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Optimization and Characterization of Tiwai *(Eleutherine americana L. Merr.)* Extract Encapsulation for Potential Bioavailability of Flavonoids

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Abstract

Tiwai *(Eleutherine americana L. Merr.)* is a traditional medicinal plant from Kalimantan, rich in bioactive compounds, particularly flavonoids, which exhibit strong antioxidant activity and therapeutic benefits. However, flavonoids in free form tend to be unstable, making encapsulation necessary to enhance their stability, bioavailability, and shelf life. This study optimized the encapsulation of Tiwai extract using the spray drying method, with parameters such as the ratio of Tiwai extract to maltodextrin (ET:MD), chitosan concentration, and drying inlet temperature adjusted using Response Surface Methodology (RSM) with Box-Behnken Design (BBD). The study provided detailed results, including an encapsulation efficiency (EE) of 93.24%, loading capacity (LC) of 95.76%, total flavonoid content (TFC) of 69.23 mg QE/g, and antioxidant activity (DPPH) of 60.01 µg/mL. A significant discrepancy was found between the quercetin content determined by HPLC (148.30 µg/g) and the TFC obtained using the $AlCl_3$ method (69.23 mg QE/g). This difference is attributed to the different principles of the two methods, as the $AlCl_3$ method reacts with flavonoids and other antioxidant compounds, whereas HPLC specifically identifies quercetin. FTIR analysis confirmed successful encapsulation, showing changes in the functional groups of the encapsulated product. These findings suggest that the optimized Tiwai encapsulation formula has potential applications in functional foods and pharmaceuticals.

Keywords**:** *antioxidant; encapsulation; flavonoids; spray drying; FTIR; HPLC*

1. Introduction

Tiwai (*Eleutherine americana* L. Merr.), also known as Dayak onion, is a traditional medicinal plant widely found in Borneo, Indonesia. It is rich in bioactive compounds, especially flavonoids such as quercetin, kaempferol, and luteolin, which exhibit strong antioxidant, anti-inflammatory, and antibacterial properties. These flavonoids play a key role in the plant's bioactivity and offer various therapeutic benefits, such as treating hypertension, diabetes, and skin infections (Adesina et al., 2016; Tiwari & Shukla, 2020; Kareem et al., 2022; Rachmawati et al., 2024). However, flavonoids in their free form are unstable, which limits their bioavailability and shelf life when used in functional foods or pharmaceuticals (Cocuron et al., 2019; Shahidi & Yeo, 2016). Therefore, encapsulation is required to improve their stability, bioavailability, and extend their shelf life in product formulations.

Encapsulation is a well-established method for protecting bioactive compounds such as flavonoids. Among the various techniques, spray drying is one of the most widely used methods. It works by converting liquid extracts into dry powder particles through rapid evaporation using a hot air stream. This process not only enhances the stability of the active compounds but also facilitates their incorporation into functional foods, nutraceuticals, and pharmaceutical formulations. Factors influencing the spray drying encapsulation process include the concentration of active ingredients, the type and concentration of coating materials, and the inlet temperature. These factors determine the efficiency, particle size, stability, and antioxidant activity of the final encapsulated product (Mudalip et al., 2021; Akbarbaglu et al., 2019; Castro-López et al., 2021; Valková et al., 2022).

While spray drying has been widely employed for encapsulating bioactive compounds, studies optimizing the encapsulation of flavonoids from Tiwai using this method are limited. Most studies have focused on single-variable optimization, which is time-consuming and does not account for potential interactions among multiple factors (Mehmood et al., 2012). In contrast, Response Surface Methodology (RSM), specifically the Box-Behnken design (BBD), is a more efficient approach for optimizing complex processes. BBD allows for the simultaneous assessment of multiple factors and their interactions, making it ideal for optimizing the encapsulation process by adjusting the ratio of Tiwai extract to maltodextrin (ET:MD), chitosan concentration, and inlet temperature. This methodology also helps maximize the range of independent variables and provides valuable data on factors influencing key responses such as encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and antioxidant activity (DPPH) (Yolmeh & Jafari, 2017; Raj & Murugesan, 2022).

This study aims to optimize the encapsulation of Tiwai extract using the spray drying method. Specifically, the study focuses on adjusting the ET:MD ratio, chitosan concentration, and inlet temperature to achieve the highest possible EE, LC, and TFC while also assessing antioxidant activity. The encapsulated product was characterized using FTIR analysis to evaluate changes in functional groups, and flavonoid content was quantified using HPLC to confirm that the encapsulation process enhanced both the stability and bioavailability of the flavonoids. These findings have potential applications in functional foods and pharmaceuticals, offering a promising solution to the instability of flavonoids in Tiwai extract.

2. Objectives

The objective of this study was to optimize the encapsulation of Tiwai *(Eleutherine americana L. Merr)* extract using Response Surface Methodology (RSM) with the Box-Behnken design (BBD) and to evaluate the stability, potential bioavailability, and antioxidant activity of the encapsulated flavonoids. The study also aimed to assess key parameters, including encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and antioxidant activity (DPPH), while characterizing functional groups through FTIR analysis and identifying flavonoids using HPLC for potential applications in functional foods and pharmaceuticals.

3. Materials and Methods

3.1 Preparation of Tiwai Extract

The seven-month harvested Tiwai onion was washed with running water and sliced using a Tupperware slicer (1 mm thickness). Drying was carried out using a microwave at 100 °C for 30 minutes based on the study by Rachmawati et al. (2024). This method was selected due to its faster drying time compared with conventional oven and freeze-drying methods, and it better preserves the bioactive flavonoids by minimizing heat-induced degradation. Pulverization was carried out using a grinder, and the powdered Tiwai was sieved using an 80-mesh sieve. For extraction, 10 g of Tiwai powder was dissolved in 100 mL of distilled water and macerated for 24 hours. Subsequently, evaporation was performed using an oven at 50 °C until a concentrated extract was obtained. The final extract yield was calculated as a percentage weight-to-weight (w/w) of the dried extract relative to the initial raw material.

