

Research Article

Response Surface Methodology for Optimizing the Concentration of Gum Arabic, Maltodextrin, and Whey Protein Isolate in *Arthrosphaera platensis* Phycocyanin Microcapsules

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Abstract

Phycocyanin is a blue-green colored phycobiliprotein present in *Arthrosphaera platensis* and has antioxidant properties. Due to its sensitivity to pH, temperature, light, oxygen, and moisture, its protection often involves microencapsulation through spray drying. This process allows it to be rapidly entrapped within the microcapsule coating material, composed of gum arabic, maltodextrin, and whey protein isolate. This study aims to determine the optimal combination concentrations of these components to optimize encapsulation performance. Optimization used the Minitab application with Response Surface Methodology and a Central Composite Design. The independent variables were the concentrations (%) of gum arabic, maltodextrin, and whey protein isolate. The response variables included yield, phycocyanin content, antioxidant activity, encapsulation efficiency, phycocyanin retention, solubility, and particle size. Scanning electron microscopy was utilized for the morphological analysis of the optimized microcapsules. Minitab analysis recommended 20 potentially optimized solutions, with the highest desirability value of 0.7656. The selected optimal formula consisted of 8.3% gum arabic, 11.7% maltodextrin, and 5.2% whey protein isolate. Its predicted response values were yield 75.30%, phycocyanin content 4.55%, antioxidant activity 48.87%, encapsulation efficiency 98.98%, phycocyanin retention 68.57%, solubility 95.15%, and particle size 212.73 nm. Validation results confirmed a yield of 81.45%, phycocyanin content of 3.60%, antioxidant activity of 52.36%, encapsulation efficiency of 94.48%, phycocyanin retention of 61.88%, and a particle size of 205.3 nm. These findings indicate that the proposed solution is effective and acceptable.

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1. Introduction

Phycocyanin is a blue-green, water-soluble phycobiliprotein derived from *Arthrospira platensis*, widely known for its antioxidant activity and therapeutic potential (Ashaolu *et al.*, 2021). The blue color is produced by a chromophore called phycocyanobilin, which binds to proteins via thioether bonds (Jaeschke *et al.*, 2021). Its antioxidant properties primarily derive from phycocyanobilin, an open-chain tetrapyrrole chromophore bound to the protein via thioether bonds (Yuan *et al.*, 2022). Phycocyanin has been used to treat various diseases, including inflammation and cancer caused by oxidative stress (Adjali *et al.*, 2022; Nege *et al.*, 2020). It is usually used as a natural colorant in foods, cosmetics, and as a synthetic coloring agent because it is nontoxic and noncarcinogenic. However, its stability during processing and in the final product is a concern for the entire food industry as it is a limiting factor in its applicability (García *et al.*, 2021). Despite its biofunctional benefits, phycocyanin's application is limited due to its sensitivity to heat, pH, light, and oxidation, which accelerates its degradation. It easily degrades, resulting in color fading and a loss of antioxidant capacity during processing and storage, thereby reducing the quality of products (Adjali *et al.*, 2022; Ribeiro and Veloso, 2021; Munawaroh *et al.*, 2020). Microencapsulation has emerged as an effective strategy to enhance the stability of phycocyanin against environmental stressors, thus extending its applicability in food and pharmaceutical formulations (Pan-utai and Iamtham, 2020).

Microencapsulation is a micro-sized packaging technology for core materials that are wrapped in a polymer layer (Li *et al.*, 2022). It can protect the core material from the environment, ensure the stability of bioactive compounds, and increase product shelf life (Ribeiro and Veloso, 2021). Spray drying encapsulation is often used because of its simple operation, high speed, and low cost (Samborska *et al.*, 2021). The selection of wall materials is based on the characteristics of the core material, such as emulsifying, solubility, and film-forming properties (Petkova *et al.*, 2022). The most commonly used wall materials for spray drying are carbohydrate- and protein-based (Petkova *et al.*, 2022). These are selected based on the characteristics needed to meet the expected quality of the final product. Carbohydrate-based wall materials are most commonly used due to their abundance, low cost, biodegradability, and biocompatibility (Ribeiro *et al.*, 2020). They can form a barrier or layer that protects the core material from oxygen. In addition, a layer of this wall material forms on the microcapsule surface during drying, preventing heat transfer toward the droplet core due to its high glass transition tem-

perature (İlter *et al.*, 2021).

Maltodextrin (MD) and Gum Arabic (GA) are the most widely used carbohydrate-based wall materials. GA is very effective due to its excellent film-forming ability, stable emulsion formation, and plasticity that prevents cracking (Alfionita *et al.*, 2022; Ribeiro *et al.*, 2020). MD has a relatively low cost, high hygroscopicity and solubility, low viscosity, and good protection against oxidation. However, due to its low emulsifying capacity (Kang *et al.*, 2019), it performs better when mixed with other wall materials that can form a stable emulsion, such as GA and whey protein isolate (WPI). Kurniasari *et al.* (2025) reported that MD alone produces lower encapsulation efficiency than a combination of MD & GA. According to Bay-san *et al.* (2021), a combination of coating materials is preferred for suitable encapsulation, desired powder properties, and high microencapsulation efficiency.

The interaction of protein-based wall materials or proteins with carbohydrates is applied in fabricating the microcapsule wall material, in which the protein fraction functions as an emulsifier and a layer-former and carbohydrates as a matrix-forming material. The most common combination in spray drying is WPI with MD or GA (Wangkulangkool *et al.*, 2023). According to İlter *et al.* (2021), protein-based wall materials are excellent for microencapsulation by spray drying because of their high functional qualities. WPI is one of the most commonly utilized wall materials, as it has a good emulsification capacity, increases the antioxidant activity of the bioactive compounds, and forms a film enveloping the core active material, which protects it from destruction by the external environment (Li *et al.*, 2022; Deng *et al.*, 2023). Zhang *et al.*, (2021) reported that WPI can enhance the storage stability of phycocyanin by encapsulating the chromophore in a protein and protecting it from oxidative attacks by external free radicals. İlter *et al.*, (2021) used a combination of carbohydrate-based wall materials (MD & GA) and protein-based wall materials (WPI & sodium caseinate) in phycocyanin microcapsules; the best combination was MD and WPI, but the encapsulation efficiency was higher using the combination of GA.

The effective coating of the core material is influenced by the encapsulation efficiency (Repka *et al.*, 2023). Different wall materials can affect the efficiency because they affect the amount of active material that can be encapsulated. Encapsulated phycocyanin has various benefits, such as increased stability, environmental protection, and controlled release (Li *et al.*, 2022). Although binary combinations of wall materials are well-documented, the use of a ternary system—combining GA, MD, and WPI—for

phycocyanin encapsulation remains underexplored. Various studies have used such a combination for the microencapsulation of turmeric oleoresin, citron extract, and anthocyanin (Mahdi *et al.*, 2020; Ribeiro *et al.*, 2020; Yousefi *et al.*, 2022). This research aimed to determine the effect of a ternary combination of carbohydrate-based wall materials (GA & MD) and protein-based wall materials (WPI) on phycocyanin microcapsules prepared by spray drying. The research related to the optimization for the use of three wall materials for encapsulation has never been done, so in this study, optimization will be carried out using Central Composite Design (CCD) based on Response Surface Methodology (RSM) to obtain microcapsules with optimal response. RSM evaluated the concentrations of GA, MD, and WPI, which will be optimized to obtain high yield values, phycocyanin content, antioxidant activity, encapsulation efficiency, phycocyanin retention, solubility, and small particle size.

2. Materials and Methods

2.1 Material

2.1.1 The equipments

The equipment used in this research included a spectrophotometer UV-VIS (Optima, Germany), an MX-S vortex (DLAB, Beijing, China), a D-500 high speed homogenizer (DLAB), a refrigerator (LG), a chiller (Modena, Jakarta, Indonesia), a YC-015 spray dryer (Shanghai Pilotech Instrument & Equipment Co. Ltd., Shanghai, China), a Nanotrac Wave II e. AAS and Micrometrix Particle Size Analyzer (PSA) (Micrometrix, PA, USA), and a JSM-6510LA scanning electron microscope (SEM) (JEOL Ltd. Tokyo, China).

