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Research Article

# Optimization of chlorophyll extraction from alpinia leaves using response surface methodology (RSM)

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***Abstract***

The optimization of the chlorophyll extraction process from Vietnamese galangal (*Alpinia* spp.) leaves was investigated using Response Surface Methodology (RSM). The synergistic effects of three independent variables, including extraction time, extraction temperature, and solid-to-solvent ratio, were evaluated through a Central Composite Design (CCD). Analysis of variance (ANOVA) revealed that the solid-to-solvent ratio had the most significant impact on the extraction yield, whereas the interactions between variables were not statistically significant ($p > 0.05$). The optimal extraction conditions were determined at a temperature of 57.5°C for 45 min with a liquid-to-solid ratio of 50:1 (mL/g), yielding a chlorophyll content of 235.56 ± 2.17 mg/100 g. The experimental results demonstrated high agreement with the predicted model (> 97%). Furthermore, the application of this method to analyze 13 real samples suggests that galangal leaves are a promising source for natural chlorophyll extraction.

***Keywords:*** *Galangal leaves, chlorophyll, extraction, optimization, Response Surface Methodology (RSM).*

## 1. INTRODUCTION

Along with the development of the food industry, food colorants have been increasingly utilized in a diverse and widespread manner to impart or enhance color, thereby improving product appeal. However, in recent years, numerous countries, including the United States, have enacted regulations to restrict and gradually phase out synthetic petroleum-based dyes due to concerns over health risks, such as allergies and behavioral disorders [1]. This trend has significantly driven the demand for identifying and utilizing safe, natural colorants sourced from local raw materials.

Chlorophyll is a family of essential green pigments found in plants, algae, and photosynthetic bacteria. Characterized by a central porphyrin ring coordinating a magnesium atom, chlorophyll not only captures light energy to drive photosynthesis but also holds significant application value in the food, pharmaceutical, and cosmetic industries due to its antioxidant properties and natural color-imparting capability [2-4].

Globally, research on the extraction of chlorophyll from plant leaves has extensively developed, with a primary focus on optimizing methods to increase extraction yield and minimize pigment degradation. Common techniques include liquid-liquid extraction (LLE), counter-current separation (CCS), ultrasound-assisted extraction, microwave-assisted extraction, and supercritical $CO_2$ extraction, which have been applied to various leaf species such as spinach, pandan, and moringa [5-9]. In Vietnam, studies have explored chlorophyll extraction from local leaves, including *Polyscias fruticosa*, *Pandanus amaryllifolius*, and *Centella asiatica* [10-12]. However, most of these works have primarily aimed at the quantitative analysis of chlorophyll content in

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food or medicinal samples. Research focusing on large-scale extraction to obtain chlorophyll as a natural food colorant remains highly limited.

Galangal leaves (*Alpinia* spp.), belonging to the family Zingiberaceae, represent a large plant genus comprising approximately 250 species native to tropical and subtropical regions of Asia. Among these, Vietnam is one of the countries with high biodiversity, where numerous species have been documented and utilized in traditional medicine. Galangal leaves are known to possess a high concentration of chlorophyll, making them a potential raw material for chlorophyll extraction [13-14].

This study was conducted to optimize the chlorophyll extraction process from galangal leaves using ultrasound-assisted extraction coupled with temperature and Response Surface Methodology (RSM), with the primary objective of maximizing chlorophyll yield at a laboratory scale. The findings of this study will provide a foundation for developing an industrial-scale chlorophyll extraction process to obtain a safe, sustainable, and natural food colorant, which holds broad application potential in the Vietnamese food industry.

## 2. MATERIALS AND METHODS

### 2.1. Research subjects

Galangal leaves were harvested from a domestic garden in Dong Anh, Hanoi. The scientific name was determined using the comparative morphological method, verified against botanical literature [15] and compared with descriptions in the reference documentation by Nguyen Quoc Binh [16]. The leaves were then washed thoroughly with water, drained, de-veined, and finely chopped under light-restricted conditions immediately prior to homogenization using a blender. The homogenized sample was stored under freezing conditions at -20°C.

### 2.2. Chemicals and equipment

Analytical-grade reagents including 96% ethanol (Vinachem, Vietnam), methanol, acetone, and diethyl ether (Merck, Germany) were used. The primary equipment and instruments utilized consisted of a Shimadzu UV-Vis spectrophotometer (UV-2401PC, Japan), an Elma 300 ultrasonic bath (Germany), an Eppendorf bottle-top dispenser (5 - 25 mL), and Eppendorf micropipettes (0.2-1 mL and 1-5 mL).

### 2.3. Research methods

#### *2.3.1. Sample preparation*

The homogenized sample (1.0 g) was transferred into a 50 mL centrifuge tube. Extraction was performed using various common solvents suitable for chlorophyll extraction, with a solvent volume ranging from 10 to 60 mL. The mixture was subjected to ultrasound-assisted extraction at temperatures between 30 and 80°C for a duration of 5 to 75 min. Following the extraction, the mixture was centrifuged at 6,000 rpm for 3 min and then filtered through filter paper. The resulting filtrate was transferred into an appropriate volumetric flask (a 50 mL volumetric flask for extract volumes less than or equal to 50 mL, or a 100 mL volumetric flask for extract volumes greater than 50 mL), and the solvent was added up to the mark. The extract was diluted 5-folds prior to measuring the absorbance at wavelengths of 649 nm and 664 nm [17].