3.2 Chemicals and Reagents

Tiwai onion was obtained from local farmers in Samarinda, Indonesia. Folin–Ciocalteu reagent was supplied by Fisher (Loughborough, UK). Sodium carbonate, quercetin, potassium ferrocyanide, and acetic acid were obtained from Scharlau (Sentmenat, Spain). Ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were purchased from Sigma (Steinheim, Germany), and iron (II) chloride tetrahydrate was obtained from Panreac Química SAU (Barcelona,

Spain). All other chemicals and reagents used were of analytical grade and commercially available.

3.3 Variable Selection and Encapsulation Process

The weight ratio of Tiwai extract to maltodextrin (X_1) ranged from 0.1375 to 0.2625 g/g, chitosan concentration (X_2) varied from 0.06 to 0.24 g, and the spray-drying inlet temperature (X_3) ranged from 70 to 80°C (Table 1). These values were selected based on a previous study of encapsulant product formulations (Liang et al., 2017). The dependent variables measured were encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and DPPH radical scavenging activity. The Tiwai extract was mixed with the encapsulating materials in varying ratios and processed at different inlet temperatures as specified in Table 1. The experimental design was generated using Design-Expert software version 13.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The compressor pressure and flow rate in the spray drying device were stabilized at –40 mmHg and 5 to 20 mL per minute, respectively.

3.4 Experiment Design

Optimization experiments were conducted using RSM for EE, LC, TFC, and DPPH methods. BBD consisted of 15 experiments including three center point experiments (Table 1). Encapsulation formula variables included the (ET:MD) ratio (X_1, g), chitosan concentration (X_2, g), and inlet temperature (SD) (X_3, ºC). The experimental data were fit with a second-order polynomial model to obtain the regression coefficient (β_0). The general second-order polynomial model used in response surface analysis is as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y is the response variable, X_i and X_j are the independent variables, n is the number of variables tested, and ε represents the error. β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic, and interaction effect coefficients, respectively.

To evaluate the predictive model for the response variables, analysis of variance (ANOVA) was conducted at a 95% confidence level to assess the effect of each factor, namely the Tiwai extract-to-maltodextrin (ET:MD) ratio, chitosan concentration, and spray-drying inlet temperature. The coefficient of determination (R^2), p-value of the regression model, and the lack-of-fit (LOF) p-value were examined to determine the adequacy of the regression model. Optimal conditions were identified based on response surface analysis (3D plots).

Table 1 Box-Behnken design matrix showing independent variables (ET:MD ratio, chitosan concentration, and inlet temperature) and measured response values (EE, LC, TFC, and DPPH) for 15 experimental runs.

Independent Variables				Responses			
Run	Ratio (ET:MD) (X_1)	Chitosan Concentration (X_2)	Inlet Temperature (SD) (X_3)	EE (%)	LC (%)	TFC (mg QE/g)	DPPH (µg/mL)
1	0.2	0.24	80	65.03	80.79	44.02	63.07
2	0.2	0.15	75	96.85	94.66	65.56	65.17
3	0.1375	0.24	75	53.36	68.00	36.12	55.62
4	0.1375	0.15	70	72.05	93.46	48.77	152.10
5	0.2625	0.15	80	65.90	94.22	44.61	124.70
6	0.2	0.15	75	95.12	95.19	67.23	63.10
7	0.1375	0.06	75	66.63	96.44	45.10	55.06
8	0.2	0.24	70	60.14	84.17	40.71	52.77
9	0.1375	0.15	80	73.86	88.65	72.54	129.30
10	0.2625	0.06	75	49.29	94.95	33.36	113.10
11	0.2	0.15	75	96.03	93.85	63.53	62.87
12	0.2	0.06	80	71.96	95.71	48.71	46.35
13	0.2	0.06	70	46.53	95.40	31.50	17.37
14	0.2625	0.15	70	69.78	93.15	47.23	74.51
15	0.2625	0.24	75	59.75	86.81	40.45	39.14

Notes. Factors: ratio Tiwai extract (ET): maltodextrin (MD) ratio (g), chitosan concentration (g), and inlet temperature spray drying (SD) (ºC). The dependent variables measured were encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and antioxidant activity (DPPH method)

3.5 Validation of Model

The optimized encapsulation conditions, namely the ratio of Tiwai extract to maltodextrin (ET:MD), chitosan concentration, and spray drying inlet temperature, were validated based on the parameters of EE, LC, TFC, and DPPH, which were obtained using RSM. All these variables were re-established in the encapsulation process at optimum conditions. Subsequently, the experimental values were compared with the predicted values of the model to assess the validity of the optimization. The functional groups in the constituent materials and encapsulated Tiwai extract were analyzed using ATR-FTIR, whereas the flavonoid profile was determined using High-Performance Liquid Chromatography (HPLC) under optimum conditions.

