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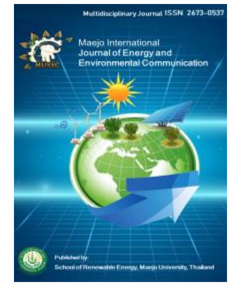

ARTICLE

Enhancing lutein extraction from marigolds through ultrasound-assisted optimization using response surface methodology

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A B S T R A C T

This research aimed to enhance and optimize the ultrasound-assisted extraction (UAE) of lutein from marigold flowers. The Response Surface Methodology (RSM) was the primary optimization technique. Three key independent variables were considered to determine the best conditions for the highest lutein yield: ultrasonic amplitude, extraction temperature, and extraction time. These variables were systematically varied following the Central Composite Design (CCD). Lutein quantitation was achieved using ultraviolet-visible spectrophotometry analysis. Using both the RSM and CCD frameworks, the study established specific ranges for the operational conditions: 21.6–38.4% for ultrasonic amplitude, 23.18–56.82 °C for extraction temperature and 3.18–36.82 minutes for extraction duration. The study determined the optimal extraction conditions to be 32.76% ultrasonic amplitude, 40.08 °C extraction temperature, and 25.82 minutes of extraction time. Under these optimized conditions, the experimental lutein yield closely matched the yield predicted by the RSM model, thus confirming the model's accuracy. The UAE demonstrated a notably superior lutein yield compared to traditional extraction techniques. The RSM is a robust tool for refining and determining optimal UAE conditions for lutein extraction from marigold flowers. With its efficiency, speed, and eco-friendliness, the optimized UAE technique presents significant potential for widespread industrial use.

1. Introduction

Post-COVID-19, the world has prioritized three developmental areas: energy, food, and health, with a particular emphasis on diet and health research. The surge in demand for healthy foods and supplements extends to beauty. Marigold flowers, rich in carotenoids, with lutein making up over 90% of these, have gained attention for their health benefits. Roberts et al. (2009) suggested that lutein offered significant protection from light-induced skin damage, particularly from the ultraviolet wavelengths, while Wang

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et al. (2006) noted that lutein supplements improved visual functions in patients with age-related macular degeneration. Epidemiological studies indicated that a high intake of lutein reduced the risk of lung cancer. Therefore, there is growing research interest in lutein extraction from various plant materials. Conventional methods of extracting lutein from natural sources rely heavily on solvents, which can be time-consuming, expensive, and potentially dangerous. Therefore, there's a push for alternative extraction techniques to address these issues (Vargas and Lopez, 1997).

Given their solubility with carotenoids, edible oils offer a viable alternative for carotenoid extraction. Li et al. (2013) utilized sunflower oil for ultrasound-assisted extraction from carrots. Other studies, like those by Sachindra & Mahendrakar (2005) and Chemat et al. (2012), explored various vegetable oils and eco-friendly solvents to achieve efficient and sustainable extraction. While oils help reduce oxidation, their high viscosity limits extraction efficiency, a challenge addressed by ultrasound-assisted extraction (UAE). This technique improves extraction due to the effects of ultrasound waves and cavitation forces, leading to tissue rupture and better compound release. Recent studies have looked into ultrasound extraction from various sources, like carrots and pomegranates, but there needs to be more research on extracting marigold carotenoids using soybean oil.

The response surface methodology (RSM) employs mathematical and statistical techniques for modeling and optimizing processes influenced by various factors to enhance a specific outcome (Ramaraj and Unpaprom, 2019). The one-factor-at-a-time approach, integral to RSM, efficiently evaluates the impact and interplay of numerous factors on target outcomes using fewer experiments (Saengsawang et al., 2020). By deploying RSM, one can glean insights into the influence of diverse parameters on extraction and pinpoint the conditions for optimal extraction (Manmai et al., 2020). Previous research (Liu et al., 2013; Wang et al., 2012; Yang et al., 2009; Yongphet et al., 2021) has frequently used RSM to predict bioactive compounds' extraction processes from natural sources.