Table 1. Values of independent variables at the three levels of the central composite design (CCD)

Independent Variable	Level Code	Min value (-1)	Max value (+1)
Gum Arabic (g)	X ₁	10	15
Maltodextrin (g)	X ₂	5	10
Whey protein isolate (g)	X ₃	4	5

2.1.2 The materials

The materials used in this study included *Arthrospira platensis* powder (Algae Biotechnology Indonesia), maltodextrin (DE 10-12, Qinhuangdao Li-hua Starch), whey protein isolate (Puro Chari Makmur Indonesia), gum arabic (Ingredion, Thailand), distilled water, ethanol (Merck, Germany), and DPPH powder (Merck, Germany).

2.1.3 Ethical approval

This study does not require ethical approval because it does not use experimental animals.

2.2 Method

2.2.1 Extraction of *A. platensis* phycocyanin

Phycocyanin extraction was conducted by dissolving 20 g of *A. platensis* powder in 200 mL of 0.1 M phosphate buffer (pH 7.0), followed by freeze-thaw cycles at -20°C for 24 hours and room temperature thawing. This cycle was repeated twice. Next, the mixture was centrifuged at 2800x g for 10 min at -4°C. The supernatant was filtered, and the phycocyanin extract was collected into a bottle wrapped in aluminum foil and stored in a chiller (Chittapun *et al.*, 2020).

2.2.2 Microencapsulation of Phycocyanin

The required amounts of GA, MD, and WPI for each treatment were placed in beaker glass, and 100 mL of distilled water was added. The mixture was stirred at 600 rpm for 30 min at 60°C until homogeneity. It was then cooled to ~45°C and stored overnight in a chiller at 15°C–20°C (Purba, 2013).

2.2.3 Spray drying

For this, 50 ml of phycocyanin extract were mixed with 100 mL of encapsulant solution (1:2 v/v) and homogenized at 12,000 rpm for 3 minutes. The mixture was then dried using a spray dryer set at inlet and outlet temperature of 110°C and 65°C, respectively. The resulting microcapsule powder was stored in lightproof plastic in a dry place (Iqbal and Hadiyanto, 2020; Pan-utai and Iamtham, 2020).

2.2.4 CCD

This study optimized the microencapsulation wall materials utilizing RSM and a CCD. The independent variables included the concentrations of GA, MD, and WPI. The dependent variables (responses) were yield, encapsulation efficiency, phycocyanin concentration, phycocyanin retention, antioxidant activity, solubility, particle size, and SEM. A total of

20 run-order treatments were conducted. The concentration of GA ranged from 10% to 15% (Adsare and Annapure, 2021; Gharibzahedi et al., 2012; Nthimole et al., 2022), maltodextrin (MD) ranged from 5% to 10% (Aminikhah et al., 2023; Mishra et al., 2014; Sablania et al., 2018; Yunilawati et al., 2018), and WPI ranged from 4% to 5% (Stănciuc et al., 2018; Zhao et al., 2023). The independent variable values at the three CCD levels are shown in Table 1.

Table 2. Formulation design of phycocyanin microcapsules using the central composite design (CCD)

Formula	GA (%)	MD (%)	WPI (%)
1	12.5	7.5	5.3
2	15	10	5
3	8.3	7.5	4.5
4	12.5	7.5	4.5
5	15	5	4
6	12.5	7.5	4.5
7	15	10	4
8	12.5	3.3	4.5
9	10	10	5
10	12.5	7.5	3.7
11	10	10	4
12	12.5	7.5	4.5
13	15	5	5
14	12.5	11.7	4.5
15	16.7	7.5	4.5
16	12.5	7.5	4.5
17	10	5	5
18	12.5	7.5	4.5
19	12.5	7.5	4.5
20	10	5	4

Description: GA = Gum Arabic; MD = Maltodextrin; WPI = Whey protein isolate.

2.2.5 Microcapsule Characteristic Analysis

2.2.5.1 Yield

The yield was calculated by comparing the weight of the microcapsule powder obtained to the total weight of the encapsulant and phycocyanin extract, then multiplying the result by 100% (Kurniasih et al., 2018). The yield was determined by applying the following formula:

$$\text{Yield} = \text{MW(g)}/(\text{WE(g)}+\text{PW(g)}) \times 100\% \dots \dots \dots (1)$$

Where :

MW = Microcapsule Weight

EW = Encapsulant Weight

PW = Phycocyanin Weight (extract).

2.2.5.2 Phycocyanin content

A total of 40 mg of phycocyanin microcapsule powder was diluted in 10 mL of 0.1 M phosphate buffer (pH 7.0), homogenized, and then the OD₆₁₅ and OD₆₅₂ were measured (Pan-utai and Iamtham, 2020; Purnamayati et al., 2016). The phycocyanin content was then calculated utilizing the following formula:

$$\text{PC (\%)} = (A_{615\text{nm}} - 0.474 A_{652\text{nm}})/5.34 \times 100\% \dots \dots \dots (2)$$

Where :

PC = Phycocyanin concentration

0.474 and 5.34 = Molar absorption coefficient of PC concentration

A₆₁₅ = Absorbance value at wavelength (λ) 615 nm

A₆₅₂ = Absorbance value at wavelength (λ) 652 nm.

2.2.5.3 Antioxidant activity

A 200 μ M DPPH solution was prepared by dissolving DPPH in 100 mL of ethanol in a volumetric flask. The solution was homogenized with a vortex and stored in an Erlenmeyer flask wrapped with aluminum foil. The microcapsule powder was dissolved in ethanol to a final concentration of 1 mg/mL. To determine the antioxidant activity, 2 mL of the microcapsule solution was mixed with 1 mL of the DPPH solution and vortexed. The mixture was then incubated at room temperature for 30 min and the OD₅₁₇ was determined using a spectrophotometer (Pan-utai and Iamtham, 2020). A blank solution consisting of DPPH and ethanol was employed as a reference. The percentage of DPPH inhibition (%DPPH) was calculated utilizing the following formula:

$$\% \text{DPPH Scavenging activity} = (A_2 - A_1) / A_2 \times 100\%. \quad (3)$$

Where :

A_1 = sample absorbance

A_2 = absorbance of blank

(2017) and Pan-utai and Iamtham (2020) with modifications. For total phycocyanin determination, 100 mg of the sample was dissolved in 10 mL of distilled water and homogenized with a vortex for 3 min. The resulting mixture was centrifuged at 13,500 rpm and

Table 3. Analysis results of the phycocyanin microcapsule parameters

Formula	Yield (%)	Phycocyanin content (%)	Antioxidant activity (%)	Solubility (%)	Encapsulation Efficiency (%)	Retention of Phycocyanin (%)	Particle Size (nm)
1	83.98	3.94	54.42	93.49	97.67	62.95	350.0
2	83.47	3.10	60.71	93.42	99.35	86.03	294.5
3	87.12	3.98	46.49	94.06	95.54	79.88	204.6
4	71.22	3.38	55.89	91.28	92.23	71.34	232.7
5	80.59	4.51	55.37	91.46	97.73	73.39	233.7
6	63.62	3.65	58.88	92.11	96.76	72.40	257.4
7	61.44	3.11	49.72	93.89	99.27	84.04	274.6
8	83.76	4.46	48.24	87.58	91.41	86.3	250.3
9	62.97	3.61	50.19	93.58	98.1	71.95	273.2
10	72.32	4.06	47.67	89.33	97.53	70.94	223.7
11	82.17	3.87	48.98	90.95	96.82	74.75	224.8
12	75.31	3.39	56.51	93.22	95.82	74.98	297.8
13	87.31	3.36	47.04	88.4	93.14	64.45	286.5
14	74.27	3.33	52.08	93.56	97.25	64.95	250.9
15	73.11	3.40	49.31	89.96	90.56	64.61	262.6
16	77.78	3.64	56.29	92.91	96.49	72.74	305.0
17	76.22	4.55	40.00	91.6	95.58	89.14	363.0
18	73.33	3.55	50.65	94.29	99.26	73.87	276.0
19	71.94	3.50	51.11	94.1	98.4	62.85	284.8
20	74.41	4.08	46.44	88.74	98.11	74.62	248.4

2.2.5.4 Encapsulation efficiency

To evaluate the efficiency of phycocyanin microencapsulation, the total and surface phycocyanin contents of the microcapsules were ascertained employing a method reported by Laokuldilok and Kanha

25°C for 5 min. The clear supernatant was collected and filtered through a 0.45 mm membrane (Millipore, MA, USA) to ascertain the phycocyanin concentration.