3.5.1 Encapsulation Efficiency and Loading Capacity

Flavonoids in the Tiwai extract encapsulant were determined using a spectrophotometric method with an aluminum chloride ($AlCl_3$) reagent. A total of 50 mg of encapsulant powder was dissolved in 10 mL of 95% ethanol and then sonicated for 30 minutes to extract the encapsulated flavonoids. The sonication solution was centrifuged at 4000 rpm for 15 min, and the supernatant obtained was used as the test sample. A total of 1 mL of supernatant was taken and added to 0.7 mL of distilled water and 0.1 mL of 5% $NaNO_2$ solution, and the mixture was allowed to stand for five minutes. Next, 0.1 mL of 10% $AlCl_3$ solution was added and allowed to stand for six minutes, followed by the addition of 0.5 mL of 1 M NaOH. The mixture was homogenized and incubated for 10 minutes. The absorbance was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer, with 95% ethanol as a blank. This was followed by calculating flavonoid content using a quercetin standard curve and expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g) (Zou et al., 2004). The EE value was calculated based on the ratio between the amount of encapsulated flavonoids and the initial amount of flavonoids used. Meanwhile, LC was calculated as the percentage of the amount of flavonoids relative to the total encapsulant weight. All tests were performed in triplicate to improve the accuracy of the results.

3.5.2 Analysis of TFC Content

An extract sample (1 mg) was dissolved in 10 mL of 95% ethanol. Subsequently, 0.7 mL of distilled water and 0.1 mL of 5% $NaNO_2$ solution were added. After 5 min, 0.1 mL of 10% $AlCl_3$ solution was introduced, followed 6 min later by 0.5 mL of 1 M NaOH. The mixture was then homogenized and incubated for 10 min. Absorbance was recorded at 510 nm, with 1 mL of 95% ethanol used as the blank. The total flavonoid content (TFC) was calculated and expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g) (Wunnakup et al., 2024; Zou et al., 2004).

3.5.3 Antioxidant Activity (DPPH)

The DPPH radical capture ability test was conducted based on a modified method from Chew et al. (2009). A total of 1 mL of plant extracts in various concentrations diluted with ethanol was mixed with 1 mL of DPPH solution (0.15 mM in ethanol). As a control, a mixture of 1 mL of DPPH solution with 1 mL of ethanol was prepared. The reaction mixture was shaken until homogeneous, then incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at a wavelength of 517 nm using an Eppendorf Bio Spectrometer Basic. Ascorbic acid was used as a positive control, and ethanol was used as a blank. The capture ability of DPPH radicals by plant extracts was calculated using the following equation:

$$\%\ \text{DPPH capture activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

where Abs control is the absorbance of the mixture of DPPH and ethanol, whereas Abs sample is the absorbance of the mixture of DPPH with the sample. Additionally, three types of controls were prepared to validate the results.

3.5.4 FTIR Analysis

FTIR spectra were scanned using a German FTIR spectrophotometer (Bruker Alpha II). Measurements were taken in the mid-infrared region 4000–600 cm^{-1} with a resolution of 4 cm^{-1}. A horizontal attenuated total reflection (ATR) accessory equipped with ZnSe crystals was used for sampling. All FTIR spectra were baseline-corrected using the spectrum of air as the background, with a new reference air spectrum collected after each scan. Spectra were recorded in absorbance mode, with each measurement performed in triplicate. These data were then used to establish correlations between FTIR spectra, antiradical activity, and flavonoid content.

3.5.5 HPLC Analysis of Flavonoids under Optimum Conditions

Analysis of flavonoids in Tiwai extract obtained under optimum conditions was carried out for three replicates using a High-Performance Liquid Chromatography (HPLC) HP1200 with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). The separation was performed using an Inertsil ODS-3 column (5.0 µm particle size; 4.6 mm × 250 mm) equipped with an Inertsil ODS-3 guard column (5.0 µm; 4.0 mm × 10 mm) from GL Sciences Inc. (Tokyo, Japan). The elution conditions used a gradient system with solvent A as 2.5% formic acid in water and solvent B as 2.5% formic acid in acetonitrile. The elution gradient profile was arranged as follows: 0 min (5% B), 13 min (11% B), 16 min (13% B), 20 min (14% B), 22 min (15% B), 25 min (20% B), 28 min (25% B), 30 min (30% B), and back to 5% B at the 40th minute. The total analysis time was 40 minutes, with a solvent flow rate of 1 mL/minute and an injection volume of 25 µL. Identification of flavonoids was carried out by comparing the retention time of sample chromatogram peaks against reference standards (Suwanpitak et al., 2025). The concentration of individual phenolic compounds was calculated using a calibration curve that had been previously made (Hongsing et al., 2024). The results were expressed in units of micrograms per gram (µg/g) of sample.

4. Results and Discussions

4.1 Response Surface Model and Analysis

The determination of variable levels in the encapsulant formula, including the ratio of Tiwai extract to maltodextrin (ET:MD), chitosan concentration, and spray-drying inlet temperature, was based on preliminary investigations. These variables were incorporated into the experimental design to evaluate their impact on encapsulated characteristics. The selected variables, ET:MD ratio, chitosan concentration, and spray-drying inlet temperature were chosen due to their significant influence on key encapsulation parameters such as encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and antioxidant activity (DPPH). These variables have been extensively studied in previous literature and are well-known for their effects on the stability and bioavailability of active compounds in encapsulated products (Hong et al., 2016; Yoksan et al., 2010). The ET:MD ratio affects the interaction between the active ingredient and the encapsulant, while chitosan concentration plays a crucial role in determining the protective capability of the encapsulation matrix. The spray-drying temperature is an essential parameter for achieving stable encapsulants. For optimization and statistical analysis, Box-Behnken design (BBD) based on Response Surface Methodology (RSM) was employed. This method facilitates regression analysis and response surface visualization, enabling the identification of optimal conditions by analyzing the interactions between the selected variables.