In our study, we incorporated the central composite design (CCD) for experimental modeling, and the resulting data was encapsulated within a polynomial equation near the optimal condition (Abdollahi et al., 2012). Our research aimed to refine the ultrasound-assisted extraction conditions for lutein from marigolds, employing soybean oil as an eco-friendly alternative to conventional organic solvents in alignment with sustainable extraction and biorefinery principles. Key variables such as ultrasonic amplitude, temperature, and duration were examined relative to extraction yield, and lutein quantification was achieved using ultraviolet-visible (UV-Vis) spectrophotometric analysis.

2. Material and methods

2.1 Materials

Marigold flowers were obtained from the Erawan Shrine in Bangkok, Thailand. The flowers were cleaned and then cut to obtain the petals, which were blended and dried at 40 °C for 24 hours. A sieve shaker separated the powder.

2.2 Preparation of the extract

The 45–150 µm particle size of milled marigold powder (5 g) was soaked in soybean oil with a liquid-to-solid ratio of 15 and then sonicated with sonicator power 750 watts (VCX 750 Sonics & Materials, Inc., USA). The sample was collected by centrifugation at the interval time and passed through the filter paper of Whatman (no. 4). The filtrate was kept in a glass bottle at 4 °C after the removal of the solid.

2.3 Analysis of the ultraviolet-visible spectroscopy

A 1-cm optical path-long quartz cell was employed to examine the absorption spectrum of each lutein extract. This measurement used a 450-nm spectrophotometer, with a 3:7 ratio of soybean oil and acetate used as blank to detect the lutein level against the mainly produced solvent. The Beer-Lambert law measured lutein content in enriched soybean oil from a lutein standard calibration curve. The line to the absorbance versus lutein concentration calibration curve depended on the Beer-Lambert law.

2.4 Design of the experiment

To further investigate the interactions of the factors, the RSM and the CCD approach were used to optimize working conditions. Based on the early results of the tests, the range and the center point values for three independent variables were provided in Table 1. At the central points, 20 treatments were created with six replicates to assess the repeatability of the approach, as provided in Table 2. The chosen independent variables were ultrasonic amplitude (X1), temperature (X2), and time (X3). This experimental design has examined the optimal working conditions for extracting marigold lutein, processed using the R Project for Statistical Computing software, version 3.4.1.

Table 1. Independent variables and values codified for the optimization of the extraction procedure

Independent variables	**Code units**	**Coded levels**				
		-1.68	-1	0	1	1.68
Ultrasonic amplitude (%)	X_1	21.6	25	30	35	38.4
Temperature (°C)	X_2	23.18	30	40	50	56.82
Time (minute)	X_3	3.18	10	20	30	36.82

3. Results and discussion

3.1 Lutein content in marigold flowers

Marigolds are rich in organic carbohydrates and other organic acids, making them a valuable biomass source. Asia dominates the global floriculture sector, where the flower trade plays a crucial role in commerce and serves as a vital revenue stream. As per Piccaglia et al. (1998), marigolds are an excellent source of lutein and its esters. While lutein is present in various fruits and vegetables, the marigold flower is the premier commercial source of pure lutein. Lutein, an oxygen-containing carotenoid, belongs to the xanthophylls group and is a natural pigment in fruits, flowers, vegetables, and algae (Maoka, 2020). Chemically, lutein is 3,3′-dihydroxy-β, α-carotene, a derivative of α-carotene without vitamin-A activity. It possesses a molecular formula of $C_{40}H_{56}O_2$ and a molecular weight of 568.88.

Lutein, a carotenoid in various foods, offers coloring and antioxidant properties, making it an ideal addition to common functional and healthy foods. It holds promise for preventing age-related macular disease and offers antioxidant protection to lipoproteins and lymphocyte DNA. Lutein consumption is associated with reduced heart disease, cancer, and macular degeneration risks (Becerra et al., 2020). Within the retina, lutein and zeaxanthin provide vital protection by shielding against blue light damage and oxidative stress. Marigold, known for its aesthetic and medicinal value, is a prime source of lutein, particularly *Tagetes erecta* and Tagetes patula species (Chitrakar et al., 2019). Marigold's year-round availability and easy cultivation make it appealing for lutein extraction. Given its potential, utilizing marigolds as a food coloring and nutrient supplement showcases its role in enhancing functional and health-promoting foods through its antioxidant and coloring benefits.