To determine the surface phycocyanin, 100

mg of the sample was extracted with 10 mL of 95% (v/v) aqueous ethanol solution. The mixture was homogenized with a vortex for 1 min, then centrifuged at 10,000 rpm and 25°C for 10 min. After phase separation, the clear supernatant was filtered through a 0.45 mm pore-size membrane (Millipore), and the surface phycocyanin was measured by absorbance. The microencapsulation efficiency was calculated by applying the following equation:

$$EE = (TP-SP)/TP \times 100\% \dots \dots \dots (4)$$

Where :

EE = Encapsulation efficiency

TP = Total Phycocyanin

SP = Surface Phycocyanin

the extract before microencapsulation (Faieta *et al.*, 2020). Phycocyanin retention was calculated utilizing the following formula:

Where :

PT = Phycocyanin Total

PE = Phycocyanin extract

2.2.5.6 Solubility

Solubility identification followed the method described by İler *et al.* (2021). A 1 g sample of the microcapsule powder was dissolved in 25 mL of distilled water and homogenized with a magnetic stirrer

Table 4. Model analysis of the response/parameters of phycocyanin microcapsules

Response	Model	Math	Significant (p<0.05)	Lack-of-Fit (p<0.05)	R ²
Yield	Quadratic	$Y_1 = 353 - 19.85 X_1 + 0.98 X_2 - 81.7 X_3 + 0.260 X_1^*X_1 + 0.211 X_2^*X_2 + 4.76 X_3^*X_3 - 0.319 X_1^*X_2 + 3.58 X_1^*X_3 - 0.17 X_2^*X_3$	0.355	0.068	0.5338
Phycocyanin content	Quadratic	$Y = 13.01 + 0.454 X_1 - 0.460 X_2 - 4.07 X_3 + 0.0060 X_1^*X_1 + 0.0176 X_2^*X_2 + 0.589 X_3^*X_3 - 0.0102 X_1^*X_2 - 0.1370 X_1^*X_3 + 0.0410 X_2^*X_3$	0.355	0.068	0.8411
Antioxidant activity	Quadratic	$Y = -19 + 7.62 X_1 - 6.36 X_2 + 16.9 X_3 - 0.381 X_1^*X_1 - 0.253 X_2^*X_2 - 5.08 X_3^*X_3 - 0.094 X_1^*X_2 + 0.789 X_1^*X_3 + 2.697 X_2^*X_3$	0.036	0.424	0.7512
Encapsulation efficiency	Quadratic	$Y = 172.8 + 3.15 X_1 - 4.25 X_2 - 35.1 X_3 - 0.115 X_1^*X_1 - 0.043 X_2^*X_2 + 3.56 X_3^*X_3 + 0.130 X_1^*X_2 - 0.326 X_1^*X_3 + 0.848 X_2^*X_3$	0.340	0.508	0.5406
Phycocyanin retention	Quadratic	$Y = -170 + 6.24 X_1 + 0.17 X_2 + 98.4 X_3 + 0.081 X_1^*X_1 + 0.153 X_2^*X_2 - 6.42 X_3^*X_3 + 0.137 X_1^*X_2 - 2.56 X_1^*X_3 - 1.37 X_2^*X_3$	0.015	0.520	0.7979
Solubility	Quadratic	$Y = -7.4 + 4.58 X_1 + 0.67 X_2 + 28.8 X_3 - 0.0459 X_1^*X_1 - 0.1273 X_2^*X_2 - 1.99 X_3^*X_3 + 0.0652 X_1^*X_2 - 0.902 X_1^*X_3 + 0.236 X_2^*X_3$	0.023	0.258	0.7766
Particle size	Quadratic	$Y = -242 + 62X_1 + 13.8 X_2 - 34 X_3 - 1.74 X_1^*X_1 - 0.78 X_2^*X_2 + 31.9 X_3^*X_3 + 3.25 X_1^*X_2 - 9.03 X_1^*X_3 - 9.91 X_2^*X_3$	0.020	0.576	0.7847

2.2.5.5 Phycocyanin retention

Phycocyanin retention is defined as the ratio of the phycocyanin content in the microcapsules after spray drying to the initial phycocyanin content in

for 30 min at room temperature. The solution was then centrifuged at 3,000 rpm for 5 min. The supernatant was transferred to a preweighed container and dried in an oven at 105°C for 5 h until constant weight. The weight of the dried sample plus the container was then

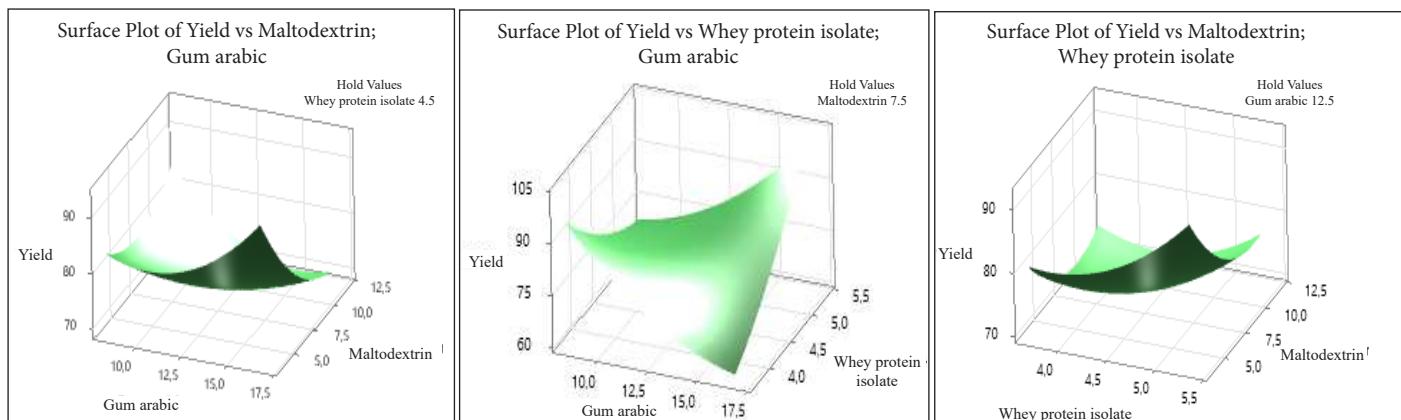


Figure 1. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate and (c) the yield parameter response.