4.2 Fitting the Model

The encapsulation formula was optimized using a second-order polynomial model. The models for EE, LC, TFC, and DPPH showed excellent fit to the experimental data, with R^2 values of 0.9572, 0.9515, 0.9682, and 0.8051, respectively (Table 2), and were statistically significant as confirmed by ANOVA (Table 2). The regression coefficients for the dependent variables were obtained by multiple linear regression as shown in Table 2. Based on the results, the linear effect of (ET:MD) ratio (X_1) was significant for LC and TFC, chitosan concentration (X_2) for LC, and SD inlet temperature for TFC. The interaction effect of X_{12} (ET:MD ratio and chitosan concentration) was found for LC, whereas X_{13} (ET:MD ratio and SD inlet temperature) was only found for TFC. The quadratic effect of X_1 (ET:MD ratio) had significant negative effects on EE and TFC, but positive effects on DPPH. The square effect of X_2 (chitosan concentration) produced significant negative effects on EE, LC, and TFC. Additionally, the square effect of X_3 (SD inlet temperature) also produced negative effects on EE.

The ANOVA results for EE, LC, and TFC indicated that the three model parameters effectively explained the variation in the experimental data for these response variables. This was evidenced by the significant F-values for the models (Table 2) and the non-significant lack-of-fit tests, confirming that the models adequately represented the experimental data. In contrast, the DPPH variable showed a non-significant model effect, suggesting that the experimental data were insufficient to accurately describe this response.

4.3 Effect of Encapsulation Formula Variables on EE

The model showed high significance values ($p < 0.01$) with the experimental data. ANOVA showed a negative ($p = 0.01$) linear effect (X_1),

quadratic effect of X_1 ($p < 0.01$), X_2 ($p < 0.001$) and X_3 ($p < 0.05$) on the EE response, as presented in Table 2. There was no significant interaction effect between X_1, X_2, and X_3 on the experimental data. The insignificant discrepancy value (F = 0.1395; $p > 0.05$) showed that the model fit the effects of the selected variables on this response with good prediction ($R^2 = 0.9572$) and was nonsignificant compared to pure error.

EE values obtained in the study were similar to those of Ding et al. (2019), who also used maltodextrin as a coating material. The results of regression analysis on each quadratic variable of the ratio (ET:MD), maltodextrin concentration, and SD inlet temperature had a significant effect on EE. This showed that the relationship between the variables and EE response was non-linear. More importantly, an increase or decrease in one of the variables does not significantly cause a linear or unidirectional change in the response (Kalam et al., 2016). Positive values determine synergistic effects on optimization, while negative values direct antagonistic effects between factors and responses (Hao et al., 2011).

The relationship between EE and process variables is shown in Figure 1a. The highest EE occurred in the range of 0.1375g to 0.200 g. Each variable and its interaction did not have a significant effect on EE in determining the Tiwai extract encapsulant formula, but a substantial impact was observed for the quadratic effect. This suggested that EE did not increase or decrease proportionally with a rise in X_1, X_2, and X_3, but through a more complex pattern (Rahman et al., 2010).

4.4 Effect of Encapsulation Formula Variables on LC

The model showed significant values ($p < 0.05$) in the experimental data. ANOVA showed significant effects of X_1 ($p < 0.05$) and X_2 ($p < 0.001$), the interaction between variables X_1 ($p < 0.05$), and a negative quadratic effect of X_2 ($p < 0.05$) on the LC response, as shown in Table 2. The nonsignificant discrepancy value (F = 1.75; $p > 0.05$) suggested that the model adequately fit the spatial influence of the variables on this response, with good prediction ($R^2 = 0.9515$). The LC values obtained were more than 80% similar to the results reported by Permanadewi et al. (2022), who used spray-drying as the encapsulation method. Additionally, regression analysis results showed higher LC at the ratio of ET:MD, as well as greater chitosan concentration up to a certain limit. The ratio of encapsulant and coating materials used played an important role in LC obtained in the encapsulation of rosemary essential oil (Turasan et al., 2015). The relationship between LC and process variables is shown in Figure 1b, where the highest LC values range from 0.12 to 0.2375% and 0.1875 to 0.2375%.

The encapsulant material ratio (ET:MD) and chitosan concentration play a crucial role in the LC of the product (Yoksan et al., 2010). The balance between extract (ET) as the active ingredient and maltodextrin (MD), including chitosan as the coating material, can significantly affect the ability of the encapsulant to effectively encapsulate and protect the active ingredient. This suggests that a greater amount of extract may increase LC. However, increasing the amount of extract beyond a certain point could weaken the structural integrity of the wrapping matrix due to its inability to stabilize the core material properly. On the other hand, increasing the content of maltodextrin and chitosan generally improves the structural stability of the matrix but reduces the proportion of extracts as active ingredients. Several studies have reported that the ratio of encapsulant material (ET) and chitosan concentration determines both encapsulation efficiency (EE) and LC. This is attributed to the significant effect on structural stability, protection of the core material, and the controlled release characteristics of the encapsulated substance (Ren et al., 2019; Soltanzadeh et al., 2021; Woranuch & Yoksan, 2013).

4.5 Effect of Encapsulation Formula Variables on TFC

ANOVA revealed significant linear effects and negative quadratic effects of X_1, X_3, X_1^2, and X_2^2 on TFC values, with $p < 0.05$ for all variables except X_2^2 ($p < 0.001$) (Table 2). Additionally, the interaction between the ET:MD ratio and spray-drying inlet temperature was highly significant ($p < 0.001$). As shown in Figure 1c, the ET:MD ratio, chitosan concentration, and inlet spray-drying temperature for TFC reached the highest point before decreasing. This trend was consistent with the EE results, where the parameters showed a strong positive correlation ($R^2 = 0.89$, $p < 0.001$) (Table 3). The highest flavonoid content was observed at an ET:MD ratio of 0.1875–0.2125 g (Figure 1c), chitosan concentration of 0.155–0.18 g, and spray-drying inlet temperature of 74–76°C (Figure 1d). The positive correlation between EE and TFC showed a unidirectional relationship between the two variables. This relationship could be due to the

better ability of the encapsulation matrix to protect or maintain flavonoids during the encapsulation process (Ydjedd et al., 2017).