Table 2. Central composite design for three variables and the observed responses

Run	X_1 (%)	X_2 (°C)	X_3 (minute)	Lutein (μg / 1 g marigold)
1	25 (-1)	30 (-1)	10 (-1)	8496.161
2	35 (1)	30 (-1)	10 (-1)	9831.132
3	25 (-1)	50 (1)	10 (-1)	8704.024
4	35 (1)	50 (1)	10 (-1)	10145.36
5	25 (-1)	30 (-1)	30 (1)	11047.7
	35 (1)	30 (-1)	30 (1)	12593.38
7	25 (-1)	50 (1)	30 (1)	11085.81
8	35 (1)	50 (1)	30 (1)	12595.98
9	21.6 (-1.68)	40 (0)	20 (0)	12761.4
10	38.4 (1.68)	40 (0)	20 (0)	12279.27
11	30 (0)	23.18 (-1.68)	20 (0)	11212.87
12	30 (0)	56.82 (1.68)	20 (0)	11233.63
13	30 (0)	40 (0)	3.18 (-1.68)	6501.672
14	30 (0)	40 (0)	36.82 (1.68)	12960.35
15	30 (0)	40 (0)	20 (0)	13209.55
16	30 (0)	40 (0)	20 (0)	13233.73
17	30 (0)	40 (0)	20 (0)	13229.04
18	30 (0)	40 (0)	20 (0)	13267.88
19	30 (0)	40 (0)	20 (0)	13230.98
20	30 (0)	40 (0)	20 (0)	13225.68

3.2 Lutein extraction and optimazations

In natural product extraction, innovative methods are sought for efficiency and sustainability. A novel technique involves extracting lutein from marigold flowers using ultrasound and soybean oil as a solvent. This approach optimizes extraction by adjusting ultrasonic factors and time (Saetang et al., 2022). Experimental results in Table 2 show the relationship between variables (amplitude, temperature, time) and lutein yield. RSM is used to understand correlations using mathematical models based on empirical data.

RSM guides extraction optimization, providing insights into how variable changes affect lutein yield (Muralidhar et al., 2001). This process aids in refining extraction for higher lutein yield, a health-promoting compound. The combination of ultrasound, soybean oil, and RSM showcases advancements in natural product extraction, contributing to the sustainable production of bioactive compounds. Research in this direction points toward a greener future in natural product extraction. The response variable and the test variables are linked to the following second-order polynomial equation by applying a multiple regression analysis on the experimental data:

$$Y = -22989.79209 + 895.46731X_1 + 658.00324X_2 + 691.36329X_3 + 0.17714X_1X_2 + 0.69886X_1X_3 - 0.60173X_2X_3 - 14.04861X_1^2 - 8.08633X_2^2 - 13.36089X_3^2$$