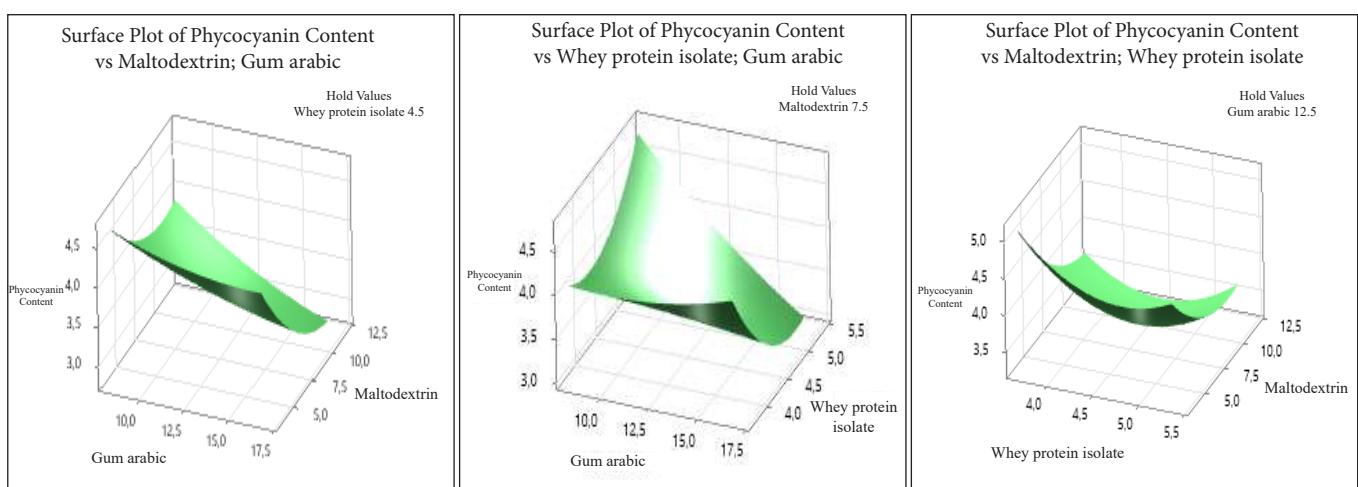


Figure 2. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate and (c) the response parameter of phycocyanin content.

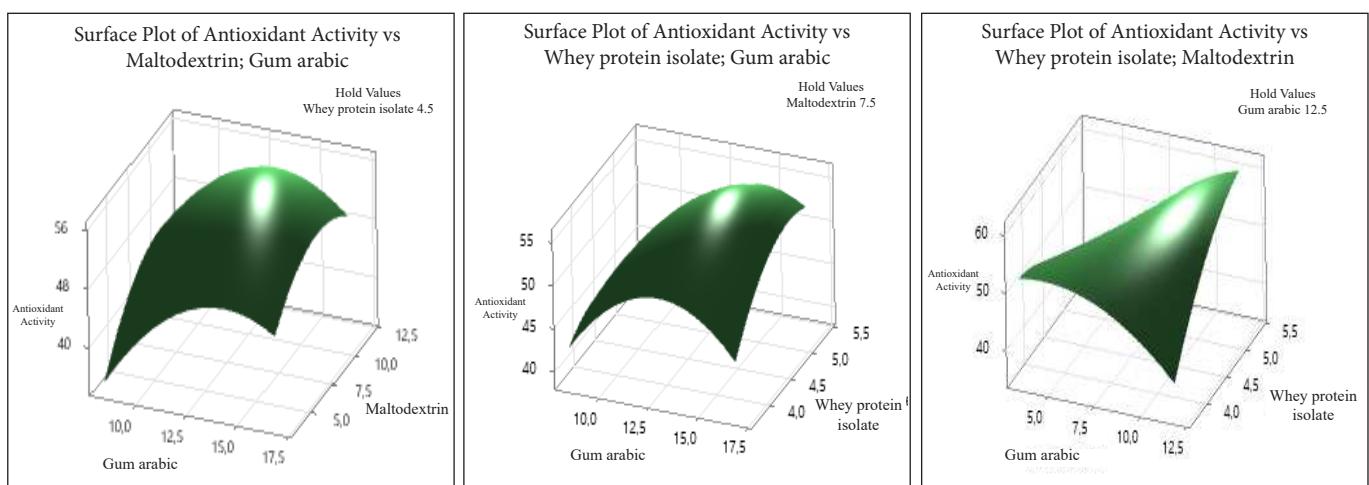


Figure 3. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate (c) of the response parameter of antioxidant activity.

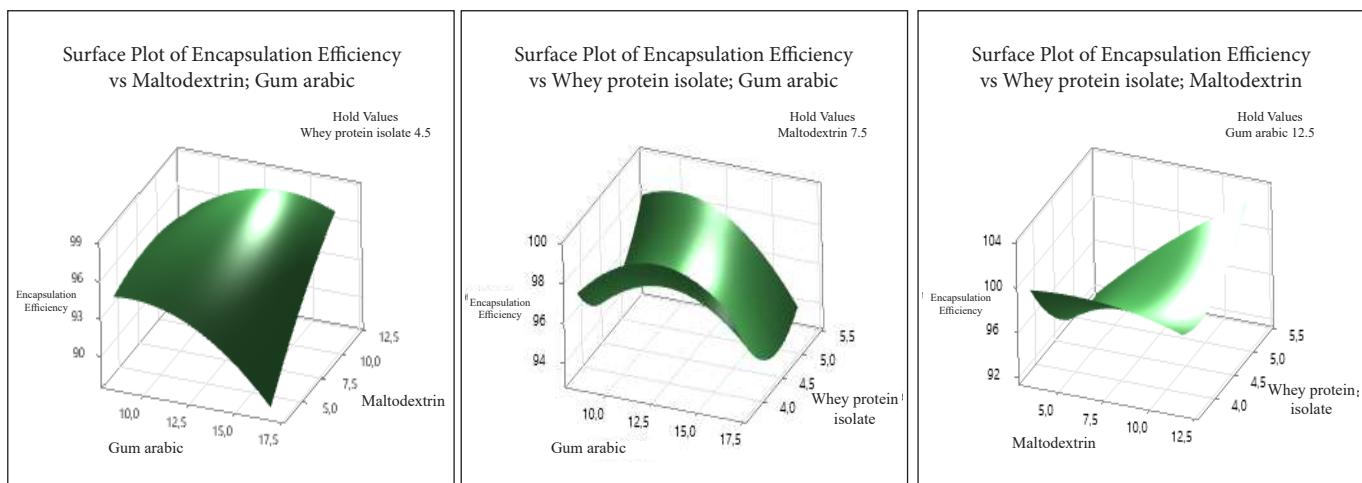


Figure 4. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate and (c) the response parameter of encapsulation efficiency.

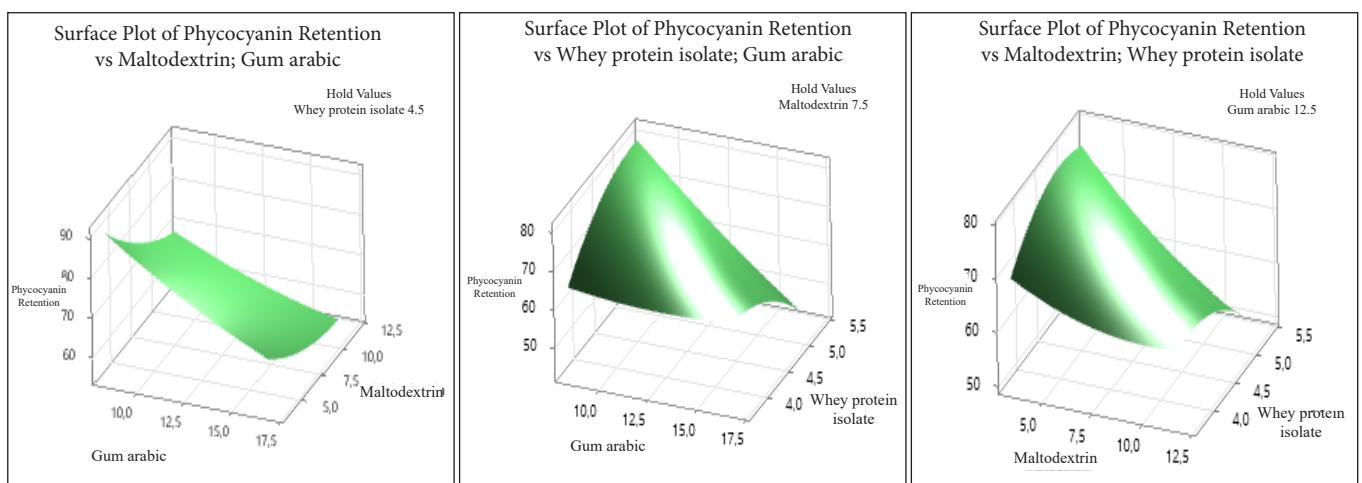


Figure 5. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate (c) response parameters of phycocyanin retention.

