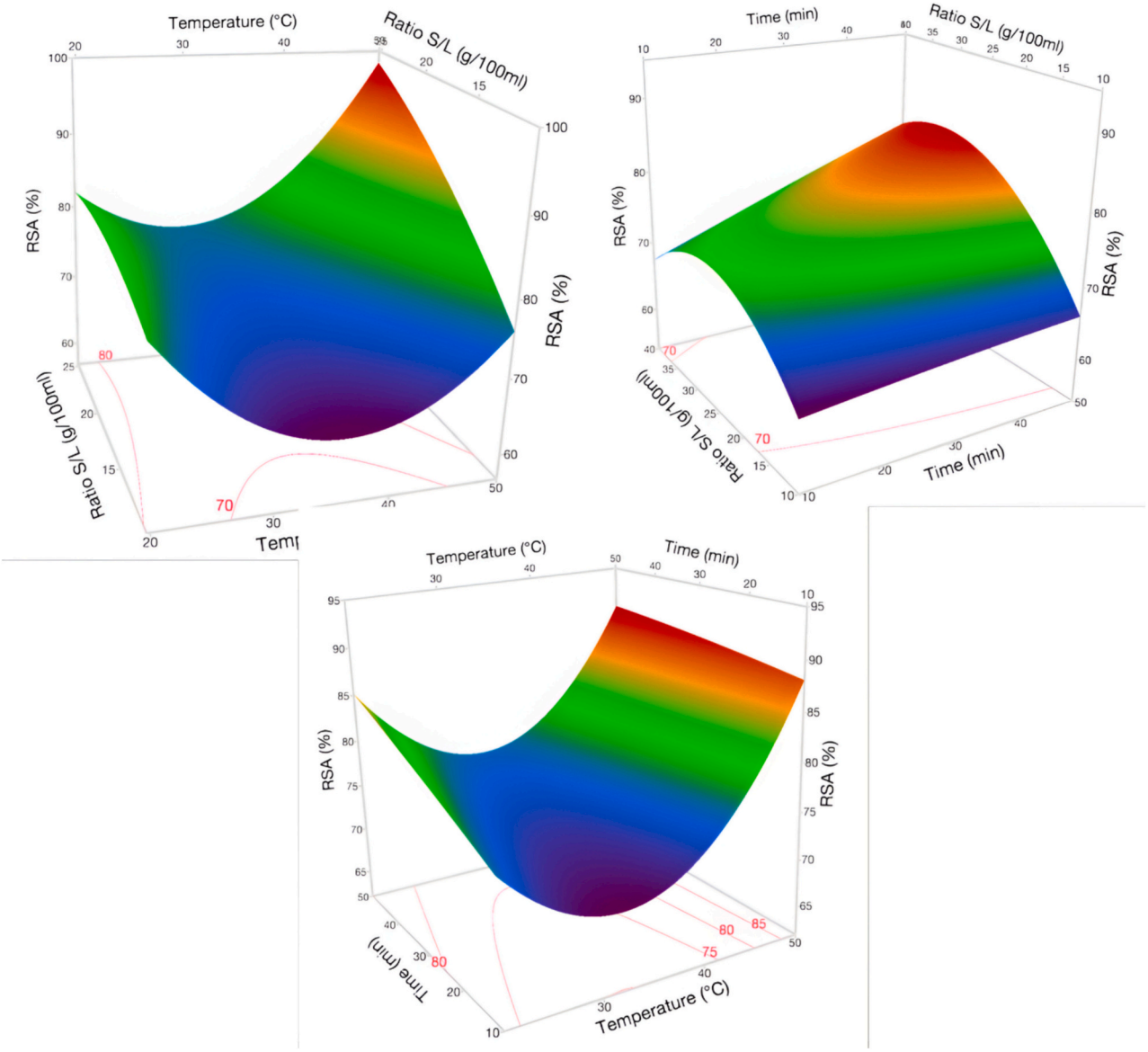

Fig. 3. : Response surfaces showing the combined effect of parameters (S/L ratio, temperature, and extraction time) on the response in RSA (DPPH %). (A) estimated S/L ratio and temperature response surface; (B) estimated S/L ratio and time response surface; (C) estimated extraction time and temperature response surface.

decreased with longer extraction periods and larger quantities of plant material for both phenolic content (Fig. 5(1)) and DPPH % (Fig. 5(2)). This implies that there exists an optimal combination of these parameters to enhance antioxidant extraction.