4.6 Effect of Encapsulation Formula Variables on DPPH

ANOVA revealed significant linear effects and negative quadratic effects of X_1, X_3, X_1^2, and X_2^2 on DPPH values, with $p < 0.05$ for all variables except X_2^2 ($p < 0.001$) (Table 2). Additionally, the interaction between the ET:MD ratio and spray-drying inlet temperature was highly significant ($p < 0.001$). As shown in Figure 1d, the DPPH value increased as the ET:MD ratio decreased, indicating a reduction in antioxidant capacity, as reflected by higher IC_{50} values (Patthamasopasakul et al., 2024). This suggests that a lower ET:MD ratio leads to reduced antioxidant activity, possibly due to a decrease in the effective encapsulation of flavonoids and other active compounds. The DPPH response, measured as the inhibitor concentration (IC_{50}), showed a quadratic effect of the ET:MD ratio (X_1^2) being significant ($p < 0.05$) (Table 2), indicating a non-linear relationship between the ET:MD ratio and antioxidant capacity. This result suggests that a balance in the ET:MD ratio is crucial for optimizing antioxidant activity, where very high or low ratios may lead to less effective encapsulation and reduced antioxidant performance. However, the model did not fully describe the observed behavior, as indicated by the lack-of-fit F-value (810.43, highly significant). This suggests that the current model does not fully capture the complexity of the system, and additional variables or a more refined model might be necessary to better predict antioxidant activity. Factors not considered by the model could include the degradation of flavonoids during the encapsulation process, interactions between flavonoids and other matrix components, and non-flavonoid antioxidant contributions that may influence the DPPH response.

The correlation of each variable can be observed in Table 3. The low correlation between TFC and DPPH was due to the contribution of other antioxidant compounds, variations in the type and structure of flavonoids, differences in measurement methods, interactions among compounds in the encapsulation matrix, and the possibility of flavonoid degradation during the encapsulation process. Similarly, Sulaiman et al. (2011) reported a low correlation between TFC and DPPH.

Table 2 Regression coefficients (β), coefficients of determination (R^2), and F-test values of the second-order polynomial models predicting EE, LC, TFC, and DPPH responses.

	Regression Coefficients (β)			
	EE	**LC**	**TFC**	**DPPH**
Intercept				
X_0	96.68	96.33	65.44	63.71
Linear				
X_1	−2.65	2.82*	−4.61*	−5.08
X_2	0.4837	−7.84***	0.3287	−2.66
X_3	3.53	−0.8513	5.21*	8.33
Cross product				
X_{12}	5.93	5.08*	4.02	−18.63
X_{13}	−1.42	1.47	−6.60*	18.25
X_{23}	−5.13	−0.9225	−3.48	−4.67
Quadratic				
X_1^2	−14.97**	−3.22	−7.32*	38.64*
X_2^2	−24.45***	−6.57*	−19.37***	−36.62
X_3^2	−11.31*	−0.7483	−4.84	17.80
R^2	0.9572	0.9515	0.9682	0.8051
F value (model)	12.44**	10.91**	16.91*	2.29
F value (lack of fit	0.1395	1.75	6.41	810.43*

Note: X_1 = ET:MD ratio (g); X_2 = chitosan concentration (g); X_3 = spray-drying inlet temperature (°C); X_1^2, X_2^2, X_3^2 = quadratic effects; X_{12}, X_{13}, X_{23} = interaction effects; EE = encapsulation efficiency (%); LC = loading capacity (%); TFC = total flavonoid content (mg QE/g); DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (µg/mL); R^2 = coefficient of determination. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

