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Study on ultrasonic extraction conditions of crocin pigment from *Gardenia jasminoides* Ellis and optimization using response surface methodology

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Abstract

Crocin is a glycosyl ester of crocetin acid and is classified as a water-soluble carotenoid. It is abundant in *Gardenia jasminoides* (Ellis GJE.) GJE is a natural ingredient widely used to impart an orange-yellow hue to traditional Vietnamese products such as cakes, jelly, and fish sauce. Crocin, a key bioactive compound in *Gardenia*, has been used in medicine for its antioxidant, anticancer, and memory-enhancing properties. In this study, we introduced an effective strategy for the extraction of crocin pigments derived from *Gardenia*. The pretreatment of *Gardenia* is crucial in the extraction process, with the most effective method involving freeze-drying *Gardenia* followed by grinding with liquid nitrogen to enhance the extraction efficiency. Second, this study focused on optimizing crocin extraction using ultrasound-assisted methods by evaluating key parameters, including ultrasonic amplitude, extraction time, material-to-solvent ratio, and solvent concentration. The crocin content reached 95.04±0.81 mg · g^{-1}dw. Finally, the optimal ultrasound-assisted extraction conditions were determined using response surface methodology, ensuring the maximum extraction efficiency of crocin (ultrasonic amplitude (60.41%), extraction time (5.95 min), solvent concentration (41.48%), and material/solvent ratio (2.7 g/100 mL). The maximum concentration of crocin from *Gardenia* was determined to be 97.05±1.00 mg · g^{-1}dw.

Keywords: *Gardenia jasminoides* Ellis, Crocin, Freeze-dried, Ultrasonic extraction, Response surface methodology

Introduction

Crocin, a type of water-soluble carotenoid pigment, primarily consists of monoglycosyl, diglycosyl, or triglycosyl polyene esters of crocetin (Alavizadeh & Hosseinzadeh, 2014). Crocin exhibits multiple pharmacological effects on the nervous system, including anxiolytic properties, antidepressant activity, and improvements in learning and memory (Ghadrdoost et al., 2011; Hosseinzadeh et al., 2012). Recent studies have also highlighted its potential in treating atherosclerosis, hyperlipidemia, and various other cardiovascular-related disorders (Liu & Qian, 2005; Xu et al., 2006).

Gardenia jasminoides Ellis has been extensively utilized as a natural colorant medicine, where it is recognized for its hemostatic, anti-inflammatory, analgesic, and antipyretic properties. The major constituents of this fruit include crocin, which is responsible for its strong coloring properties due to its high water solubility. As a result, *gardenia* fruit is extensively utilized as a natural coloring agent in the food industry (Lee et al., 2005). Extraction serves as a crucial initial step in isolating various bioactive compounds from Gardenia, enabling their potential applications in different industries (Chen et al., 2009).

Ultrasound-assisted extraction (UAE) has been extensively utilized for extracting proteins, bioactive compounds, and other valuable components from various food sources. Moreover, UAE enhances the efficiency of bioactive compound extraction by promoting the disruption of plant cell wall structures through

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acoustic cavitation, thereby facilitating the release of intracellular constituents (Liu et al., 2022). According to previous studies, UAE enables the rapid extraction of bioactive compounds while operating at lower temperatures, resulting in reduced energy consumption and minimal solvent usage. As a non-thermal extraction technique, UAE is particularly effective in preserving the functionality of bioactive compounds, especially natural pigments. However, key extraction parameters, including ultrasonic amplitude, extraction time, solvent type, and liquid-to-solid ratio, must be thoroughly investigated and optimized for each specific product to achieve maximum extraction efficiency (Kumar et al., 2021).

Response Surface Methodology (RSM) is a comprehensive mathematical and statistical approach used for empirical modeling and optimization, allowing the evaluation of interactions between multiple independent variables that influence a dependent variable or response (Nguyen et al., 2020). The optimization of crocin extraction conditions using ultrasound has been widely studied, demonstrating significant advantages in enhancing extraction efficiency and yield (Nguyen et al., 2022).