#### *2.3.2. Determination of total chlorophyll content*

According to the method described by Lichtenthaler (1987) [17], the total chlorophyll content in the extract was determined using molecular absorption spectrophotometry based on characteristic wavelengths. The absorbance values (A) were recorded at wavelengths of 649 nm and 664 nm using a UV-Vis spectrophotometer. The concentrations of chlorophyll a ($C_a$), chlorophyll b ($C_b$), and total chlorophyll ($C_{total}$) in the diluted extract were calculated using the following equations (expressed in mg/L):

$$C_a = 13.36A_{664} - 1.28A_{649} \quad (1)$$

$$C_b = 27.43A_{649} - 8.12A_{664} \quad (2)$$

$$C_{total} = 5.24A_{649} + 22.24A_{664} \quad (3)$$

Where: $A_{664}$ is the absorbance measured at a wavelength of 664 nm.

$A_{649}$ is the absorbance measured at a wavelength of 649 nm.

The actual chlorophyll content in galangal leaves (expressed in mg/100 g of fresh weight) was determined using the following equation:

$$\text{Content (mg/100 g)} = \frac{C_{total} \times D \times V}{m \times 1000} \times 100 \quad (4)$$

Where: $V$ is the final volume of the volumetric flask used for the extract (mL); $m$ is the mass of the solid sample (g); $D$ is the dilution factor of the sample during spectrophotometric measurement ($D$ = 5); *1000* is the conversion factor from mL to L.

*2.3.3. Investigation of factors affecting the extraction process*

2.3.3.1. Investigation of extraction solvents

The effects of solvent polarity and safety on the chlorophyll extraction process were investigated using four types of solvents: Acetone, 96% ethanol, methanol, and diethyl ether. Other experimental conditions were kept constant, including the ultrasound-assisted extraction method, a solvent-to-sample ratio of 25:1, an extraction temperature of 40°C, and an extraction time of 20 min.

2.3.3.2. Investigation of solvent-to-solid ratio

After determining the optimal solvent system, the effect of the solvent volume-to-solid mass ratio on the extraction yield was evaluated to optimize the diffusivity of chlorophyll. The experiment was conducted by keeping the sample mass constant (1 g) and varying the solvent volume from 5 mL to 60 mL in increments of 10 mL (corresponding to ratios from 5:1 to 60:1 v/w). Other experimental conditions were kept constant, including the ultrasound-assisted extraction method, an extraction temperature of 40°C, and an extraction time of 20 min.

2.3.3.3. Investigation of extraction temperature

To determine the optimal temperature threshold for the diffusion process while ensuring chlorophyll stability, experiments were carried out across a temperature range of 30°C to 80°C. The temperature increments were set at 10°C during the initial stage and narrowed to 5°C during the later stage (70°C to 80°C) to capture detailed physicochemical changes of the pigment. Other experimental conditions were kept constant, including the ultrasound-assisted extraction method, a solvent-to-sample ratio of 25:1, and an extraction time of 20 min.

2.3.3.4. Investigation of extraction time

To elucidate the dependence of cavitation efficiency and mass transfer kinetics on the extraction time, experiments were conducted across a time range of 5 to 35 min with 5-min increments, along with an additional investigation point at the 60-min mark to evaluate the equilibrium state of the system. Other experimental conditions were kept constant, including the ultrasound-assisted extraction method, a solvent-to-sample ratio of 25:1, and an extraction temperature of 40°C.

*2.3.4. Optimization experimental design*

Design-Expert 13 software was utilized for experimental design and statistical data analysis. A Central Composite Design (CCD) with three independent variables was selected, and the specific level values of these factors are presented in **Table 1**.

***Table 1.*** *Experimental factors and their corresponding levels*

| Factor | Level | | |
|---|---|---|---|
| | **-1** | **0** | **+1** |
| $X_1$ (Extraction temperature - °C) | 40 | 57.5 | 75 |
| $X_2$ (Extraction time - min) | 15 | 45 | 75 |
| $X_3$ (Liquid-to-solid ratio - mL/g) | 10 | 30 | 50 |

The total number of experiments in the CCD matrix is calculated using the following equation:

$$N = N_f + N^* + N_o \quad (5)$$

Where: $N_f$ is the number of factorial (cube) points ($N_f = 2^{n-q}$, where n is the number of factors and q is the fractional roll factor); $N^*$ is the number of axial (star) points, $N^* = 2n$; $N_o$ is the number of center points, $N_o > 1$.

With n = 3 and q = 0, the components of the experimental design are determined as: $N^* = 6$; $N_f = 8$; $N_o = 6$.

Consequently, the experimental matrix was deployed with a total of 20 runs (N = 20) using galangal leaf samples, which included 6 replicates at the center point to evaluate the reproducibility of the method. The axial distance from the center was established at d = 1.682, corresponding to a rotatable central composite design for three independent variables. All experiments within the model were conducted in duplicate under identical conditions.