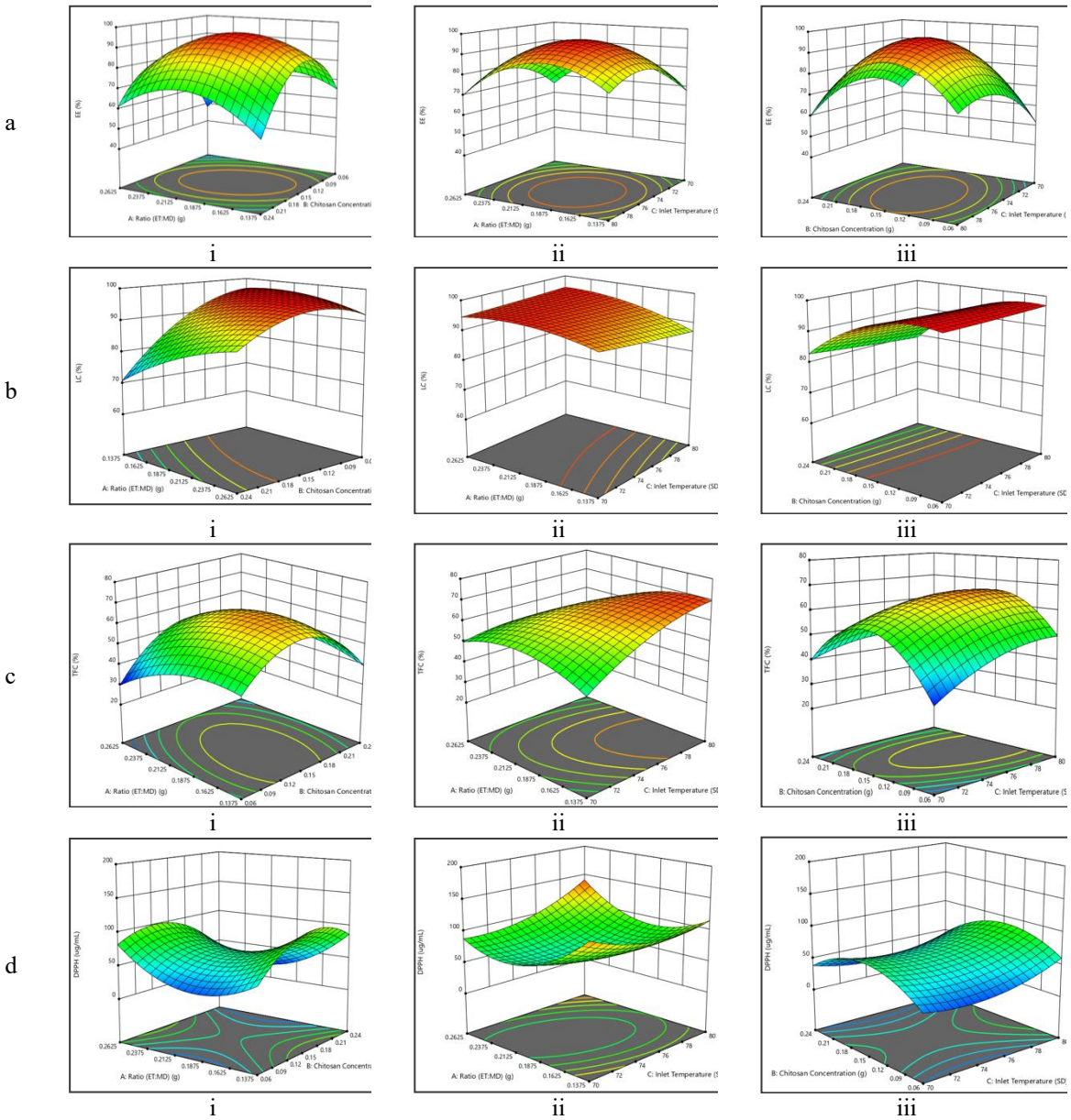

Figure 1 Three-dimensional (3D) response surface plots showing the effect of encapsulation variables on: a) encapsulation efficiency (EE, %), b) loading capacity (LC, %), c) total flavonoid content (TFC, mg QE/g), and d) antioxidant activity (DPPH, µg/mL). Within each panel: (i) ET:MD ratio (X_1) vs. chitosan concentration (X_2) at fixed inlet temperature (X_3 = 75°C); (ii) ET:MD ratio (X_1) vs. inlet temperature (X_3) at fixed chitosan concentration (X_2 = 0.15 g); (iii) chitosan concentration (X_2) vs. inlet temperature (X_3) at fixed ET:MD ratio (X_1 = 0.20 g).

Table 3 Pearson correlation matrix among encapsulation response variables: encapsulation efficiency (EE), loading capacity (LC), total flavonoid content (TFC), and antioxidant activity (DPPH).

	EE	**LC**	**TFC**	**DPPH**
EE	1.00	0.35	0.89**	0.09
LC		1.00	0.27	0.15
TFC			1.00	0.26
DPPH				1.00

Notes. Level of significance * $p < 0.05$; ** $p < 0.01$; EE = Encapsulation efficiency; LC = Loading Capacity; TFC = Total Flavonoid Content (mg QE/g); DPPH = 2,2- diphenyl-1-picrylhydrazyl radical scavenging ability (µg/mL)

Table 4 Comparison of predicted and experimental values for EE, LC, TFC, and DPPH under optimal encapsulation conditions, with percentage coefficient of variation (%CV).

Dependent Variable	Predicted Value	Experiment Value	%CV
EE (%)	95.97	93.24	2.86
LC (%)	97.53	95.76	1.81
TFC (mg QE/g)	65.55	69.23	5.62
DPPH (μg/mL)	63.55	60.01	5.56

Note. EE = encapsulation efficiency; LC = loading capacity; TFC = total flavonoid content; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; %CV = percentage coefficient of variation. Predicted values were generated using Design-Expert software version 13.0 based on the optimized Box–Behnken design.

4.7 Encapsulation Formula Parameter Optimization and Model Validation

Optimum conditions were identified by maximizing the overall desirability using Design-Expert software version 13.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The optimization aimed to achieve the highest possible EE, LC, and TFC values, while minimizing DPPH. The resulting optimal formulation parameters were an ET:MD ratio of 0.195 g, chitosan concentration of 0.130 g, and spray-drying inlet temperature of 75°C, yielding a desirability score of 0.805. These conditions were applied in the encapsulation process, and the responses were determined and validated following standard procedures. The experimental results closely matched the predicted values, with coefficients of variation (CV) ranging from 1.81% to 5.62% (Table 4), confirming the reliability and reproducibility of the optimized encapsulation model. Under optimal conditions for the Tiwai extract encapsulant formula, additional measurements of functional groups were made for comparison with the raw material. The quercetin compound content of the final product was also identified using the HPLC method.

4.8 Scanning of FTIR Spectra

Figure 2 shows the FTIR spectra of the encapsulant samples of Tiwai extract (TEE), TE, with Tiwai extract and a mixture of maltodextrin and chitosan (TE+CM) scanned in the mid-infrared region corresponding to wave numbers 4000–650 cm^{-1}. Each peak in FTIR spectra corresponds to the stretching and bending vibrations of functional groups that absorb radiation, causing vibrational transitions between bonds. The shape of the FTIR spectra in the three samples shows the presence of functional groups that differ in absolute intensity. This is because the FTIR spectra describes the interaction of infrared light with molecules in the sample, leading to a characteristic absorption pattern (Chen et al., 2012; Siddique, 2024).