In this study, raw material pretreatment was performed using convection drying, freeze-drying, coarse grinding, and liquid nitrogen grinding. Based on the obtained results, UAE conditions were applied to pretreated *Gardenia* materials to maximize crocin yield. Key extraction parameters, including extraction time, temperature, ultrasonic amplitude, and solvent-to-material ratio, were investigated and optimized. The primary objective of this study was to maximize crocin pigment extraction using UAE following various drying and grinding pretreatment techniques.

Materials and Methods

Raw materials

Gardenia fruit was collected in January 2024 from Quang Nam province, Vietnam. The fruits were first sorted and peeled. Subsequently, one portion of the material was dried using a convection dryer (CD) at 70℃ for 24 h (Nguyen et al., 2022), while another portion was freeze-dried (FD) at −45℃ for 24 h. Finally, the dried samples were processed using two different comminution methods: (1) coarse grinding with a grinder and (2) homogenization in liquid nitrogen (Nguyen et al., 2020).

Extraction procedures for crocin using the ultrasound-assisted method

UAE was conducted using a VC 750 ultrasonic processor (Sonics & Materials, Inc., Newtown, CT, USA) operating at a frequency of 20 kHz±50 Hz with an output power of 750 W. A 1 g portion of *Gardenia* powder was placed into the extraction vessel and mixed with an appropriate volume of extraction solvent (100 mL). Ultrasound-assisted extraction was conducted at 50% amplitude for 6 min (Nguyen et al., 2022). Following ultrasonic treatment, the extracts were subjected to vacuum filtration and subsequently centrifuged at 6,000 rpm for 15 min (Samy et al., 2017). The resulting crude extracts (CE) were then collected and stored for quantification of crocin.

Crocin determination

Crocin yield was determined by UV-Vis spectrophotometry at

Fig. 1. Pretreatment of materials: CD1: convection drying followed by coarse grinding; FD1: freeze-drying followed by coarse grinding; CD2: convection drying followed by liquid nitrogen grinding; FD2: freeze-drying followed by liquid nitrogen grinding.

440 nm wavelength (Nguyen et al., 2022).

$$a = \frac{A.M.V.F}{\varepsilon.l} \quad (1)$$

where:

a is the crocin yield;

A is the absorbance value measured at 440 nm;

F is the dilution;

M is the molar mass of crocin (M = 977 g/mol);

l is the optical path length of the pigment layer (1 cm);

ε is the molar extinction coefficient of crocin = 89.000 L/ (cm · mol).

Experimental design and evaluation

After investigating the factors affecting the crocin extraction process, the pigment content was optimized using the response surface methodology (RSM). The optimization included ultrasonic amplitude (50–70%), extraction time (4–8 min), solvent concentration (20–60%), and material-to-solvent ratio (2 g/100 mL to 4 g/100 mL) in the FD2 sample.

In this study, a three-level, four-factor Box–Behnken Design (BBD) was applied to determine the optimal combination of variables for crocin extraction from FD2. A total of 54 experiments were conducted and designed, including six central points. The factors were systematically varied at high and low levels. Additionally, the medium levels of all four factors were used to assess the linear relationship between the high and low levels of the tested variables (Table 1). In this method, the measured response variable was the crocin concentration. The software Statgraphics Centurion XVI and the ANOVA procedure (p<0.05) were utilized in this study.

Table 1. Levels of factors and coding in the BBD experimental design

Factor	Coding level		
	−1	0	1
Ultrasonic amplitude (%)	50	60	70
Extraction time (min)	4	6	8
Solvent concentration (%)	40	60	80
Material-to-solvent ratio (g/100 mL)	2	3	4

Results and Discussion

Effect of different raw material pretreatment methods on crocin extraction yield

Pretreatment plays a crucial role in improving extraction efficiency by modifying the physical and chemical properties of solid samples. Among various pretreatment methods, freezing and drying are widely acknowledged as thermophysical techniques that can be effectively applied to solid matrices to extraction. These methods enhance the structural integrity of the material, thereby improving the overall yield and quality of extracted compounds (Phalla et al., 2022). This study investigated various pretreatment techniques to improve the efficiency and yield of crocin pigment extraction from *gardenia* fruit. The applied methods included convector drying and freeze-drying, followed by grinding, employing both coarse grinding and liquid nitrogen-assisted grinding. The impact of these pretreatment techniques on the yield and efficiency of crocin extraction was systematically evaluated, as illustrated in Fig. 2.