*2.3.5. Method validation*

The precision of the method was evaluated using two parameters: Repeatability and intermediate precision (intra-laboratory reproducibility). Repeatability was determined by analyzing replicate samples (n = 6) of galangal leaves and other potential leafy by-products under identical experimental conditions. Intermediate precision was assessed by repeating the analytical procedure at different times (on different days) to verify the stability of the method under varying environmental conditions.

To evaluate the efficiency of the proposed extraction method, the chlorophyll content obtained via the optimized method (RSM-UAE) was compared against the standard method TCVN 13283:2021 [18] across three distinct matrices with different chlorophyll concentration ranges: Galangal, lettuce, and mugwort leaves. The relative deviation percentage was calculated using the following equation:

$$\text{Relative deviation (\%)} = \frac{Mean_{UAE} - Mean_{TCVN}}{Mean_{TCVN}} \times 100 \qquad (6)$$

Where: $Mean_{UAE}$ is the mean chlorophyll content obtained by the proposed research method.

$Mean_{TCVN}$ is the mean chlorophyll content obtained by the standard method TCVN 13283:2021.

## 3. RESULTS AND DISCUSSION

### 3.1. Investigation of factors affecting the extraction process

The preliminary investigation stage was conducted to evaluate the effects of four independent variables (extraction solvent, solvent volume-to-sample mass ratio, temperature, and time) on the chlorophyll extraction yield. The screening results enabled the determination of preliminary optimal variation thresholds, thereby narrowing the scope of research and establishing a solid foundation for constructing the experimental matrix according to the CCD of the RSM.

*3.1.1. Investigation of extraction solvents*

According to previous studies on chlorophyll extraction [7], [17], acetone, 96% ethanol, methanol, and diethyl ether are common and recommended solvents for extracting chlorophyll from plant leaves. The results regarding the effect of extraction solvents on the chlorophyll content are presented in **Figure 1.**

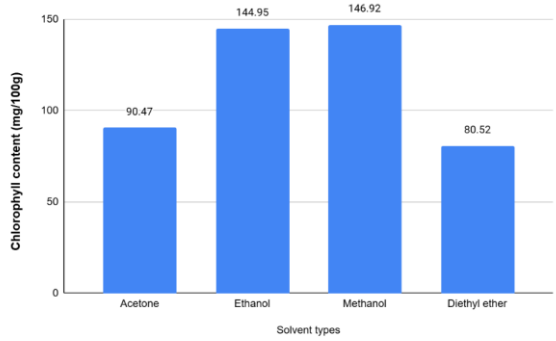


***Figure 1.*** *Effect of extraction solvents on chlorophyll content*

The results presented in **Figure 1** indicate that methanol and 96% ethanol were the two most efficient extraction solvents, with chlorophyll contents reaching 146.92 and 144.95 mg/100 g, respectively. Due to their polar protic nature and strong hydrogen-bonding capability, these two solvents easily disrupt the protein-lipid interactions within the thylakoid membrane to solubilize chlorophyll [19]. In contrast, acetone and diethyl ether exhibited significantly lower extraction efficiencies due to weaker interactions [20]. Although the chlorophyll yields obtained using methanol and ethanol showed no significant difference, 96% ethanol was selected due to its safety, ease of residue removal, and high feasibility for potential scale-up to industrial applications [21].

*3.1.2. Investigation of solvent-to-solid ratio*

To determine the effect of the solvent volume-to-sample mass ratio, investigation experiments were conducted across a range of 10:1 to 60:1. The resulting chlorophyll contents are illustrated in **Figure 2**.

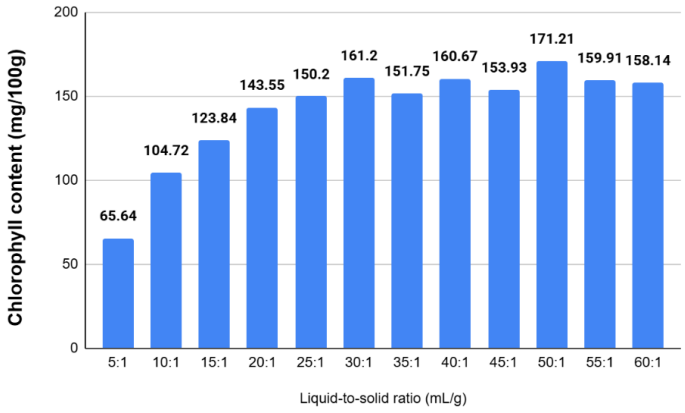


***Figure 2.*** *Effect of different solvent-to-solid ratios on chlorophyll content*

The experimental results in **Figure 2** indicate that the liquid-to-solid ratio strongly influences the extraction efficiency. The chlorophyll content increased continuously from a ratio of 5:1 (65.64 mg/100 g) and reached its maximum at 50:1 (171.21 mg/100 g). This upward trend is elucidated by Fick's laws of diffusion: A larger solvent volume creates a higher concentration gradient, thereby accelerating the mass transfer of chlorophyll from the plant cells into the liquid phase [19], [22], [23]. However, when the ratio exceeded 50:1, the chlorophyll content exhibited a slight downward trend (decreasing to 158.14 mg/100 g at a ratio of 60:1). This phenomenon could be attributed to the system reaching an equilibrium state, while an excessive solvent volume dilutes the extract and increases the risk of target compound oxidation [22]. Based on these findings, the variation ranges from 10:1 to 50:1 was selected for the RSM design to optimize extraction efficiency while minimizing solvent costs [20].