Where,

Y = the extraction yield of lutein; and

X_1, X_2, and X_3 = the experimental coded variables

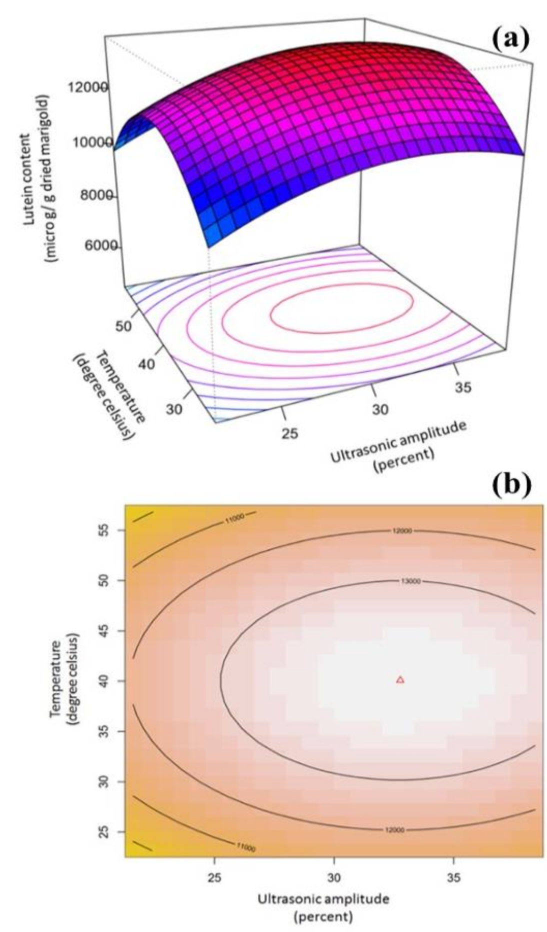

Figure 1. Three-dimensional response surface graphs alongside two-dimensional contour diagrams illustrate variables' influence on response Y: (a) lutein levels as influenced by ultrasonic amplitude and temperature at a constant time; (b) lutein extraction rate based on ultrasonic amplitude and time at a set temperature

Table 3 provides the regression coefficients of the student's t-test statistics and probability values for the significance of the variables for each term in the model. The p-value is employed as a tool for monitoring the significance of the coefficients and the strength of interactions between the independent variables. The related variables will become more significant if the absolute t-value grows and the p-value becomes lower (Quanhong & Caili, 2005). As the p-values become smaller, the coefficients become more significant. This table shows that linear X_2 and X_3 coefficients were significant at $p < 0.05$ and $p < 0.01$, respectively. The X_2^2 and X_3^2 quadric terms were significant with extremely small p-value, such as $p < 0.01$ or $p < 0.001$, respectively. The remaining terms of the coefficient ($p > 0.05$) were not considered significant.

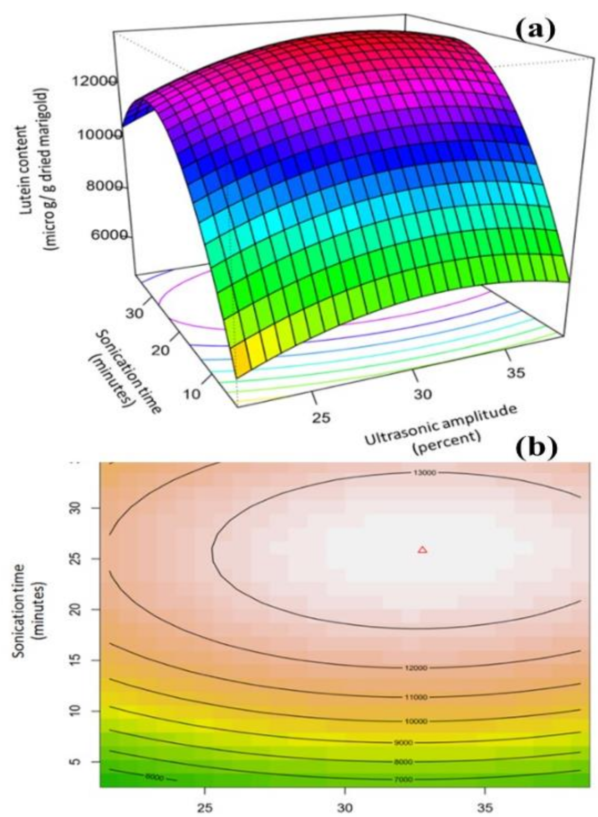

Figure 2. 3D response surface plots and 2D contour plots that show the variables' effects on the response Y: (a) and (b) extraction rate of lutein with the ultrasonic amplitude and time at a fixed temperature