The interpretation of the FTIR spectra in terms of wave numbers, functional groups, and transmittance is shown in Table 5. Wave number (a) 3276.34 cm^{-1} shows a low stretching of –OH (hydroxyl bond) in TEE. This may be caused by the heating process during spray drying leading to a decrease in free hydroxyl groups or converting into ether or ester groups (Akbarbaglu et al., 2019). Flavonoids with a carbonyl group (C=O) at the ketone position in ring C (e.g., flavones and flavonols such as quercetin) and aromatic compounds such as rings A and B show C=C vibration at 1638.78 cm^{-1} peak (Chen et al., 2022; Kolekar et al., 2019). Moreover, the lower transmittance intensity in TEE (63.6%) compared with TE+CM (70.6%) indicates that the flavonoid content in TEE is higher than TE+CM. This occurs because of free or active carbonyls (Horincar et al., 2019), including the coating material in TE+CM that can reduce the availability of flavonoid aromatic groups to absorb infrared light due to imperfect homogenization (Rahmadi et al., 2022).

The peak at 1413.8275 cm^{-1} is associated with C–H deformation vibrations derived from aliphatic or aromatic structures and stretching vibrations of C–O groups in compounds with carboxylic (–COO–) or ester groups (Yang et al., 2016a; Yang et al., 2016b). However, this peak is not detected in the encapsulant of Tiwai extract due to the reaction of active groups with the coating material (maltodextrin and chitosan) during the encapsulation process (Rahmadi et al., 2022). The peak at 1357–1361.10 cm^{-1}, attributed to C–H bending (aromatic/aliphatic), such as $–CH_2$ and $–CH_3$ (Russo et al., 2014), the transmittance of 90.4% in TEE shows low absorption. This indicates that the content of aromatic/aliphatic C–H groups is less active compared with TE and TE+CM. Furthermore, C–O/C–N and C–O stretching functional groups at peaks 1149.93, 1017.69, and 926.69 cm^{-1} are undetectable or minor in TE. These functional groups include C–O stretching derived from ether and ester bonds, or carbohydrate compounds such as maltodextrin. C–N stretching is

commonly found in amine or amide compounds such as chitosan, and C–O Stretching (glycosidic linkage) is derived from glycosidic bonds in polysaccharides (maltodextrin) or glycosides (Fornaro et al., 2016). The peak at 1076.7795 cm^{-1} is related to C–O–C stretching vibration, which likely corresponds to glycosidic linkages or ether bonds found in the encapsulant material, such as maltodextrin and chitosan. The vibrations observed in the FTIR spectra support the presence of flavonoids in the Tiwai extract encapsulant, which are responsible for the antioxidant activity.

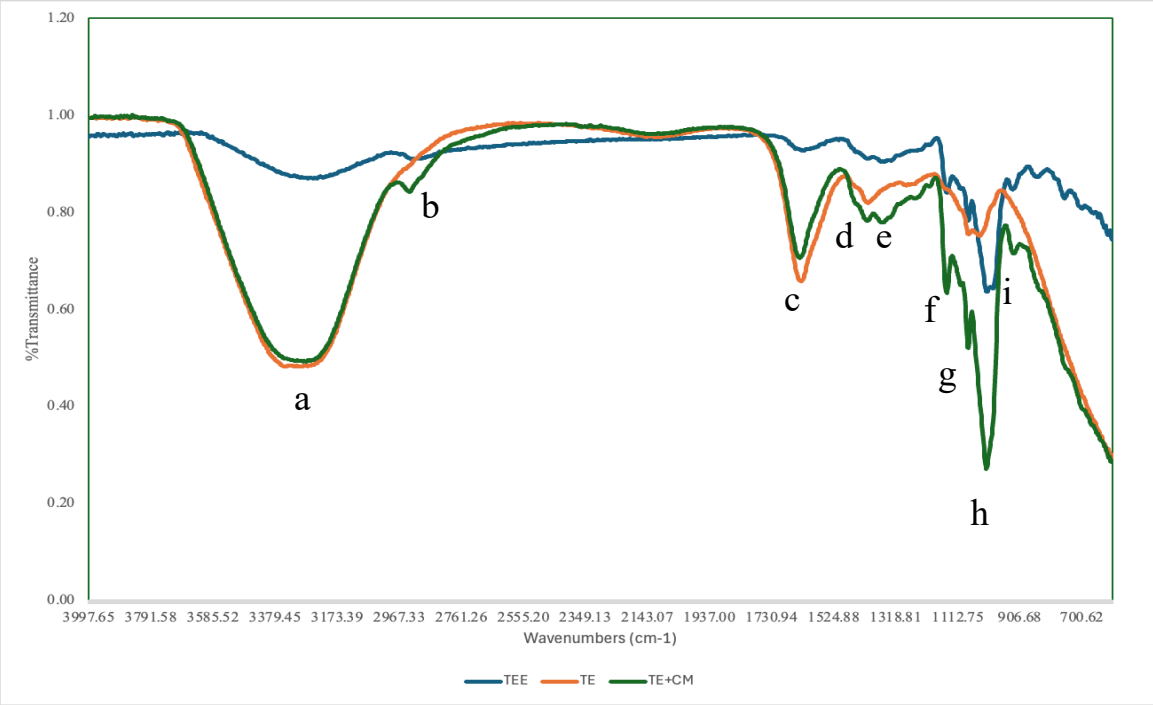

Figure 2 ATR-FTIR spectra of Tiwai extract encapsulant (TEE), Tiwai extract (TE), and Tiwai extract with coating material (TE+CM) scanned at wavenumbers 4000−650 cm^{-1} using attenuated total reflectance (ATR) mode. Labeled peaks (a–i) correspond to functional group assignments listed in Table 5.