*3.1.3. Investigation of extraction temperature*

To determine the appropriate variation range for the RSM design, the effect of temperature on chlorophyll content was investigated from 30°C to 80°C. The results are presented in **Figure 3**.

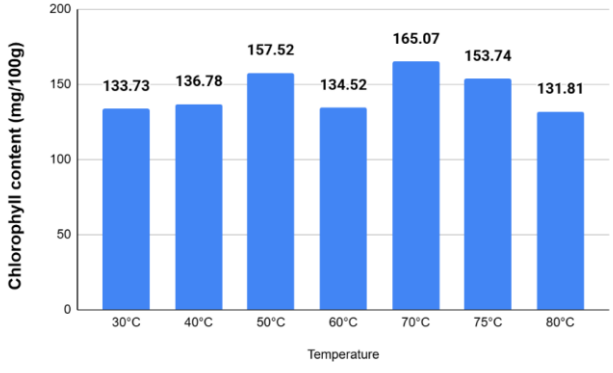


***Figure 3.*** *Effect of different extraction temperatures on chlorophyll content*

The results in **Figure 3** indicate that the chlorophyll content gradually increased with temperature and reached its maximum at 70°C (165.07 mg/100 g). This upward trend follows the Arrhenius law: Higher temperatures elevate kinetic energy, thereby promoting the diffusion of chlorophyll from the thylakoid membrane into the solvent. However, when the temperature exceeded 70°C, the chlorophyll content dropped sharply (a 20% reduction at 80°C) due to thermal degradation, during which the $Mg^{2+}$ ion in the porphyrin ring is replaced, leading to the formation of pheophytin [20]. The temperature range of 40 - 75°C was selected for the RSM design because it encompasses the high-efficiency region and the preliminary optimal point. This

range not only enables accurate model predictions but also avoids excessively high temperatures that cause severe degradation of the target compound, ensuring both economic efficiency and process stability for practical applications [19].

*3.1.4. Investigation of extraction time*

The effect of extraction time on chlorophyll content was investigated across a range of 5 to 75 min. The results are presented in **Figure 4**.

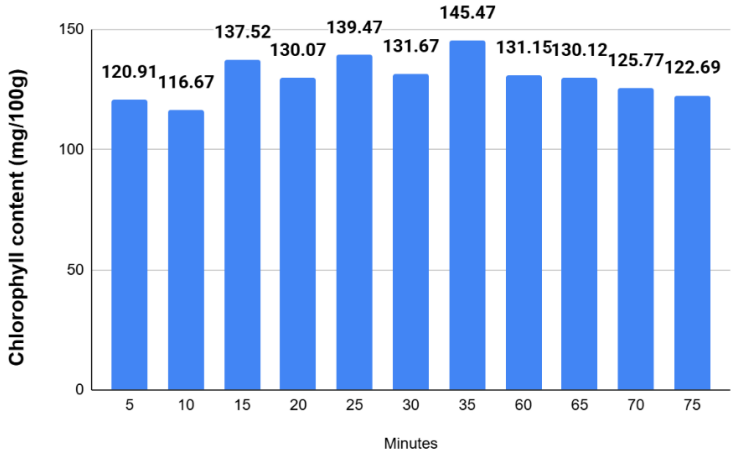


***Figure 4.*** *Effect of different extraction times on chlorophyll content*

The experimental results in **Figure 4** indicate that the chlorophyll content reached its maximum at 35 min (145.47 mg/100 g) [19]. During the initial stage from 10 to 35 min, the extraction efficiency increased sharply due to the diffusion of chlorophyll from plant cells into the solvent, which obeys Fick's laws [20]. However, when extending the extraction time beyond 35 min, the chlorophyll content exhibited a slight downward trend, decreasing to 131.15 mg/100 g at 60 min and 122.69 mg/100 g at 75 min. This decrease is attributed to oxidative degradation and pheophytinization caused by prolonged exposure to extraction temperature and dissolved oxygen. Consequently, the time range of 15 to 75 min was selected for the RSM design.

**3.2. Optimization of extraction conditions**

The chlorophyll content obtained under different experimental conditions is summarized in **Table 2**. Additionally, the statistical parameters regarding the individual effects and the interactions between the factors on the extraction efficiency are presented in detail in **Table 3**.

***Table 2.*** *CCD matrix and experimental results*

| Run | Temp. (°C) | Time (min) | LSR (mL/g) | Content (mg/100 g) | Run | Temp. (°C) | Time (min) | LSR (mL/g) | Content (mg/100 g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 40 | 15 | 10 | 109.31 | 11 | 40 | 75 | 10 | 106.39 |
| 2 | 75 | 75 | 50 | 228.80 | 12 | 40 | 15 | 50 | 233.51 |
| 3 | 75 | 15 | 10 | 150.98 | 13 | 57.5 | 45 | 63.64 | 223.76 |
| 4 | 86.93[(2)] | 45 | 30 | 178.34 | 14 | 57.5 | 45 | 30 | 194.36 |
| 5 | 57.5 | 95.45 | 30 | 205.11 | 15 | 57.5 | 45 | 30 | 194.82 |
| 6 | 57.5 | 45 | 30 | 192.38 | 16 | 28.07[(2)] | 45 | 30 | 194.02 |
| 7 | 57.5 | -5.45[(1)] | 30 | | 17 | 75 | 75 | 20 | 123.49 |
| 8 | 75 | 15 | 50 | 227.53 | 18 | 40 | 75 | 50 | 217.58 |
| 9 | 57.5 | 45 | 30 | 192.24 | 19 | 57.5 | 45 | 30 | 199.72 |
| 10 | 57.5 | 45 | -3.64[(1)] | | 20 | 57.5 | 45 | 30 | 201.51 |