The plots of the 3D-response surface and 2D contour graphically reflect the regression equation. The outcomes of the extractive output of lutein are shown in Figure 1 as impacted by ultrasonic amplitude (X_1), temperature (X_2), and time (X_3). For removing lutein with changing ultrasonic amplitude and temperature in the fixed time, the 3D-response-surface plot and the 2D-contour plot were created, as shown in Figure 1. The lutein yield raised at a fixed temperature, with an increased ultrasonic amplitude between 21.60% and 32.76%. This effect might be owing to enhanced cavitation and the mechanical impact of ultrasound that enhanced the contact area between marigold powder and soybean oil and led the soybean oil to penetrate the matrix of the marigold. The collapse of cavitation bubbles at high amplitude values is severer (Suslick and Price, 1999). Nevertheless, the rise in ultrasonic amplitude governed a yield deterioration, possibly owing to plant material degradation, with amplitude levels above 32.76%. Extraction yield increases with the fixed ultrasonic amplitude of 23.18 °C to 40.08 °C and with an increase in temperature of 40.08 °C to 56.82 °C, contributing to a visible opposite trend. The effect might be related to the fact that a rise in the temperature improves the solubility and diffusion coefficients of the extracted material and lowers the solvent viscosity, making its transit through the solid substrate mass more facilely. At high temperatures, however, lutein can be degraded (Ahmad et al., 2013).

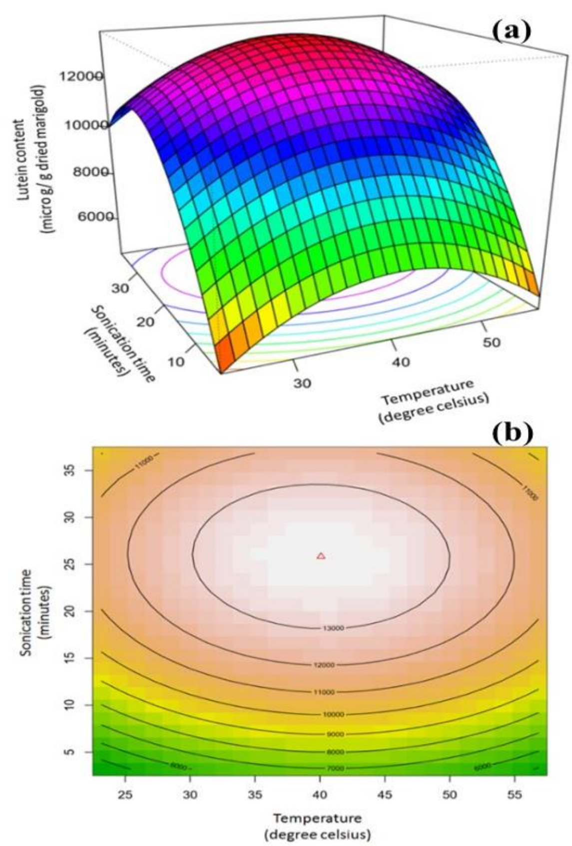

Figure 3. 3D response surface plots and 2D contour plots that show the variables' effects on the response Y: (a) and (b) extraction time is fixed, the extraction by different temperatures and times

The extraction rate of lutein in accordance with the ultrasonic amplitude and time at a fixed temperature in the 3D-response-surface and 2D-contour plots (Figure 2). Lutein yield rose at a fixed ultrasonic amplitude, from 3.18 to 25.82 minutes, with an increased sonication time. In contrast, increasing time above 25.82 minutes, the lutein yield decreased. The fact that the extraction process takes time for two stages may be ascribed to this. The first stage, characterized by a quick pace, includes a cellular structural insertion of the solvent and then the dissolution of the soluble components in the solvent. The second stage involves the extrinsic diffusion of soluble components from the solution into the bulk of the solution by means of a porous structure of the remaining solids and their transfer (Goula, 2013). However, in a long sonication time, this effect may give the opposite trend because of an accumulation of heat in the extraction process, which may cause the degradation of lutein content. At a fixed sonication time, the lutein yield increased with increasing ultrasonic amplitude from 21.6% to 32.76%, whereas with increasing ultrasonic amplitude from 32.76% to 38.40%, the lutein yield decreased.