Table 5 ATR-FTIR spectral assignments showing wave numbers, functional groups, and percent transmittance (%T) values for Tiwai extract encapsulant (TEE), Tiwai extract (TE), and Tiwai extract with coating material (TE+CM).

Code	Wave Number (cm^{-1})	Functional Group	TEE (%T)	TE (%T)	TE+CM (%T)
a	3276.34	O–H stretching (hydroxyl)	87.0	48.1	49.2
b	2910 – 2932	C–H stretching (aliphatic/aromatic)	91.0	95.6	84.2
c	1638.78	C=O stretching (carbonyl) / C=C (aromatic)	63.6	65.7	70.6
d	1413.82	C–H bending / C–O (carboxylate)	0	75.4	78.3
e	1357 – 1361.10	C–H bending (aromatic/aliphatic)	90.4	75.1	77.9
f	1149.93	C–O / C–N stretching	84.7	0	63.3
g	1076.7795	C–O–C stretching	76.8	75.6	52.0
h	1017.6902	C–O stretching (ether) / C–N (amine)	92.8	81.9	27.1
i	926.6942	C–O stretching (glycosidic linkage)	87.3	0	71.5

Note: %T = percent transmittance extracted from ATR-FTIR spectra following standard baseline correction. A value of 0 indicates that the corresponding absorption peak was absent or undetectable in that sample. TEE = Tiwai extract encapsulant; TE = Tiwai extract; TE+CM = Tiwai extract + coating material.

4.9 HPLC Analysis of Flavonoids

Qualitative and quantitative analysis of flavonoid compounds in the optimized Tiwai extract encapsulant (TEE) was performed by reverse-phase HPLC under gradient elution conditions. Quercetin was identified as the predominant flavonoid based on its retention time (18.37 min), which matched that of the quercetin reference standard (18.41–18.47 min) under identical chromatographic conditions. Quantification was carried out using a calibration curve constructed from quercetin standard solutions at concentrations of 100, 200, and 500 µg/mL. The quercetin content in the TEE was determined to be 148.30 µg/g.

This value (0.148 mg/g when converted to consistent units) was substantially lower than the TFC of 69.23 mg QE/g measured by the $AlCl_3$ colorimetric method. This discrepancy is attributed to the fundamentally different analytical principles of the two methods. The $AlCl_3$ colorimetric method reacts broadly with flavonoids and other antioxidant compounds — including proteins, carbohydrates, and unsaturated fatty acids — thereby providing a higher collective estimate of total flavonoid-equivalent content (Sultana et al., 2024; Santos-Sánchez et al., 2019; Gulcin, 2020; Munteanu & Apetrei, 2021). In contrast, HPLC is highly selective and specifically identifies and quantifies individual compounds such as quercetin, yielding a more precise but necessarily lower value (Deshmukh & Patil, 2024; Jain & Shaikh, 2016; Nguyen et al., 2023). Similar findings were reported by Sultana et al. (2024), who observed that $AlCl_3$-based derivatization in HPTLC analysis consistently overestimated flavonoid content relative to chromatographic quantification due to the broad reactivity of $AlCl_3$ with non-flavonoid molecules. These results collectively emphasize that method selection critically determines the apparent flavonoid content, and that HPLC and $AlCl_3$-based colorimetric results should not be directly compared without acknowledging their differing analytical scopes.

The results were in line with the FTIR analysis, which showed significant changes in key functional groups in the flavonoid structure, such as a decrease in the intensity of free hydroxyl groups (–OH), carbonyl groups (C=O), and aromatic bonds (C=C) in the encapsulant sample. This indicated a chemical interaction between the active compound (such as quercetin) and the coating material (maltodextrin and chitosan) during the encapsulation process, which could inhibit quercetin in its free form. The absence of the C–H group at wave number 1413.8275 cm^{-1} and the appearance of new functional groups such as C–O/C–N stretching further confirmed structural changes in the encapsulant attributable to molecular interactions during encapsulation.

5. Conclusion

In conclusion, formulation and optimization of Tiwai extract encapsulant using the spray drying method were successfully carried out using Box-Behnken Design–Response Surface Methodology (BBD-RSM). The ET:MD ratio, chitosan concentration, and inlet temperature were shown to have significant effects on EE, LC, TFC, and DPPH. Optimum condition produced encapsulant with high efficiency (EE 93.24%; LC 95.76%), adequate flavonoid content (TFC 69.23 mg QE/g), and antioxidant activity (DPPH 60.01 µg/mL). In line with the results, FTIR analysis showed changes in functional group structure due to the interaction of active compound with coating matrix, which strengthened the evidence of successful encapsulation. Quercetin content measured by HPLC (148.30 µg/g) showed a selective profile of active compounds that was not detected by the conventional colorimetric method.

6. Abbreviations

Abbreviation	Full term
$AlCl_3$	Aluminum chloride
ANOVA	Analysis of variance
ATR	Attenuated total reflectance
BBD	Box–Behnken design
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EE	Encapsulation efficiency
ET	Tiwai extract
ET:MD	Ratio of Tiwai extract to maltodextrin
FTIR	Fourier transform infrared spectroscopy
HPLC	High-performance liquid chromatography
LC	Loading capacity
MD	Maltodextrin
RSM	Response surface methodology
SD	Spray drying
TEE	Tiwai extract encapsulant
TE	Tiwai extract
TE+CM	Tiwai extract with coating material
TFC	Total flavonoid content
QE	Quercetin equivalent

Abbreviation	Full term
IC_{50}	Half maximal inhibitory concentration
CV	Coefficient of variation

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8. CRediT Statement

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