*Note: [(1)] For negative values of the investigated parameters, the chlorophyll content was assigned a value of 0; [(2)] for temperature values were rounded and set to a resolution of 0.5°C.*

***Table 3.*** *Regression coefficients and p-values of the model factors*

| Model factors | Coefficent | *p*-value |
|---|---|---|
| Temperature | 0.9016 | 0.3702 |
| Time | 1.63 | 0.2379 |
| Liquid-to-solid ratio | 214.45 | 0.0001 |
| Temperature × Time | 0.0588 | 0.8145 |
| Temperature × Liquid-to-solid ratio | 3.11 | 0.1157 |
| Time × Liquid-to-solid ratio | 0.2695 | 0.6177 |
| Temperature$^2$ | 2.15 | 0.1810 |
| Time$^2$ | 1.20 | 0.3046 |
| Liquid-to-solid ratio$^2$ | 24.99 | 0.0011 |

The analysis results indicated that the investigated factors were statistically significant at $p < 0.05$. Among these parameters, the liquid-to-solid ratio exerted a substantially greater effect compared to extraction temperature and extraction time (**Figure 5**). This phenomenon can be elucidated by the enhancement of the concentration gradient between the solid and liquid phases as the solvent volume increases, thereby providing a stronger driving force to accelerate the mass transfer diffusion process. However, the extraction efficiency is not infinitely proportional to the solvent volume. The presence of the quadratic effect indicates that upon exceeding the optimal threshold, the extraction yield gradually approaches an equilibrium state; thus, further increasing the solvent ratio merely results in unnecessary solvent consumption.

The reduced regression model, which includes only the statistically significant factors, is expressed by the following equation (7):

$$C_{total}\ (mg/100\ g) = 0.1785 + 2.4266 \times A - 0.5817 \times B + 6.5465 \times C - 0.0018 \times AB - 0.0191 \times AC + 0.0033 \times BC - 0.0141 \times A^2 + 0.005 \times B^2 - 0.0490 \times C^2 \quad (7)$$

Where:

A: Temperature (°C)

B: Time (min)

C: Liquid-to-solid ratio (mL/g)

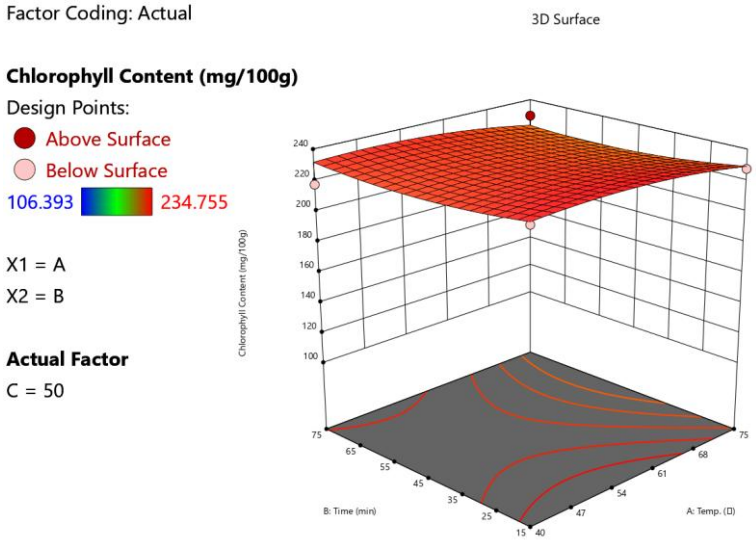


***Figure 5.*** *Response surface plot illustrating the interactive effect of extraction temperature and extraction time on chlorophyll content (with a fixed liquid-to-solid ratio of 50:1)*

The partial derivatives of the regression equation with respect to each independent variable were calculated and set to zero to determine the optimal extraction conditions: an extraction temperature of 57.5°C, an extraction time of 45 min, and a liquid-to-solid ratio of 50:1.

To evaluate the reliability of the optimization model, verification experiments were conducted in triplicate using galangal leaf samples. The experimental results demonstrated that the average total chlorophyll content reached 235.56 ± 2.17 mg/100 g, with an experimental variance at the optimal point of $S_{op}^2 = 0.40$.

When compared to the predicted value from the model (229.55 mg/100 g), the adequacy of the model was evaluated using the Fisher's criterion (*F*-test). The calculated results showed that the experimental *F*-value ($F_{calc}$ = 0.026) was significantly lower than the critical F-value ($F_{crit\ (0.95;\ 2;\ 5)}$ = 5.79). This confirms that the difference between the experimental and theoretical values is not statistically significant at a 95% confidence level, demonstrating that the mathematical model is completely compatible with the experimental data.