Table 3. Results of regression coefficients from the data of the CCD experiments

Term	Coefficient	t-value	p-value
Intercept	-22989.79209	2.2956	0.04459
X_1	895.46731	1.8502	0.094021
X_2	658.00324	3.1293	0.010703
X_3	691.36329	3.6585	0.0044
X_1X_2	0.17714	0.0367	0.971412
X_1X_3	0.69886	0.1450	0.887618
X_2X_3	-0.60173	0.2496	0.807923
X_1^2	-14.04861	1.9523	0.079442
X_2^2	-8.08633	4.5037	0.001137
X_3^2	-13.36089	7.4413	0.00002207

In Figure 3, when the extraction time is fixed, the extraction rate of lutein is influenced by different temperatures and times. The extraction yield increased with the increasing extraction temperature from 23.18 °C to 40.08 °C. The lutein yield decreased with the increasing extraction temperature from 40.08 °C to 56.82 °C. At a fixed temperature, lutein yield increased from 3.18 minutes to 25.82 minutes, with increasing sonication time. Meanwhile, the lutein yield decreased as the sonication time increased from 25.82 minutes to 36.82 minutes.

3.3 UAE: pioneering energy efficiency in extraction

Ultrasound-assisted extraction (UAE) has emerged as a transformative technique in the realm of energy conservation during extraction processes. Compared to traditional extraction methods, which are notorious for their high energy consumption, UAE offers a more energy-efficient alternative. A key advantage of UAE is its expedited processing time, achieved through its direct impact on the material. Additionally, by operating at ambient to moderate temperatures, UAE avoids the energy-intensive heating associated with other methods, leading to significant energy savings (Balicki et al., 2020).

When it comes to energy conservation, UAE excels. It channels energy precisely towards disrupting cellular structures or enhancing material permeability, ensuring maximum energy utilization with minimal waste (Liu et al., 2013). Furthermore, its ability to effortlessly regulate temperature further conserves energy by preventing excessive heating. As for energy efficiency, ultrasound's prowess in penetrating materials elevates the extraction process's efficacy, curbing energy wastage. Equipment designed for UAE also requires less maintenance, indicating its long-term energy efficiency. Notably, the reduced solvent demand inherent to UAE not only supports environmental sustainability but also saves the energy typically allocated to solvent handling.

Another distinctive feature of UAE is its scalability. It can be adapted to large-scale industrial operations without a proportionate increase in energy usage (Belwal et al., 2020). In summary, while conventional extraction methods have been marred by inefficiencies and high energy demands, the advent of UAE signifies a paradigm shift towards greater energy conservation, efficiency, and sustainability. This innovation not only optimizes operational costs but also nudges industries towards greener practices.

4 Conclusion

This study pinpointed the ideal conditions for the ultrasound-assisted extraction of lutein from marigold flowers: an ultrasonic amplitude of 32.76%, a temperature setting of 40.08 °C, and a precise extraction time of 25.82 minutes. These findings are groundbreaking, suggesting a notable enhancement in the efficiency of lutein extraction. The empirical evidence showcases that ultrasound is not just a viable method for lutein extraction from marigold, but it also brings forth a gamut of benefits like cost reduction, faster extraction times, and an increase in overall yield. One cannot overlook the environmental implications, either. The traditional methods of extraction, which typically rely on prolonged durations and elevated temperatures, invariably contribute to higher energy consumption. In contrast, by leveraging the optimized ultrasound-assisted technique, industries stand to benefit from slashed energy expenses, embodying a leap towards greener and more sustainable operational standards. Recognizing lutein's pivotal role in the cosmetic, nutraceutical, and food sectors, the transition of this optimized technique from the lab's confines to full-fledged industrial realms heralds an era of sustainability. This transition couples energy efficiency with cost-effectiveness in the extraction processes, providing multifaceted advantages that positively influence both our environment and economic efficiency.

Conflict of Interest Statement

The authors confirm that there are no financial conflicts or personal affiliations that could be perceived as influencing the findings presented in this study.

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