According to the Fig. 2, the results present the crocin yield in *Gardenia* fruit extracted under various pretreatment conditions. The findings demonstrate that pretreatment methods significantly affected crocin accumulation during ultrasonic extraction, emphasizing their crucial role in maximizing pigment recovery.

The quantities of crocin extracted with CD ranged from 33.55±0.51 mg · g^{-1}dw to 55.33±0.54 mg · g^{-1}dw, while with FD

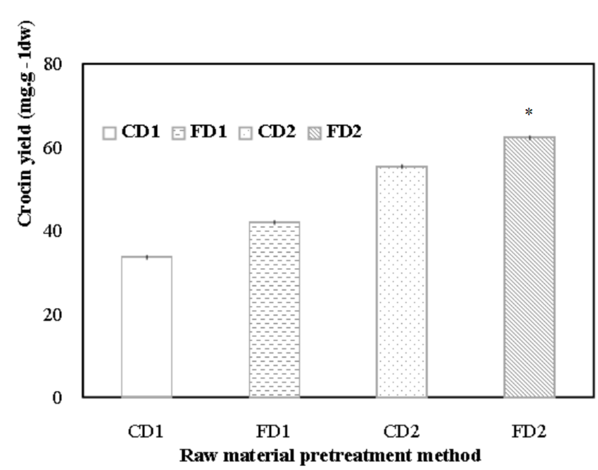

Fig. 2. Effect of different raw material pretreatment methods on crocin extraction yield: CD1: convection dryer and coarse grinding; FD1: freeze-dried and coarse grinding; CD2: convection dryer and liquid nitrogen grinding; FD2: freeze-dried and liquid nitrogen grinding. Data are expressed as the mean±SD (n=3). Significantly different results with p<0.05 are indicated by (*) for each pretreatment material.

they changed from 41.92±0.43 mg · g^{-1}dw to 62.23±0.50 mg · g^{-1}dw. After verifying ANOVA (p<0.05) showed that there is a significant difference between the pretreatment methods, the highest crocin extraction yield (62.32±0.50 mg · g^{-1}dw) was obtained from the freeze-dried sample, followed by grinding with liquid nitrogen (p<0.05), indicating the effectiveness of this pretreatment combination in enhancing crocin recovery.

The impact of freeze-drying on *Gardenia* fruit showed that freeze-dried samples are more extractable, particularly when ground in liquid nitrogen to reduce particle size. Freeze-dried materials have been widely utilized in numerous studies on extraction, the extraction of bioactive compounds (Kunal et al., 2015; Sun et al., 2015; Oprica et al., 2019; Krakowska-Sieprawska et al., 2022), leading to the conclusion that they can enhance the extraction process. There are significant differences between freeze-drying and conventional drying, making freeze-drying a more effective method for sample preparation. The convective drying method often exposes the product to high temperatures, causing chemical and physical alterations. This is particularly crucial for delicate plant extracts, where such changes can significantly impact the final product's quality. The primary distinctions lie in the water removal mechanism, the extent of dehydration—freeze-drying removes up to 98% of water, whereas conventional drying eliminates only 70–80% and nutrient retention, with freeze-dried products preserving substantially higher levels of vitamins and overall nutritional value compared to those dried by convective drying (Jiang et al., 2019; Oprica et al., 2019; Elshaafi et al., 2020; Krakowska-Sieprawska et al., 2022). Moreover, cryogenic grinding is an innovative technique developed to maintain the integrity of volatile and heat-sensitive compounds in various materials. By utilizing ultra-low temperatures, approximately −196℃, this process enables extreme particle size reduction, thereby enhancing extraction efficiency (Hemery et al., 2011; Balbino et al., 2019). Consequently, the pretreatment of materials plays a crucial role in influencing both the extraction efficiency and the recovery of crocin yield from *gardenia* fruit. In the following experiments, the FD2 sample was utilized to investigate the factors influencing extraction and to optimize the process in the following study.