Additionally, the plot illustrating the correlation between the experimental and predicted values derived from the regression equation (**Figure 6**) indicates that the coefficient of determination reached $R^2$ = 97.50%. This index reflects a strong correlation and high reliability of the model in predicting the chlorophyll content under various extraction conditions.

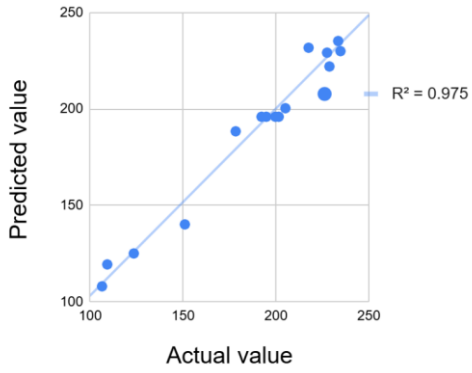


***Figure 6.*** *Correlation between actual and predicted values*

### 3.3. Method Validation

*3.3.1. Repeatability and intermediate precision*

Regarding repeatability, the analysis of six replicates under identical optimal conditions yielded an average value of 235.495 mg/100 g with a relative standard deviation ($RSD_r$) of 0.92%. This $RSD_r$ value is substantially lower than the maximum acceptable limit specified by the AOAC guidelines (which requires an $RSD_r < 3.7\%$ for an analyte concentration level of 0.1%) [24].

For intermediate precision, the evaluation was performed on six samples across three different days to assess the chronological variation of the method. The results recorded an average content of 233.712 mg/100 g with an $RSD_R$ of 3.22% (**Table 4**). Although environmental and temporal factors contributed to an increased variance compared to the repeatability study, this result remains well within the permissible limits of the AOAC standards (which requires an $RSD_r < 6.0\%$ for a 0.1% concentration level) [24].

***Table 4.*** *Method validation results*

| Run | Repeatability | | | Intermediate precision | | |
|---|---|---|---|---|---|---|
| | Content (mg/100 g) | $RSD_r$ | AOAC limits | Content (mg/100 g) | $RSD_R$ | AOAC limits |
| 1 | 233.787 | 0.92% | < 3.7% | 231.098 | 3.22% | < 6% |
| 2 | 235.283 | | | 236.325 | | |
| 3 | 235.175 | | | 235.283 | | |
| 4 | 232.871 | | | 235.175 | | |
| 5 | 239.015 | | | 231.012 | | |
| 6 | 236.746 | | | 212.338 | | |

*3.3.2. Comparison between the proposed method and the standard method TCVN 13283:2021*

The comparative results between the proposed method (RSM-UAE) and the standard method TCVN 13283:2021 are presented in **Table 5.**

The research results exhibited a consistent positive deviation compared to the standard method TCVN 13283:2021 across all three sample matrices (Galangal leaves: + 9.88%; Lettuce: + 9.72%; Mugwort leaves: + 9.68%). This discrepancy stems from the fundamental differences in extraction techniques: The TCVN standard method utilizes acetone as the solvent at room temperature, whereas the proposed method employs ultrasound-assisted extraction (UAE) combined with thermal activation. Furthermore, the TCVN protocol requires multiple liquid-liquid extraction steps (using petroleum ether and water to remove residual acetone), which potentially leads to analyte loss while being significantly more solvent- and time-consuming. Consequently, while the TCVN procedure is intended for laboratory-scale chlorophyll quantification, it is unsuitable for the extraction of natural colorants on an industrial scale (**Table 5**).

***Table 5.*** *Comparison of extraction results between the RSM-UAE method and the standard method*

| Sample matrix | Optimized results by RSM-UAE (mg/100 g) | Results according to TCVN 13283:2021 (mg/100 g) | Deviation from TCVN |
|---|---|---|---|
| Galangal leaves | 222.05 ± 2.03 | 200.11 ± 9.83 | + 9.88% |
| Lettuce | 50.69 ± 0.53 | 45.82 ± 2.39 | + 9.72% |
| Mugwort leaves | 99.27 ± 0.96 | 89.66 ± 2.93 | + 9.68% |

### 3.4. Real sample analysis

The conditions for extracting chlorophyll from galangal leaves (*Alpinia* spp.) were successfully optimized in this study. Among plant leaves, this specific matrix possesses a highly rigid tissue structure and is rich in essential oils, making it one of the most challenging matrices to extract. Due to the structural similarity of the thylakoid membrane and the unaltered physicochemical properties of the chlorophyll molecule across different species, the optimized parameters for diffusion kinetics and thermal stability can be uniformly applied. Consequently, the optimal extraction conditions established for galangal leaves can be extended to other plant matrices. The optimized protocol was subsequently applied to analyze 13 commercial plant samples collected from markets in Hanoi, including: *Polyscias fruticosa* (Dinh Lang), *Cnidoscolus aconitifolius* (chaya), *Chrysanthemum coronarium* (crown daisy), *Moringa oleifera* (moringa), *Pandanus amaryllifolius* (pandan), *Spinacia oleracea* (spinach), *Artemisia vulgaris* (mugwort), *Brassica juncea* (mustard greens), *Musa* × *paradisiaca* (banana leaves), *Brassica oleracea* L. var. *acephala* (kale), *Lactuca sativa* (lettuce), *Brassica oleracea* var. *gongylodes* (kohlrabi leaves), and *Nasturtium officinale* (watercress). Each sample was analyzed in duplicate, and the results are illustrated in **Figure 7**.