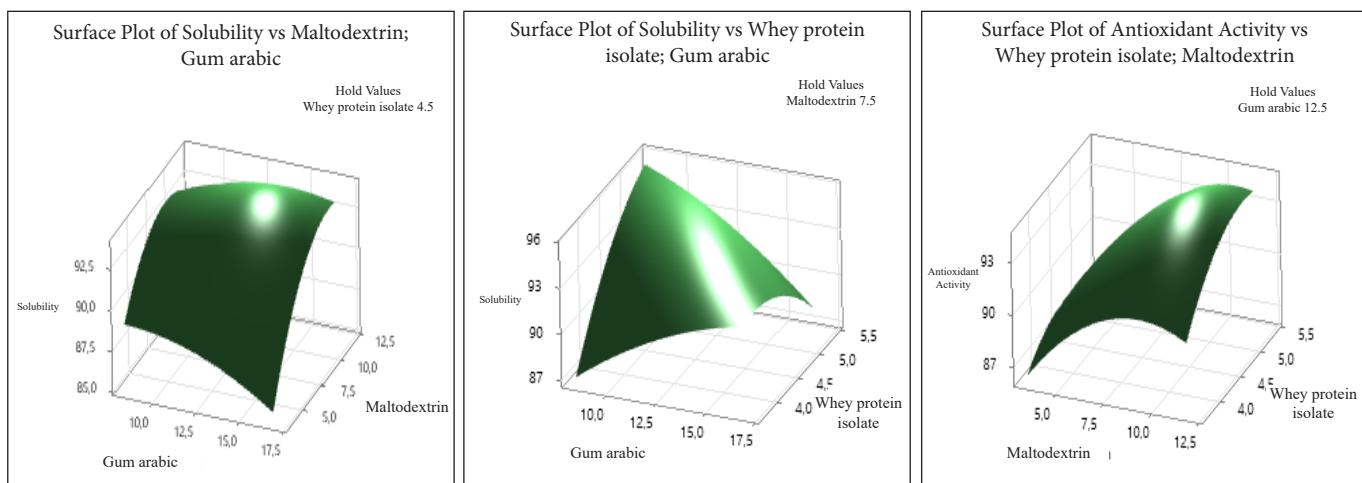


Figure 6. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate (c) of the solubility parameter response.

recorded. The powder solubility was calculated employing the following formula:

$$\text{Solubility} = \text{DS/SP} \times 100\% \dots \dots \dots (6)$$

Where :

DS = Dried Supernatant

SP = Sample Powder

2.2.5.7 Particle size

Particle size was analyzed following the method of [Liang et al. \(2013\)](#) using a PSA. A total of 0.01 g sample of the microcapsule powder was diluted in 5 mL of distilled water, and a portion of this solution was transferred into a tube with a maximum length of 15 mm. The data were displayed on a computer screen.

Table 5. Response verification of the optimum formulation for phycocyanin microcapsules

Response	Optimum formula				95%		95%	
	Prediction	Verification	STDEV	Confidence level		Predict level		
				Low/low	High	Low/low	High/high	
Yield (%)	75.30	81.45	4.3494	46.1	104.5	42.4	108.2	
Phycocyanin content (%)	4.55	3.60	0.6718	3.518	5.591	3.385	5.723	
Antioxidant activity (%)	48.87	52.36	2.4713	34.19	63.54	32.31	65.42	
Encapsulation efficiency (%)	98.98	94.48	3.1784	88.57	109.38	87.23	110.72	
Phycocyanin retention (%)	68.57	61.88	4.7320	47.77	89.37	45.11	92.03	
Solubility (%)	95.19	93.38	1.2827	89.30	101.08	88.54	101.83	
Particle size (nm)	212.73	205.3	5.2538	102.8	322.7	88.7	336.8	

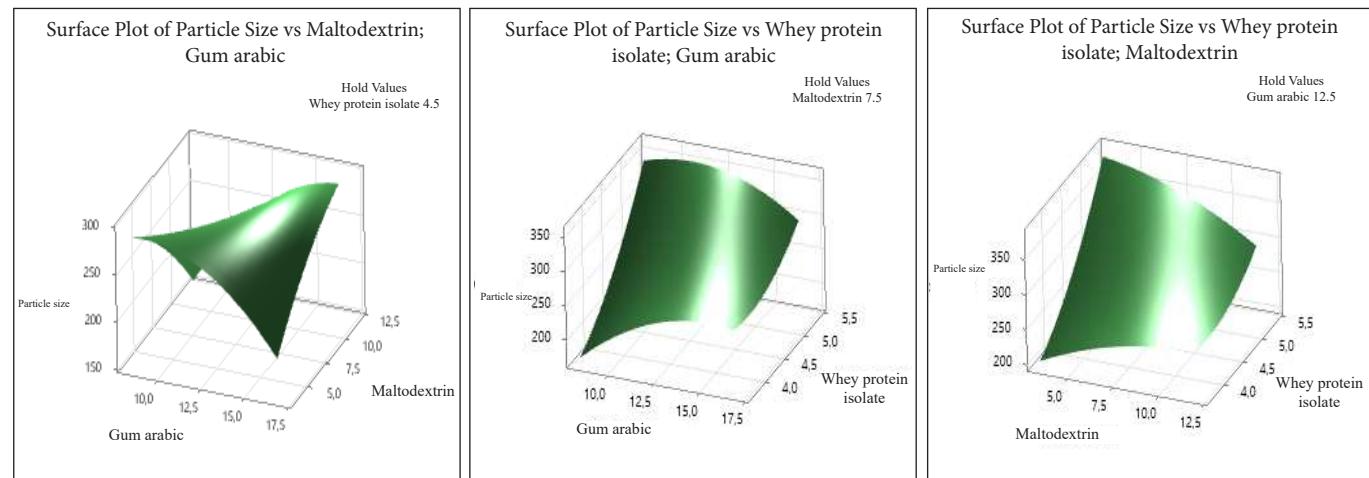


Figure 7. Surface plot of gum arabic, maltodextrin (a); gum arabic, whey protein isolate (b); maltodextrin, whey protein isolate and (c) the response parameter of particle size.

2.2.5.8 Scanning electron microscopy (SEM)

Particle morphology was observed using SEM according to the method of Ho *et al.* (2021). The microcapsule powder samples were evenly distributed on the aluminum stubs and coated with gold using an Ion Sputter Coater (Hitachi, Tokyo, Japan). The gold-coated samples were then examined using a SU3500 SEM (Hitachi, Japan) at 1,000x; 5,000x; and 10,000x.

2.3 Analysis Data

This research employed the CCD method of the RSM to optimize the combination variables (GA, MD, and WPI). The microcapsule characteristics (response) analyzed were yield, phycocyanin content, antioxidant activity (Pan-utai and Iamtham, 2020), encapsulation efficiency (Laokuldilok and Kanha 2017; Pan-utai and Iamtham, 2020), encapsulation efficiency (Laokuldilok and Kanha 2017; Pan-utai and Iamtham, 2020), phycocyanin retention (Faieta *et al.*, 2020), solubility (İlter *et al.*, 2021), particle size (Liang *et al.*, 2013). Validation was performed by comparing the predicted results generated by the Minitab 21 software with the actual analysis results at the optimum point. The Minitab 21 software predicts values based on the analysis, identifying the optimum conditions.