Effect of extraction conditions on crocin yield

Extraction conditions play a crucial role in determining the yield of crocin from *gardenia* fruit. Several factors significantly impact the crocin content. After reviewing previous studies, we surveyed the following influencing factors: concentration of solvent (ethanol: H_2O; from 20:80 to 80:20, v:v), material/solvent ratio (from 1 g/100 mL to 5 g/100 mL), ultrasonic amplitude (from 40 to 80%), and extraction time (from 2 to 10 min) (Nguyen & Pham, 2016; Yingpeng et al., 2018). The results of each experiment are presented in Fig. 3.

According to Fig. 3, the values indicate that the highest crocin extraction yield was achieved using an ethanol-water solvent system at a volume ratio (v:v) of 40:60 (88.04±0.45 mg · g^{-1}dw), with a *Gardenia* raw material ratio of 3 g/100 mL of solvent (88.45±0.68 mg · g^{-1}dw), an ultrasonic amplitude of 60% (94.95±0.91 mg · g^{-1}dw), and an extraction time of 6 min (95.04±0.81 mg · g^{-1}dw), respectively.

The structure of crocin comprises two distinct components: a hydrophilic glycosyl group and a hydrophobic polyene segment derived from crocetin acid. This unique composition allows crocin to dissolve effectively in a solvent system containing both water and alcohol. However, an excessive amount of water may hinder the solubility of the hydrophobic part, whereas an excessive amount of alcohol may reduce the solubility of the hydrophilic part (Shi et al., 2016; Nguyen et al., 2022). In addition, the extraction efficiency of crocin increases as the raw material ratio increases. However, when the mass ratio of the sample continues to rise, the contact surface area between the raw material and the solvent decreases, or the amount of solvent may become insufficient to dissolve the pigment compounds in the raw material, ultimately resulting in a decline in extraction efficiency (Nguyen et al., 2022). The ultrasonic method enhances the extraction efficiency of crocin pigment, with the highest crocin content observed at an ultrasonic amplitude of 60%. However, when the intensity is increased to 80%, the crocin content decreases. This decline is attributed to the excessive ultrasonic amplitude, which induces the breakdown and decomposition of some fused crocin pigments. Higher ultrasonic amplitude also leads to increased heat generation, further contributing to pigment degradation (Wang et al., 2012; Kutlu et al., 2022; Lipeng et al., 2023). Extraction time is influenced by factors such as the type of raw materials, the solvent used, and the ultrasonic amplitude. Generally, a longer extraction time enhances efficiency. However, beyond a certain threshold, further extending the extraction time does not improve efficiency and may alter the pigment structure or extract unwanted compounds that affect color quality. If the ultrasound duration is too short, the solvent may not sufficiently