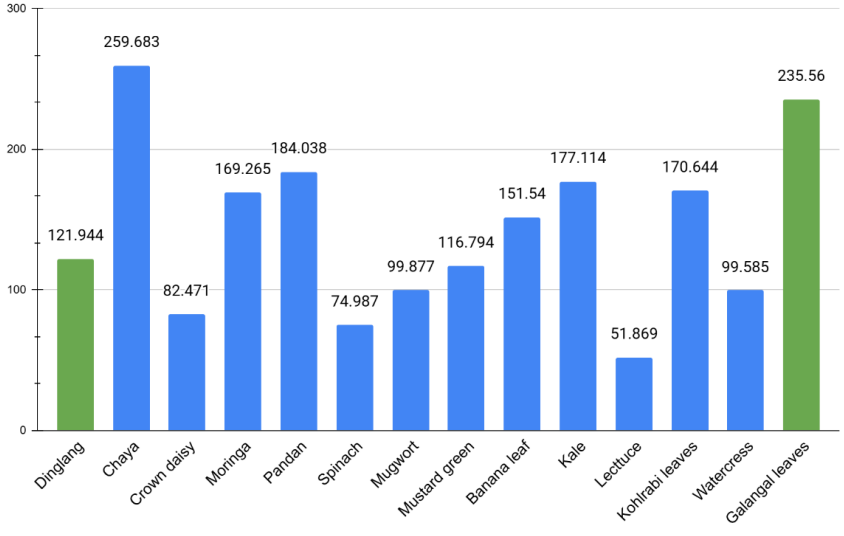


***Figure 7.*** *Chlorophyll content of real plant samples*

The average total chlorophyll content across the analyzed samples ranged from 51.87 mg/100 g to 259.68 mg/100 g. Among these, chaya (*Cnidoscolus aconitifolius*) exhibited the highest chlorophyll content, reaching 259.68 mg/100 g. This was followed by a group of leaves with high chlorophyll concentrations exceeding 150 mg/100 g, which included pandan (*Pandanus amaryllifolius*) at 184.04 mg/100 g, kale (*Brassica oleracea* L. var. *acephala*) at 177.11 mg/100 g, kohlrabi leaves (*Brassica oleracea* var. *gongylodes*) at 170.64 mg/100 g, moringa (*Moringa oleifera*) at 169.26 mg/100 g, and banana leaves (*Musa* × *paradisiaca*) at 151.54 mg/100 g.

Conversely, lettuce (*Lactuca sativa*) had the lowest chlorophyll content among all analyzed samples, at only 51.87 mg/100 g. Other samples, such as spinach (*Spinacia oleracea*) at 74.99 mg/100 g and crown daisy (*Chrysanthemum coronarium*) at 82.47 mg/100 g, were also categorized into the low-to-medium group, with chlorophyll levels below 100 mg/100 g. The remaining samples, including Dinh Lang (*Polyscias fruticosa*), mugwort (*Artemisia vulgaris*), mustard greens (*Brassica juncea*), and watercress (*Nasturtium officinale*), maintained stable chlorophyll concentrations ranging from 99.59 mg/100 g to 121.94 mg/100 g.

## 4. CONCLUSION

This study successfully optimized the chlorophyll extraction process using RSM. The determined optimal extraction parameters were an extraction temperature of 57.5°C, an extraction time of 45 min, and a liquid-to-solid ratio 50:1 mL/g. The experimental results confirmed that the model possessed high adequacy and strong predictive capability, with the actual chlorophyll yield being in close agreement with the predicted value. These optimized laboratory-scale conditions provide a solid foundation for further research into industrial-scale chlorophyll extraction aimed at producing natural colorants to replace synthetic alternatives.

## REFERENCES

[1]. U.S. Food and Drug Administration, "HHS, FDA to phase out petroleum-based synthetic dyes in nation's food supply," Apr. 22, 2025. [Online]. Available: https://www.fda.gov/news-events/press-announcements/hhs-fda-phase-out-petroleum-based-synthetic-dyes-nations-food-supply

[2]. American Chemical Society, "Chlorophyll," *Molecule of the Week*, 2019. [Online].

[3]. H. Scheer, "Chlorophylls: A personal snapshot," *Molecules*, vol. 27, no. 3, pp. 1093, 2022.

[4]. R. Mandal and G. Dutta, "From photosynthesis to biosensing: Chlorophyll proves to be a versatile molecule," *Trends in Analytical Chemistry* , vol. 154, pp. 116738, 2022.

[5]. M. Sitarek-Andrzejczyk, J. Dobrzyński, P. Orliński, and J. L. Przybył, "Balancing yield and stability: optimizing leaf pigment extraction to minimize chlorophyll degradation," *Planta*, vol. 263, no. 1, 2026.

[6]. S. B. Kim, J. Bisson, J. Brent Friesen, G. F. Pauli, and C. Simmler, "Selective chlorophyll removal method to degreen botanical extracts," *Journal of Natural Products.*, vol. 83, no. 6, pp. 1846–1858, 2020.