3. Results and Discussion

3.1 Results

3.1.1 The effects of Gum Arabic, Maltodextrin, and Whey Protein Isolate combination

The effects of GA, MD, and WPI combination on the yield, phycocyanin content, antioxidant activity, encapsulation efficiency, phycocyanin retention, solubility, and particle size are summarized in Table 3. The yield ranged from 61.44% to 87.31%, phycocyanin content from 3.10% to 4.55%, antioxidant activity from 40.00% to 60.71%, encapsulation efficiency from 90.56% to 99.35%, phycocyanin retention from 62.85% to 89.14%, solubility from 87.58% to 94.29%, and the particle size from 204.6 to 363.0 nm. ANOVA revealed that the selected model for all responses was quadratic. This model indicated that all responses were markedly influenced by GA, MD, and WPI, as well as their interactions. The model was significant, with p-values <0.05 for antioxidant activity ($p = 0.036$), phycocyanin retention ($p = 0.015$), solubility ($p = 0.023$), and particle size ($p = 0.020$). However, the model was insignificant ($p > 0.05$) for yield ($p = 0.355$), phycocyanin content ($p = 0.355$), and encapsulation efficiency ($p = 0.340$). The lack-of-fit F-values for all responses had p-values > 0.05 , including yield ($p = 0.068$), phycocyanin content ($p = 0.068$), antiox-

idant activity ($p = 0.424$), encapsulation efficiency ($p = 0.508$), phycocyanin retention ($p = 0.508$); solubility ($p = 0.258$), and particle size ($p = 0.576$). These insignificant F-values indicate that the model fits the response data well (Purwoto and Christi, 2017).

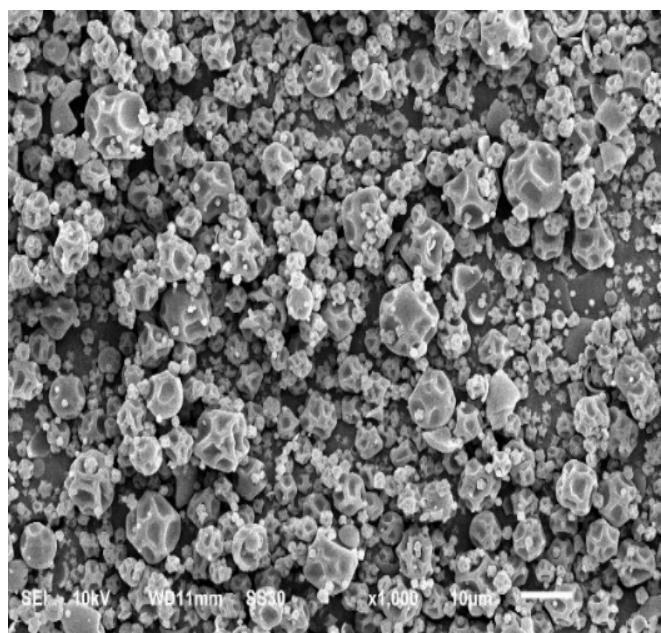


Figure 8. Morphology of optimum formulation of phycocyanin Microcapsules with central composite design.

3.1.2 The optimum formula

The Minitab program suggested 20 potential optimization solutions, from which we selected the optimum formula for validation. As shown in Table 3, the program predicted the optimum formula would have a response of 75.30% for yield, 4.55% for phycocyanin content, 48.87% for antioxidant activity, 98.89% for encapsulation efficiency, 68.57% for phycocyanin retention, 95.15% for solubility, and 212.73 nm for particle size. A validation of the optimum formula resulted in an actual yield of 81.45%, a phycocyanin content of 3.60%, an antioxidant activity of 52.36%, an encapsulation efficiency of 94.48%, a phycocyanin retention of 61.88%, and a particle size of 205.3 nm.

3.2 Discussion

3.2.1 Yield

Yield represents the amount of the microcapsule product obtained after spray drying. It also indicates the effectiveness of the microencapsulation process. The RSM equation used to optimize the concentration of the encapsulation materials for the yield response is as follows:

$$Y_1 = 353 - 19.85 X_1 + 0.98 X_2 - 81.7 X_3 + 0.260 X_1 * X_1 + 0.211 X_2 * X_2 + 4.76 X_3 * X_3 - 0.319 X_1 * X_2 + 3.58 X_1 * X_3 - 0.17 X_2 * X_3 \quad \text{Equation (1)}$$

Equation (1) reveals that the yield response increases at higher MD concentrations in linear. Quadratic effect of WPI can increase yield and interaction between GA and WPI can increase yield. Figure 1 illustrates the surface plot depicting the relationship between GA, MD, and WPI on the yield response. MD has robust binding properties, enabling it to bind to more suspensions and enhance yields (Hasna *et al.*, 2018). It also helps reduce the emulsion's viscosity. Combining MD with GA and WPI facilitates the drying process. Although GA has a high viscosity, WPI stabilizes the emulsion, and together, enhances product output. During drying, water molecules from the core material and encapsulant evaporate more readily, optimizing drying efficiency (Purnomo *et al.*, 2014).

In this study, the yield of phycocyanin microcapsules ranged from 61.44% to 87.12%, significantly greater than those made with MD and carrageenan, which ranged from 18% to 29% (Purnamayati *et al.*, 2018). The improvement in yield can be attributed to an increase in the glass transition temperature of the powder due to the addition of the GA, MD, and WPI. These high-molecular-weight materials reduce powder stickiness, enhancing the product yield during spray drying. A combination of these three encapsulants elevated the yield by >50%, which is consistent with previous studies which reported a yield increase of ~66.0%–76.6% when utilizing these three for the microencapsulation of palm fruit anthocyanins (Santana *et al.*, 2016). Additionally, microcapsule yield is influenced by factors such as the ratio of core to protective material; feed solid concentrations; surfactant use; feed and drying air flow rates; and inlet and outlet temperatures (Arpagaus *et al.*, 2017).

3.2.2 Phycocyanin content

The phycocyanin content refers to the amount of phycocyanin present in all parts of the microcapsule. This parameter determines the effectiveness of the encapsulation process for retaining phycocyanin across different combinations of GA, MD, and WPI. A combination of these encapsulant materials directly affects the encapsulation efficiency. The RSM equation for optimizing encapsulant concentrations concerning the phycocyanin content is as follows:

$$Y_2 = 13.01 + 0.454 X_1 - 0.460 X_2 - 4.07 X_3 + 0.0060 X_1 * X_1 + 0.0176 X_2 * X_2 + 0.589 X_3 * X_3 - 0.0102 X_1 * X_2 - 0.1370 X_1 * X_3 + 0.0410 X_2 * X_3 \quad \text{Equation (2)}$$

Equation (2) shows that the phycocyanin content response was directly correlated to the GA concentration in linear, quadratic effect of WPI can increase phycocyanin content and interaction of MD and WPI can increase phycocyanin content. Figure 2 illustrates the surface plot indicating the relationship between GA, MD, and WPI on phycocyanin content. GA inclusion in the encapsulant material likely enhances phycocyanin retention because it functions as an emulsifier and film former (Wyasu and Okereke, 2012). A thicker, more durable microcapsule wall helps trap more phycocyanin. In this study, the phycocyanin content was higher than the values reported using MD and alginate, which ranged from 0.05% to 2.42% (Kurniasih *et al.*, 2018). Dewi *et al.* (2016) reported that phycocyanin microcapsules made with MD and carrageenan had phycocyanin contents of ~0.71%–2.83%. Iqbal and Hadiyanto (2020) suggested that the phycocyanin content in microcapsules depends on the ratio between the encapsulant and the core material. For instance, a higher maltodextrin–phycocyanin ratio (1:4) produced a lower phycocyanin content of 0.24%, compared to a lower ratio (2:1), which resulted in 0.86% yield. Furthermore, Purnamayati *et al.*, (2018) explained that the inlet temperature also affects the phycocyanin concentration. The microcapsules dried at an inlet temperature of 90°C had a phycocyanin content of 1.729%, while those dried at 130°C had a lower content of 1.08%.