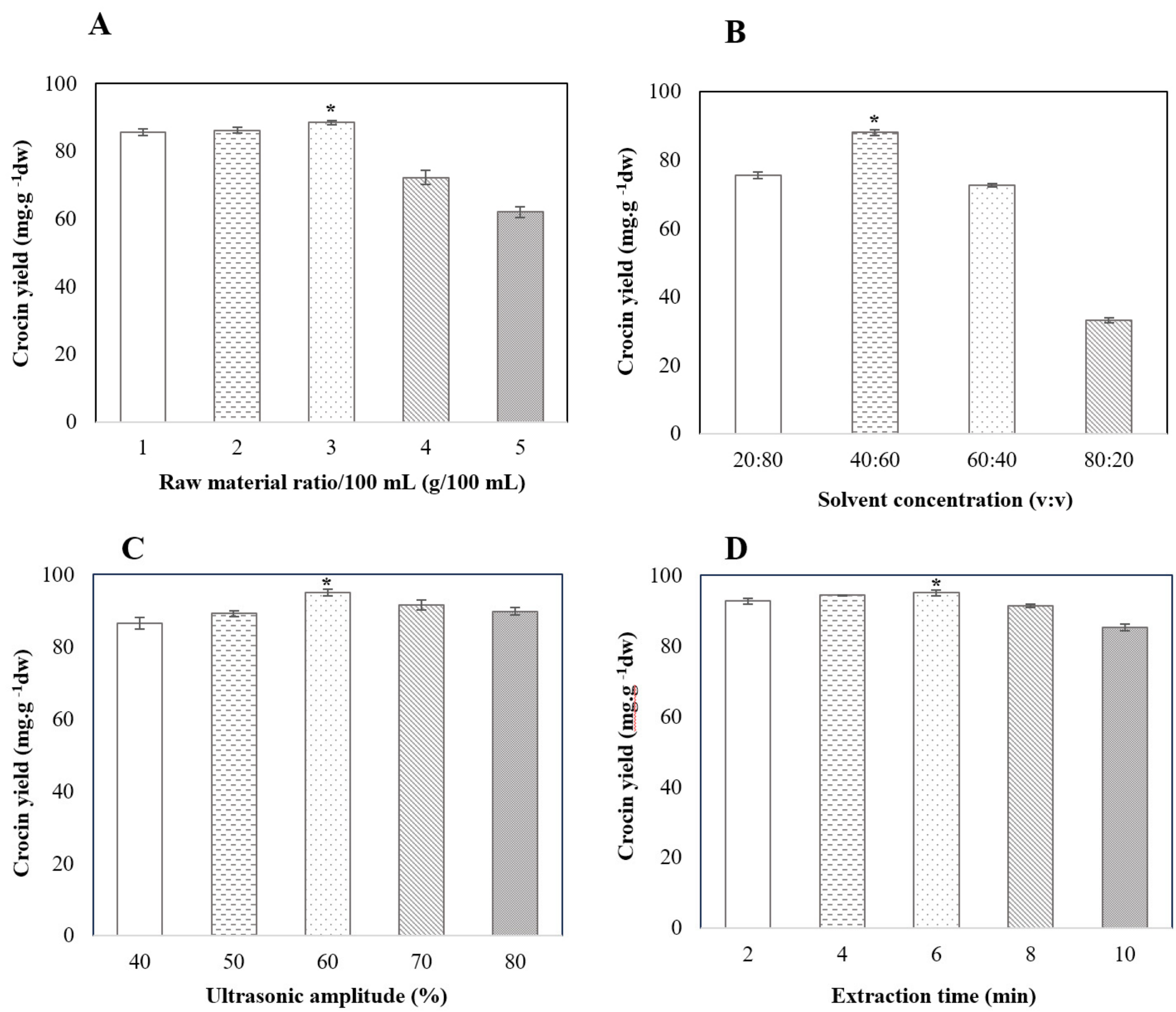

Fig. 3. Effects of extraction factors on crocin yield: A: solvent concentration; B: raw material ratio; C: ultrasonic amplitude; D: extraction time. Data are expressed as the mean±SD (n=3). Significantly different results with $p<0.05$ are indicated by (*) for each factor.

penetrate the cells to dissolve crocin, resulting in low extraction efficiency. Conversely, if the ultrasound time is excessively prolonged, crocin may undergo oxidation, negatively impacting the extraction yield (Naczk & Shahidi, 2004; Silva et al., 2007; Xin-Sheng et al., 2012). To achieve a high crocin content, we further investigated and optimized the aforementioned factors to enhance the extraction efficiency of this pigment.

Optimization of crocin extraction using response surface methodology (RSM)

Experimental design

The experimental design levels of the independent variables used in the extraction of FD2 are presented in Table 1. The responses, represented by crocin yield, obtained for each experiment are summarized in Table 2.