[7]. A. M. Shehata, S. M. Abdel-Hameed, A. F. Anter, and R. R. Abdelsalam, "Ultrasound assisted extraction enhances phytochemical profile and functional properties of moringa leaf extract with protection against gentamicin induced nephrotoxicity," *Scientific Reports.*, vol. 15, no. 1, 2025.

[8]. O. J. S. Gomes, A. Leitão, H. C. de Sousa, L. M. Gando-Ferreira, and M. E. M. Braga, "Bioactive compounds extraction from *Moringa oleifera* leaves: A comparative study of vacuum-assisted and bed-stirred extractions," *Journal of Food Science.*, vol. 90, no. 11, pp. e70700, 2025.

[9]. M. Yildirim, M. Erşatır, S. Poyraz, M. Amangeldinova, N. O. Kudrina, and N. V. Terletskaya, "Green extraction of plant materials using supercritical $CO_2$: Insights into methods, analysis, and bioactivity," *Journal of Supercritical Fluids*, vol. 188, pp. 105857, 2024.

[10]. N. Q. Dung, D. L. T. Thu, N. T. Thuy, N. T. Hong, and T. T. Kim, "Kinetic study on chlorophyll and antioxidant activity from *Polyscias fruticosa* (L.) Harms leaves via microwave-assisted extraction," *Molecules*, vol. 26, no. 12, pp. 3762, 2021.

[11]. N. H. K. Nguyen, N. T. Diem An, P. K. Anh, and T. T. Truc, "Microwave-assisted extraction of chlorophyll and polyphenol with antioxidant activity from Pandanus amaryllifolius Roxb. in Vietnam," *IOP Conference Series: Materials Science and Engineering* ., vol. 1166, no. 1, pp. 012039, 2021.

[12]. D. B. T. Thien, V. N. Boi, N. D. Nghia, and D. X. Cuong, "Effect of various solvents and extraction methods on polyphenol, chlorophyll, and antioxidant activities of Centella asiatica grown in south-center, Vietnam," *International Journal of Pharmaceutical Research*, vol. 13, no. 3, 2021.

[13]. C. T. Le *et al.,* "Reconstruction of the evolutionary biogeography reveals the origins of Alpinia Roxb. (Zingiberaceae): A case of 'out-of-Asia' migration to the Southern Hemisphere," *Acta Bot. Brasilica*, vol. 36, pp. e2021abb0255, 2022.

[14]. N. Phuong Hanh and N. Quoc Binh, "Distribution of Alpinia (Zingiberaceae) and their use pattern in Vietnam," *Journal of Biodiversity & Endangered Species*, vol. 02, no. 02, 2014 (in Vietnamese).

[15]. "eFloras.org Home." Accessed: Jun. 27, 2026. [Online]. Available: http://www.efloras.org/

[16]. N. Q. Binh, N. P. Hanh, N. D. Trong, D. H. Chung, and N. T. Thanh, "New record of a plant species in Northern Vietnam belong to genus *Alpinia* Roxb. – Zingiberaceae for flora of Vietnam," *VNU Journal of Science:Natural Sciences and Technology.*, vol. 35, no. 3, 2019 (in Vietnamese).

[17]. H. K. Lichtenthaler, "Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes," *Methods Enzymol*, vol. 148, pp. 350–382, 1987.

[18]. Foodstuff - Determination of total chlorophyll content by spectrophotometric method, TCVN 13283:2021, 2021 (in Vietnamese).

[19]. W. Kong *et al.*, "Optimization of ultrasound-assisted extraction parameters of chlorophyll from Chlorella vulgaris residue after lipid separation using response surface methodology," *Journal of Food Science and Technology.*, vol. 51, no. 9, pp. 2006, 2012

[20]. A. Bucić-Kojić *et al.,* "Study of solid–liquid extraction kinetics of total polyphenols from grape seeds," *Journal of Food Engineering.*, vol. 81, no. 2, pp. 236–242, 2007.

[21]. B. Chand, M. Kumar, S. Prasher, A. Sharma, and M. Kumar, "Aprotic and protic solvent for extraction of chlorophyll from various plants: Chemical characteristic and analysis," *Journal of Physics: Conference Series.*, vol. 2267, no. 1, 2022.

[22]. Dianursanti, A. R. Siregar, Y. Maeda, T. Yoshino, and T. Tanaka, "The Effects of Solvents and Solid-to-Solvent Ratios on Ultrasound-Assisted Extraction of Carotenoids from Chlorella vulgaris," *International Journal of Technology*, vol. 11, no. 5, pp. 941–950, 2020.

[23]. D. Naviglio, P. Scarano, M. Ciaravolo, and M. Gallo, "Rapid Solid-Liquid Dynamic Extraction (RSLDE): A Powerful and Greener Alternative to the Latest Solid-Liquid Extraction Techniques," *Foods 2019, Vol. 8, Page 245*, vol. 8, no. 7, pp. 245, 2019.

[24]. AOAC International, "Appendix F: Guidelines for standard method performance requirements," in *Official Methods of Analysis of AOAC International*, 20th ed., Rockville, MD, USA: AOAC International, 2016, pp. 1–18. [Online]. Available: https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf.

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