3.2.3. Antioxidant activity

Antioxidants are compounds that either accept or donate electrons, enabling them to prevent the formation of free radicals during oxidation reactions. A compound is considered to have antioxidant activity if it can donate hydrogen atoms to DPPH free radicals. The RSM equation utilized to optimize the encapsulant material concentration for the antioxidant activity response is as follows:

$$Y_3 = -19 + 7.62 X_1 - 6.36 X_2 + 16.9 X_3 - 0.381 X_1 * X_1 - 0.253 X_2 * X_2 - 5.08 X_3 * X_3 - 0.094 X_1 * X_2 + 0.789 X_1 * X_3 + 2.697 X_2 * X_3 \quad \text{Equation (3)}$$

Equation (3) indicates that the antioxidant activity was directly proportional to the WPI concentrations in linear, quadratic effect of MD can decrease antioxidant activity and interaction of MD and WPI can increase antioxidant activity. Figure 3 illustrates the surface plot showing the relationships between GA, MD, or WPI on the antioxidant activity. A combination of these encapsulant materials plays a crucial role in maintaining antioxidant stability and preserving phycocyanin levels during spray drying (Dewi *et al.*,

2016). According to Agustina et al. (2020), elevated phycocyanin levels correspond to greater antioxidant capacity, enhancing the ability of antioxidants to donate electrons and suppress free radical formation. Encapsulation provides an additional layer of protection, preventing degradation caused by oxygen.

In this study, phycocyanin extract coated with a mixture of GA, MD, and WPI yielded antioxidant activities ranging from 40.00% to 60.71%, as measured by the DPPH inhibition method. Zhang et al., (2022) found that combining carbohydrates and proteins, such as GA and WPI, enhanced the antioxidant activity in *Spirulina* chlorophyll microcapsules. Such an increase can be attributed to the Maillard reaction, which produces melanoidins that contribute to the antioxidant properties. The reaction occurs in the protein component under high drying temperatures (Wang et al., 2011), with melanoidin formation helping to protect the active ingredients from oxidation and thereby improving product stability. Furthermore, the WPI's ability to prevent oxidation is associated with its sulphydryl (-SH) groups, which reduce free radicals in spray-dried powders (Gad et al., 2011; Ton et al., 2016; Premi and Sharma, 2017).

3.2.4 Encapsulation efficiency

Encapsulation efficiency is a key parameter for evaluating the effectivity of the microencapsulation process in trapping or retaining the core material—phycocyanin extract. It is a crucial indicator of microencapsulated particles, contributing to better stability and longer shelf life (Timilsena et al., 2020). The RSM equation for optimizing the encapsulant concentration to improve the encapsulation efficiency is as follows:

$$Y_4 = 172.8 + 3.15 X_1 - 4.25 X_2 - 35.1 X_3 - 0.115 X_1 * X_1 \\ - 0.043 X_2 * X_2 + 3.56 X_3 * X_3 + 0.130 X_1 * X_2 \\ - 0.326 X_1 * X_3 + 0.848 X_2 * X_3 \quad \text{Equation (4)}$$

Equation (4) suggests that the encapsulation efficiency was positively correlated with the GA concentration in linear, quadratic effect of WPI can increase encapsulation efficiency, and interaction of MD and WPI can increase encapsulation efficiency. Figure 4 illustrates the surface plot indicating the relationship between GA, MD, or WPI on encapsulation efficiency. Dewi et al. (2017) explained that the encapsulation efficiency is influenced by the type of polymer used, which can affect the hydrophobic characteristics of the emulsifier. Certain polymers, such as GA and WPI, are known for their ability to emulsify and maintain emulsion viscosity. The combination of GA, MD, and WPI employed in this study resulted in encapsulation

efficiencies ranging from 90.56% to 99.35%. These results surpass those of previous studies, such as the encapsulation efficiency of 41.42% achieved using 9.2% MD and 0.8% alginate (Kurniasih et al., 2018) and 86.9% with a 1:1 combination of GA and WPI (İlter et al., 2021).

GA can achieve high encapsulation efficiency (Yousefi et al., 2022). As a highly branched sugar heteropolymer with a small amount of protein covalently attached to its carbohydrate chains, GA functions as an excellent film-forming agent, effectively encapsulating molecules, by forming a protective matrix around the core material, shielding it from air. The surface-active properties of GA help safeguard reactive or volatile core materials (Cilek et al., 2012) ultrasonication time and core to coating ratio on encapsulation of phenolic compounds extracted from sour cherry pomace. For this study, maltodextrin and gum arabic were chosen as coating materials. Different maltodextrin/gum arabic ratios (10:0, 8:2, 6:4). Additionally, proteins and polysaccharides serve as a bridge that allows bioactive components to maintain stability within complex food matrices (Zhang et al., 2022).

Generally, two main types of interactions occur between proteins and polysaccharides: non-covalent complexation and covalent bonding. Non-covalent bonds create bioadhesive states that facilitate the formation of micromaterials, while covalent interactions produce protein-polysaccharide conjugates with excellent amphiphilic properties, enabling them to encapsulate bioactive components (Sadiyah et al., 2022). When the microstructure of such conjugates is established, they provide superior encapsulation performance. Additionally, the encapsulation efficiency is also affected by the spray drying inlet temperatures. Purnamayati et al. (2018) demonstrated that using an inlet temperature of 90°C resulted in a higher encapsulation efficiency of 29.623% compared to 18.457% at 130°C. This suggests that lower inlet temperatures help preserve the phycocyanin content. Furthermore, the core-encapsulant material ratio is critical for achieving high encapsulation efficiency. Iqbal and Hadiyanto (2020) reported that a phycocyanin-maltodextrin ratio of 1:2 produced the maximal encapsulation efficiency of 61.53%.

3.2.5 Phycocyanin retention

Phycocyanin retention measures the effectiveness of spray drying in encapsulating the *A. platensis* phycocyanin extract. It was determined by comparing the total phycocyanin content after microencapsulation with the initial phycocyanin content before microencapsulation. A successful encapsulation method

relies on high retention of the core material and minimal core material present on the surface of the powder particles. The RSM equation for optimizing the encapsulant material concentration to maximize the encapsulation efficiency is as follows:

$$Y_5 = -170 + 6.24 X_1 + 0.17 X_2 + 98.4 X_3 + 0.081 X_1 X_1 + 0.153 X_2 * X_2 - 6.42 X_3 * X_3 + 0.137 X_1 * X_2 - 2.56 X_1 * X_3 - 1.37 X_2 * X_3 \quad \text{Equation(5)}$$

Equation (5) shows that phycocyanin retention was directly proportional to the concentrations of WPI in linear, quadratic effect of MD can increase phycocyanin retention and interaction between GA and MD can increase phycocyanin retention. Figure 5 illustrates the surface plot of the relationship between these encapsulant materials and phycocyanin retention. Proper encapsulant levels play a crucial role in stabilizing the emulsion, directly impacting retention. Enhanced solid content facilitates skin formation and accelerates drying, thereby improving retention. This is also influenced by the emulsion viscosity—higher solid content elevates viscosity, which reduces internal mixing, due to which the core components are less likely to migrate to the surface, allowing for more effective film formation and enhanced retention.

According to [Charve and Reineccius \(2009\)](#), the film-forming and emulsification abilities of the encapsulant materials can vary significantly in terms of retention, even when they are acceptable. Retention during drying with the WPI may be attributed to the excellent emulsifying and binding properties of β -lactoglobulin, the main whey protein. In this study, the retention of phycocyanin utilizing a combination of GA, MD, and WPI ranged from 62.85% to 89.14%. These results are comparable to those reported by [Santana et al. \(2016\)](#), who observed retention values of 86.1%–95.1% (GA:MS:WPC) and 79.6%–91.0% (GA:MS:SPI) in microcapsules containing anthocyanin from palm fruits. Similarly, [Faieta et al. \(2020\)](#) reported retention values >70% for anthocyanin spray drying with MD and trehalose. [Diaz et al. \(2015\)](#) observed 71.62% retention using GA to encapsulate blackberry anthocyanins. [Charve and Reineccius \(2009\)](#) also noted a volatile retention rate of 87% when using WPI to encapsulate flavors during spray drying.