From the results in Table 2, it is evident that in experiment No. 38, the crocin content reached its highest value. This was achieved with a raw material-to-solvent ratio of 3 g/100 mL, a solvent concentration of 40%, an ultrasonic amplitude of 60%, and an ultrasound time of 6 min, yielding a maximum crocin content of 96.51 mg · g^{-1}dw. Conversely, in experiment No. 36, with a raw material-to-solvent ratio of 3 g/100 mL, a solvent concentration of 20%, an ultrasonic amplitude of 70%, and an ultrasound time of 6 min, the crocin content reached its lowest value of 54.86 mg · g^{-1}dw. At the central points, where the raw material-to-solvent ratio was 3 g/100 mL, the solvent concentration was 40%, the ultrasonic amplitude was 60%, and the ultrasound time was 6 min, the crocin content ranged from 93.70 to 96.51 mg · g^{-1}dw.

Table 2. Extraction conditions and responses for crocin contents obtained for the BBD

No.	Raw material ratio (g/100 mL)	Solvent concentration (%)	Ultrasonic amplitude (%)	Extraction time (min)	Crocin yield (mg · g^{-1}dw)
1	2	60	60	6	80.94
2	2	40	50	6	72.00
3	2	40	60	8	77.85
4	2	20	60	6	68.52
5	2	40	60	4	79.62
6	2	40	70	6	82.62
7	2	60	60	6	79.76
8	2	40	50	6	72.08
9	2	40	60	8	77.53
10	2	20	60	6	69.69
11	2	40	60	4	78.61
12	2	40	70	6	82.29
13	3	40	60	6	95.58
14	3	60	50	6	67.32
15	3	60	60	8	65.80
16	3	60	60	4	64.75
17	3	40	50	4	63.05
18	3	40	70	8	59.28
19	3	40	70	4	60.54
20	3	40	60	6	93.70
21	3	20	70	6	62.52
22	3	60	70	6	71.17
23	3	40	60	6	95.77
24	3	20	60	4	70.01
25	3	40	50	8	56.45
26	3	20	60	8	68.54
27	3	20	50	6	71.37
28	3	40	60	6	94.53
29	3	60	50	6	66.52
30	3	60	60	8	66.82
31	3	60	60	4	66.69
32	3	40	50	4	63.47
33	3	40	70	8	60.38
34	3	40	70	4	61.91
35	3	40	60	6	94.18
36	3	20	70	6	54.86
37	3	60	70	6	71.56
38	3	40	60	6	96.51
39	3	20	60	4	70.75
40	3	40	50	8	57.30
41	3	20	60	8	69.93
42	3	20	50	6	71.98
43	4	20	60	6	69.03
44	4	60	60	6	67.04
45	4	40	70	6	71.89
46	4	40	60	4	65.68
47	4	40	50	6	70.01
48	4	40	60	8	68.80
49	4	20	60	6	70.61
50	4	60	60	6	68.54
51	4	40	70	6	71.71
52	4	40	60	4	65.93
53	4	40	50	6	70.33
54	4	40	60	8	69.50

Response surface methodology

After obtaining the responses from each experiment (Table 2), response surface analysis was conducted within the experimental design investigated. Based on the responses and the effects of the independent variables on the estimates, the optimal conditions for crocin extraction and yield are summarized in Table 3. Three- dimensional graphs illustrating the influence of these parameters on the overall desirability of crocin concentration are presented in Fig. 4: (A) Solvent concentration/raw material ratio; (B) ultrasonic amplitude/raw material ratio; (C) Solvent concentration/extraction time. The convex shape of the graphs suggests that the optimal conditions for crocin extraction were identified with a high desirability value of 91%.

Under these optimized conditions, the highest crocin yield obtained was approximately 95.65 mg · g^{-1}dw, when the independent variables were set at 2.7 g/100 mL (raw material ratio), 5.95 min (extraction time), 60.41% (ultrasonic amplitude) and 41.48% (solvent concentration).

The extraction yield values of crocin obtained throughout the study are presented and summarized in Table 3. After optimization using RSM, the crocin extraction yield increased by 2.01 mg · g^{-1}dw compared to the yield obtained without optimization. However, the raw material ratio significantly decreased to 2.7 g/100 mL from 3 g/100 mL.