3.6. *Experimental validation of optimal conditions*

The empirical values closely matched the predicted values derived from the final selected model, with no notable difference between experimental and predicted responses (Table 5). Hence, we can infer that the three extraction factors optimized through response surface methodology reliably predict phenolic content yield and antioxidant activity in our extracts.

4. Discussion

Response surface methodology (RSM) employs designed experiments to model how independent variables affect system performance, facilitating optimization of process conditions. Efficient design gathers ample information with fewer experiments, maximizing resource utilization (Bezerra et al., 2008; Du et al., 2021; Khataee et al., 2011; Slimani et al., 2022; Venkata Rao & Murthy, 2018; Witek-Krowiak et al., 2014). Central composite design is a top choice for optimizing extraction parameters, accurately predicting key variables like yield or purity (Rahman et al., 2021; Vishnumulaka et al., 2008).

This study employed the central composite design to investigate the influence of various factors on the extraction of bioactive content from *C. sativus* flowers. Sunflower oil, replacing traditional solvents, was used in ultrasound-assisted extraction, showing promise as a method for extracting bioactive content like carotenoids. Several studies have demonstrated its effectiveness in this regard (Lara-Abia et al., 2022; Nie et al., 2021; Veillet et al., 2010).

Sunflower oil was identified as an eco-friendly solvent due to its high extraction effectiveness, minimal toxicity, sustainable sourcing, and higher boiling point compared to traditional organic solvents. In extraction yield experiments, it demonstrated superior performance compared to other vegetable oils (Chemat & Vian, 2014; Goula et al., 2017; Razi Parjikolaei et al., 2015; Sachindra & Mahendrakar, 2005).

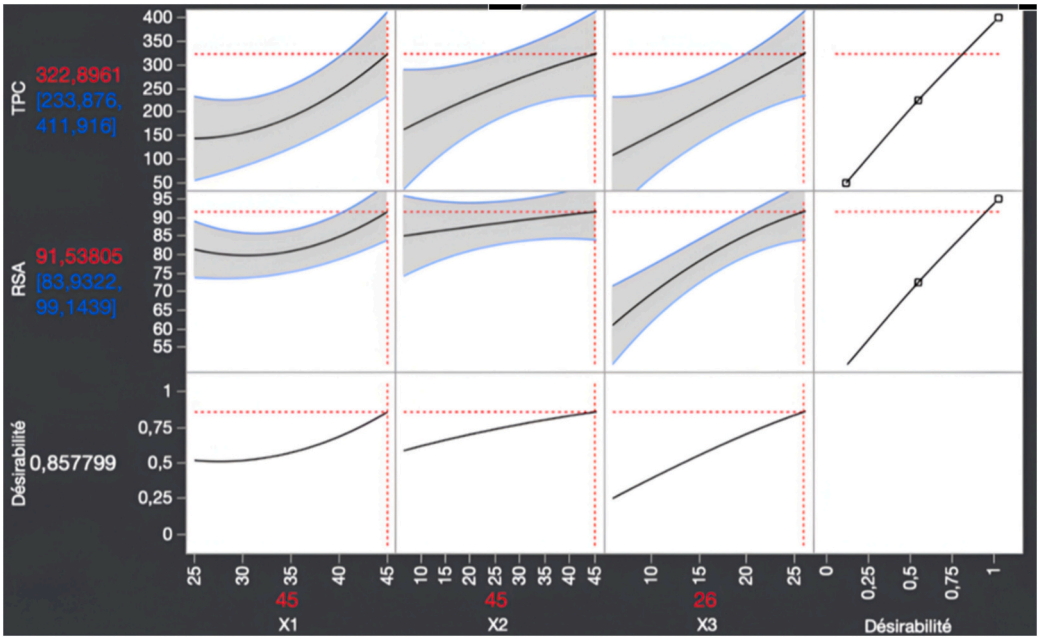

Fig. 4. Desirability plots showing the precise proportions of S/L ratio, temperature, and time leading to the optimal value for TPC (Phenols content mg GAE/ Kg DM) and RSA (DPPH %) for flower saffron-enriched oils.