3.2.6 Solubility

Solubility is a key parameter used to assess the performance of the microcapsule powders upon reconstitution. High water solubility enables easier release of the active ingredients during applications. The RSM equation for optimizing the encapsulant material

levels concerning the solubility response is as follows:

$$Y_6 = -7.4 + 4.58 X_1 + 0.67 X_2 + 28.8 X_3 - 0.0459 X_1 * X_1 - 0.1273 X_2 * X_2 - 1.99 X_3 * X_3 + 0.0652 X_1 * X_2 - 0.902 X_1 * X_3 + 0.236 X_2 * X_3 \quad \text{Equation (6)}$$

Equation (6) indicates that the solubility was directly proportional to the concentrations of whey protein isolate in linear, quadratic effect of GA can decrease solubility and interaction between MD and WPI can increase solubility. Figure 6 illustrates the surface plot suggesting the relationship between these three encapsulant materials and their solubility. Solubility is influenced by the encapsulant type. Higher MD concentrations enhance solubility because MD can bind to hydrophobic compounds and is highly water-soluble, forming a uniformly dispersed solution system ([Ayu et al., 2016](#)). [Yuliawaty and Susanto \(2015\)](#) also noted that MD's hydroxyl groups interact with water during dissolution, resulting in elevated solubility levels as more free hydroxyl groups are available. WPI, as an effective emulsifier, helps suspend compounds in both the oil and water phases. This property can improve microcapsule dissolution when the WPI concentration increases ([Hasna et al., 2018](#)). A higher solubility value generally reflects better product quality and facilitates wider applicability.

[İlter et al. \(2021\)](#) reported that phycocyanin microcapsules encapsulated with MD and WPI had a solubility of $79.52 \pm 2.53\%$, while a combination of MD and GA yielded a lower solubility of $67.92 \pm 0.96\%$. However, in this study, a combination of GA, MD, and WPI resulted in solubility values >90%. This finding is consistent with that of [Mahdi et al. \(2020\)](#), who reported a solubility of $91.26 \pm 4.26\%$ for microencapsulated finger orange extracts using the same combination of wall materials. Similarly, [Santana et al. \(2016\)](#) observed elevated solubility values for palm fruit anthocyanin microcapsules produced with a blend of GA, MD, and WPI ($\text{GA:MS:WPC} = 1/6:2/3:1/6 = 93.5 \pm 2.9\%$ and $\text{GA:MS:SPI} = 1/6:2/3:1/6 = 92.8 \pm 2.7\%$).

3.2.7 Particle size

Microencapsulation is a technique that involves employing coatings to encapsulate microscopic particles, typically ranging from 1 to 1000 μm in size ([Lodhi et al., 2021](#)). The particle size has a remarkable impact on other microencapsulation characteristics. Smaller particles can lead to higher encapsulation efficiency, better particle morphology, and faster release rates. The RSM equation applied to optimize the encapsulant concentration for the particle size response

is as follows:

$$Y_7 = -242 + 62X_1 + 13.8X_2 - 34X_3 - 1.74X_1^2 - 0.78X_2^2 + 31.9X_3^2 + 3.25X_1X_2 - 9.03X_1X_3 - 9.91X_2X_3 \quad \text{Equation(7)}$$

Equation (7) shows that the particle size was directly proportional to GA and MD concentrations rise in linear, quadratic effect of WPI can increase particle size and interaction between GA and MD can increase particle size. **Figure 7** illustrates the surface plot representing the relationship between GA, MD, or WPI on the particle size response. The particle size is influenced by the type of encapsulant material. **Shamaei et al. (2017)** the effects of wall material formula and spray drying conditions on physicochemical properties of walnut oil microcapsules were investigated. Three different wall materials including skim milk powder (SMP) explained that variations in the molecular structure and physicochemical properties of encapsulant materials, such as surface activity and molecular weight, can affect particle size. GA, for instance, is highly viscous and forms a more viscous emulsion. **Fernandes et al. (2014)** found that GA as a wall material resulted in large particle sizes. **Jafari et al. (2008)** further explained that larger particles have higher encapsulation efficiency.

However, adding MD helps reduce the particle size because of its low viscosity at high concentrations. **İlter et al. (2021)** demonstrated that utilizing a combination of GA and WPI in phycocyanin microcapsules distributed the particles more homogeneously compared to MD and WPI. Phycocyanin microcapsules using a 50:50 combination of GA and MD produced particles with a size of 54.4 μm (**Pan-utai and Iamtham, 2020**), while MD and alginate produced a much smaller sized particle of 3.10 nm (**Kurniasih et al., 2018**). In this study, microcapsules composed of GA, MD, and WPI had small particle sizes ranging from 204.6–363.0 nm.

3.2.8. Optimization and Validation Results

The measurement results for each parameter were evaluated employing ANOVA, and polynomial regression equations were derived for each response. The optimization process aimed to identify the best combination of model parameters to achieve the desired outcomes. Each parameter was standardized simultaneously to produce a desirability value, representing the target or ideal response level. This desirability value reflects the relative importance of each response. The simultaneous desirability value obtained was 0.7656, determined using the Minitab software (<https://www.minitab.com/en-us/products/>

minitab/), based on the responses of the seven phycocyanin microcapsule parameters. This desirability value, ranging from 0.63 to 0.80, indicated that the results were acceptable. The optimal solution included 8.3% GA, 11.7% MD, and 5.2% WPI, producing microcapsules that met 76.56% of the desired target (desirability).

The next step was a verification test to confirm the values predicted by the Minitab software against the actual results under optimum conditions. It showed that the actual yield and antioxidant activity values exceeded the predicted ones. However, the existent values for particle size, phycocyanin content, solubility, encapsulation efficiency, and phycocyanin retention were slightly lower than the projected ones. The SD between the predicted and actual values ranged from 0.6781 to 5.2538, indicating that they were closely aligned. The verification results were within the 95% prediction and confidence intervals, confirming that the optimization was accurate and reliable. The response verification of the optimum formulation of phycocyanin microcapsules is shown in **Table 5**.

3.2.9 Morphology of the Microcapsules Fabricated Applying the optimized formula

The morphological characteristics of the optimized microcapsules were a round shape, a uniform size, a smooth surface, and no flocculation. Most microcapsules were round, with slight dents or indentations and a shrunken appearance. This surface shrinkage can result from the rapid evaporation of water during spray drying and is common in polymeric coatings derived from polysaccharides (**Purwaningsih et al., 2013**). Additionally, no clumping or cracking was observed. The particle shape can be influenced by the uneven shrinkage during drying and the enhanced surface protein content (**Hasrini et al., 2017**). According to **Tonon et al. (2009)**, surface shrinkage can occur due to low inlet temperatures, which slow down heat transfer and result in particles with more deformed crusts.

4. Conclusion

The concentrations of the three encapsulant ingredients (GA, MD, and WPI) in the phycocyanin microcapsules influenced the resulting characteristics. However, not all responses produced significant predictive models with a combination of these three ingredients. Simultaneous optimization identified the optimum encapsulant formulation for phycocyanin microcapsules: 8.3% GA, 11.7% MD, and 5.2% WPI. The desirability value of 0.7656 indicates that the optimum formulation can produce phycocyanin micro-

capsules meeting 76.56% of the desired targets. This value falls within the range of acceptable quality and suggests that the formulation can be effectively applied to the product.

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Author's Contribution

The contributions of each author were as follows: Puspa Pebriyanti carried out the experiment, collected data, analyzed the data for the research; Mrs. Siti Ari Budhiyanti as research coordinator, conceptualizing the idea, and designed the research; and Mrs. Nurfitri Ekantari conceptualizing the idea, designed the research, and analyzed the data. All authors wrote and contributed the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

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