With the aid of response surface methodology, the maximum desirability (D) was determined to be 91%, suggesting that the model could predict 91% of the response variability. According to the study by Nguyen et al. (2022), the desirability for crocin yield from *Gardenia jasminoides* Ellis through soaking was determined to be 92.94% using RSM (Nguyen et al., 2022). Similarly, Sarfarazi et al. (2022) desmonstrated that maximum desirability for crocin extraction from *Saffron* using UAE was calculated to be 95%. In the present study, the high desirability (D) reflects a highly reliable result, attributed to the cumulative effects of all the analyzed factors. Each parameter had a distinct influence on the obtained responses, with their interactions playing a crucial role not only in the ultrasound process but also in the extraction. These interactions were optimized to achieve the highest yield of crocin.

The results of this study are summarized and presented in Table 3. RSM further confirmed that ultrasound enhanced crocin yield from freeze-dried *Gardenia* and grinding with liquid nitrogen. The application of RSM demonstrated the feasibility and reproducibility of this process, emphasizing the necessity of conducting similar

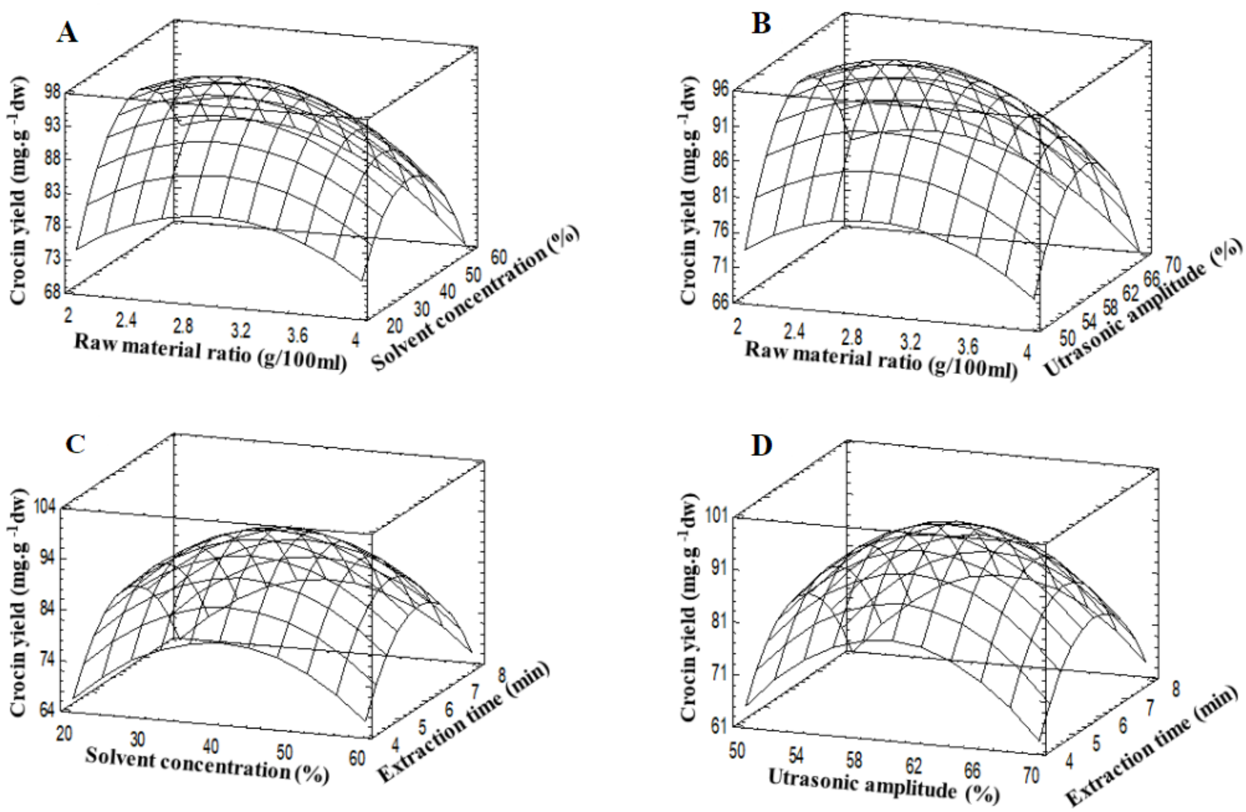

Fig. 4. Estimated response surfaces according to extraction time, solvent concentration, ultrasonic amplitude and raw material ratio parameters. A: crocin yield (mg · g^{-1}dw) as a function of solvent concentration and raw material ratio. B: crocin yield (mg · g^{-1}dw) as a function of ultrasonic amplitude and raw material ratio. C: crocin yield (mg · g^{-1}dw) as a function of solvent concentration and extraction time. D: crocin yield (mg · g^{-1}dw) as a function of ultrasonic amplitude and extraction time.

Table 3. Summary of the results for crocin extraction yield without optimization and optimization from FD2

	Without optimization	Optimization	
		Prediction values	After optimization
Raw material ratio (g/100 mL)	3	2.70	2.70
Time (min)	6	5.95	5.95
Solvent concentration (%)	40	41.48	41.48
Ultrasonic amplitude (%)	60	60.41	60.41
Crocin yield (mg · g^{-1}dw)	95.04±0.81	95.65	97.05±0.63

The mean±standard deviation was obtained within five replicates.

investigations for each material studied. After optimization using RSM, the crocin extraction yield improved compared to the yield obtained without optimization, while the raw material ratio significantly decreased (2.7 g/100 mL instead of 3.0 g/100 mL). It can contribute to reducing material costs and enhancing economic efficiency. According to the literature, crocin values for *Gardenia