According to the tendency observed in the response surface plot (Figs. 2 & 3), the highest phenol content could be achieved with a higher extraction temperature which concurs with the obtained data in Table 3. Increasing the temperature from 25 °C to 45 °C led to increase in the phenolic content yield of sunflower oil, but exceeding 45 °C resulted in a decrease, influenced our decision to use 45 °C as the optimal temperature for extracting antioxidants from saffron flower. Higher temperatures enhance extraction efficiency by improving solubility, diffusion, and penetration into cellular tissues (Boonkird et al., 2008; Goula et al., 2017; Purohit & Gogate, 2015; Tiwari et al., 2010). However, temperatures beyond a certain threshold can denature thermolabile antioxidants (Dong et al., 2010; Rostagno et al., 2007; Spigno et al., 2007).

High temperatures can damage antioxidants by breaking chemical bonds within them, leading to loss of function. This process also oxidizes the antioxidants themselves, rendering them inactive. Isomerization reactions can occur at high temperatures, converting antioxidants into less effective forms. Antioxidants are often stabilized with carrier molecules, but high temperatures can denature these carriers, releasing or deactivating the antioxidants (Blokhina et al., 2003; Mollica et al., 2020).

Optimizing extraction time is crucial for cost reduction and energy efficiency, considering the potential degradation of bioactive compounds under prolonged ultrasonic exposure (Mehmood et al., 2019; Shen et al., 2023). Some researchers aimed to enhance the nutritional properties of olive oil through the infusion of olive leaves using ultrasound waves. Their research revealed that soaking olive oil for 45 min significantly boosted its nutritional content (Achat et al., 2012).

Boosting the solid-liquid ratio (S/L) from 0.1:1 to 0.26:1 initially increased the phenol concentration and DPPH activity of sunflower oil. However, pushing it to 0.31:1 led to a drop in both, suggesting reduced solubility due to heightened oil saturation. This finding resonates with previous research (Kaderides et al., 2015; Sachindra & Mahendrakar, 2005; Zou et al., 2013).

Employing vegetable oils as solvents provides a more efficient method for extracting desired molecules from plants (Handayani et al., 2008; Kang & Sim, 2008; Ordóñez-Santos et al., 2015; Portillo-López et al., 2021). Enriched oils, derived through this process, find diverse applications in areas such as food, aquaculture, and cosmetics. Enriched oils refer to oils that have been enhanced with extra nutrients, like vitamins, minerals, or antioxidants (Oubannin et al., 2024; Sandhya et al., 2023; Szabó et al., 2023; Vidal et al., 2022).

Vegetable oils play a protective role by acting as a barrier during extraction, preventing the oxidation and degradation of target molecules. This protective function is crucial as it helps maintain the quality and effectiveness of these target molecules over time (Achat et al., 2012; Sachindra & Mahendrakar, 2005).

Conclusion

The study examined how different ultrasound-assisted extraction conditions impact phenol content and antioxidant activity extraction from saffron flower. It employed central composite design and response surface methodology for experimental design and optimization. Three variables were evaluated: temperature (°C), time (min), and S/L ratio (g/100 mL). Sunflower oil served as the green extraction solvent. Optimal conditions were found to be a solid-to-liquid ratio of 26 g/100 mL, a temperature of 45 °C, and a duration of 45 min. These conditions resulted in an effective extraction of phenol at a concentration of 317.15 mg/Kg and achieving an antioxidant activity level of 89.34%.

Using vegetable oils in ultrasound-assisted plant extraction enhances solvation and shields against phenol degradation. This eco-friendly approach yields potent phenolic content and nutraceuticals. Evaluating the oil's oxidative stability is advised. From a perspective, the analysis of the stability of enriched oils revolves around examining how effectively natural extracts act as antioxidants to inhibit oxidation. This, in turn, enhances the overall quality and extends the shelf life of the oils.

CRediT authorship contribution statement

Chaimae Slimani: Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Chaimae Rais:** Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Farid Mansouri:** Writing – review & editing, Visualization, Validation, Investigation. **Saadia Rais:** Validation, Methodology, Investigation, Data curation. **Meryem Benjelloun:** Writing – review & editing, Validation, Investigation, Data curation. **Riaz Ullah:** Writing – review & editing, Validation, Investigation, Funding acquisition. **Zafar Iqbal:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Data