jasminoides* Ellis range from 10.08 to 36.97 mg · g^{-1}dw (He et al., 2006; Huang et al., 2022) with reported values of 4 and 20 mg · g^{-1}dw in some cases (Nguyen et al., 2022). For *saffron*, the crocin yields range from 178.06% to 270.65% (Mostapha et al., 2024). In our study, crocin yields varied from 33.55 mg · g^{-1}dw to 97.05 mg · g^{-1}dw, depending on the material pretreatment and extraction conditions. Therefore, this study highlights the impact of different pretreatment methods on crocin extraction and provides valuable insights into the ultrasonic extraction yields of crocin from *Gardenia jasminoides*.

Conclusion

This study highlights the effectiveness of material pretreatment and ultrasound-assisted extraction for optimizing crocin extraction from *Gardenia jasminoides*. In the initial phase, freeze-drying was identified as the most effective pretreatment technique, while subsequent grinding with liquid nitrogen further enhanced concentration.

The application of response surface methodology enabled the identification of key factors influencing the extraction process at various levels. A strong correlation between predicted and experimental results validated the accuracy and reliability of this model in optimizing crocin production from *Gardenia*. The optimized process significantly improved crocin extraction yield, achieving the highest values previously reported for this material.

The crocin-rich extracts obtained through this method have promising applications in various human-centered industries, including medicine, cosmetics, and food production. However, further research is needed to enhance the quality of the extract through purification techniques such as chromatography or filtration. Additionally, future studies should focus on assessing the thermal stability of crocin and its bioactive compounds.

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Conflict of interests

No potential conflict of interest relevant to this article was reported.

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Data availability

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authorship contribution statement

Conceptualization: Nguyen Huu Phuoc T.
Data curation: Nguyen Huu Phuoc T, Mai Thi Phuong C.
Formal analysis: Nguyen Huu Phuoc T.
Methodology: Nguyen Huu Phuoc T, Tran Thi Ngoc L.
Validation: Nguyen Huu Phuoc T, Tran Thi Kim H.
Investigation: Nguyen Huu Phuoc T.
Writing - original draft: Nguyen Huu Phuoc T.
Writing - review & editing: Nguyen Huu Phuoc T, Tran Thi Kim H, Tran Thi Ngoc L, Mai Thi Phuong C.

Ethics approval

Not applicable.

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