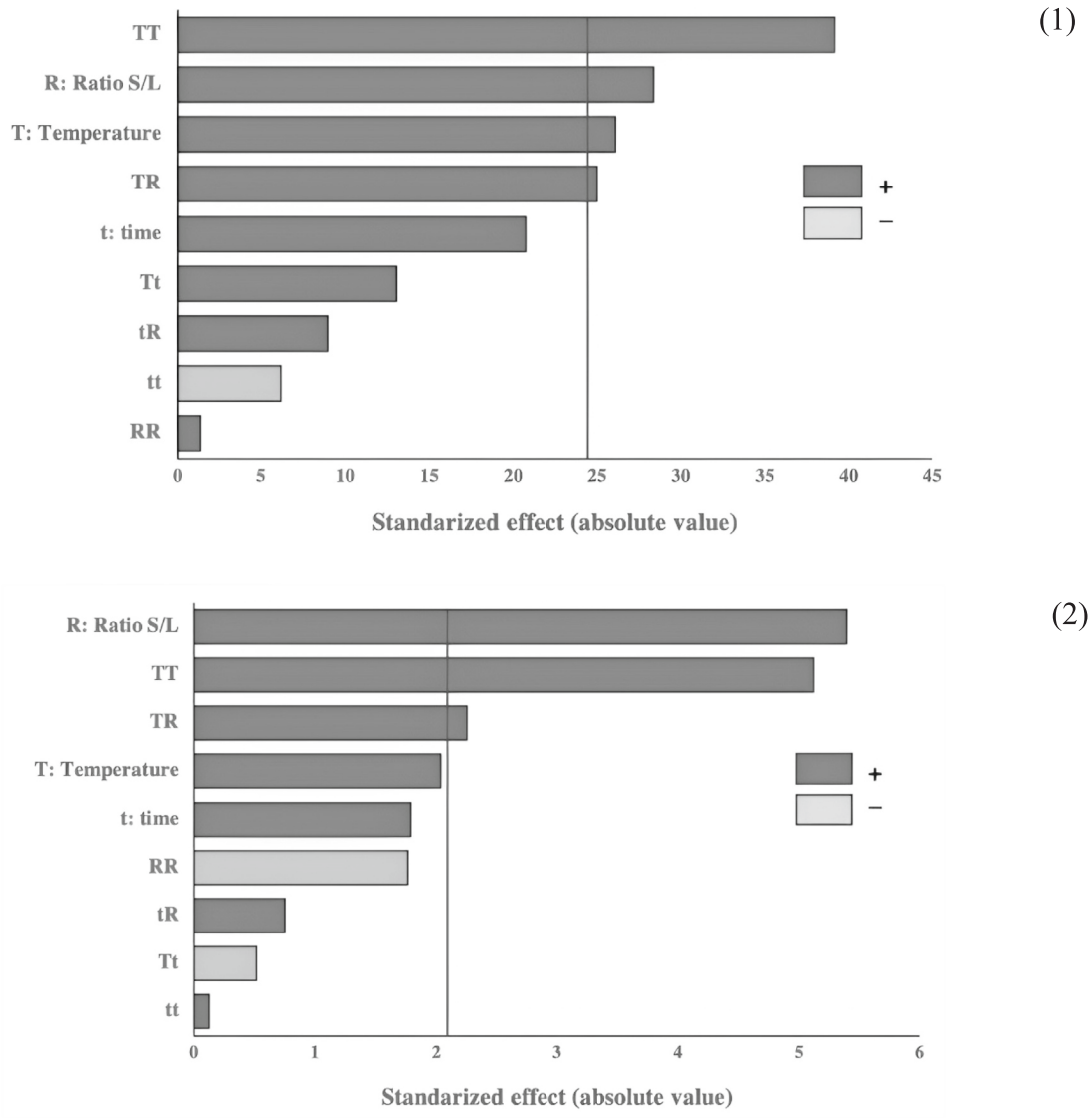

Fig. 5. Pareto diagrams of standardized effects of the two responses, those values exceeding the line, represent the elements that have a significant contribution to the model. (1): Phenolic content; (2): DPPH %.

Table 5
Predicted and observed values for optimal extraction conditions.

	Independent variables			Phenolic content (mg/ Kg)		DPPH %	
	Temperature (°C)	Time (min)	S/L Ratio (g/mL)	Predicted value[a]	Observed value[b]	Predicted value[a]	Observed value[b]
Flowers	45	45	26/100 mL	322.90	317.15 ± 1.63	91.54	89.34 ± 0.73

a: The predicted value;
b: The observed value is given with the standard deviation of the response in our experimentation.

curation. **Khang Wen Goh:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Data curation. **Learn-Han Lee:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Data curation. **Abdelhakim Bouyahya:** Writing – review & editing, Validation, Investigation, Formal analysis. **Abderrahim Lazraq:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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