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Microencapsulation of Phycocyanin from Spirulina platensis by Freeze-Drying: Optimization of Maltodextrin–Soy Protein Matrices for Enhanced Stability and Antioxidant Functionality

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Abstract. Phycocyanin, a natural blue pigment from Spirulina platensis, exhibits strong antioxidant activity but is highly unstable under light, heat, and pH variations, limiting its practical applications. This experimental study addresses the lack of systematic optimization data on maltodextrin–soy protein isolate (SPI) wall matrices for phycocyanin microencapsulation via freeze-drying. Phycocyanin was extracted using phosphate buffer and encapsulated at different maltodextrin:SPI ratios (9:1, 8:2, and 7:3). Each formulation was analyzed in triplicate (n = 3) for encapsulation efficiency (EE), phycocyanin retention, moisture content, particle size, and antioxidant activity (DPPH assay). The 8:2 ratio exhibited the best performance with EE of 88.5%, phycocyanin content of 0.710 mg·mL^{-1}, and particle size of 70.2 µm. Moderate antioxidant activity was observed (IC_{50} = 102.29 ppm). ANOVA confirmed that the polymer ratio significantly affected all parameters ($p < 0.05$). Overall, the optimized maltodextrin–SPI microcapsules enhanced the stability and antioxidant functionality of phycocyanin under laboratory conditions, supporting their potential application as bioactive ingredients in functional food and pharmaceutical formulations.

Keywords: freeze-drying, microencapsulation, maltodextrin–soy protein, functional ingredients

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1. Introduction

Spirulina platensis, a nutrient-rich blue–green microalga, is recognized as a promising source of bioactive compounds due to its high content of proteins, carbohydrates, essential fatty acids, vitamins, minerals, and pigments such as phycobiliproteins, particularly phycocyanin [1]. Phycocyanin is a natural blue pigment with remarkable antioxidant, anti-inflammatory, anticancer, and antidiabetic properties, making it highly valuable for food, pharmaceutical, cosmetic, and nutraceutical applications [2]

However, the industrial utilization of phycocyanin remains limited due to its poor chemical stability. The pigment is highly sensitive to temperature, light, pH, and oxygen, leading to denaturation and rapid color degradation [3]. Therefore, developing stabilization strategies to maintain its bioactivity during processing and storage is essential.

Among various preservation techniques, microencapsulation has proven effective in protecting unstable bioactive compounds from environmental degradation, enhancing stability, and prolonging shelf life [4]. Compared with liposomal or nanoencapsulation methods, freeze-drying (lyophilization) offers a simpler, solvent-free, and cost-effective approach suitable for heat-sensitive molecules such as phycocyanin [5]. Although spray drying is widely applied in industrial settings due to its scalability and low cost, it exposes the pigment to high thermal stress, often resulting in color fading and loss of activity [6]. In contrast, freeze-drying operates under low temperature and vacuum conditions, minimizing thermal denaturation, preserving color integrity, and maintaining protein stability [1].

The success of microencapsulation largely depends on the selection and proportion of wall materials. Maltodextrin is commonly used due to its neutral taste, high solubility, and good film-forming ability but has limited emulsifying properties [7]. To overcome this limitation, it is often combined with proteins such as soy protein isolate (SPI), which provides excellent emulsification, film formation, and compatibility with food and pharmaceutical matrices [8]. Notably, SPI is classified as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration, though potential allergenicity should still be considered during formulation [9].

Despite several studies on phycocyanin microencapsulation, few have systematically optimized maltodextrin–SPI ratios via freeze-drying and examined the resulting physicochemical and antioxidant characteristics [10]. Previous research has primarily focused on single-wall materials or spray-drying systems, leaving a gap in understanding how carbohydrate–protein combinations influence encapsulation efficiency and stability in freeze-dried matrices [11].

Therefore, this study aimed to optimize the maltodextrin–SPI ratio for phycocyanin microencapsulation via freeze-drying and to evaluate the resulting encapsulation efficiency, physicochemical stability, and antioxidant capacity of the microcapsules. It was hypothesized that an intermediate ratio of maltodextrin to SPI would yield improved encapsulation efficiency and stability due to balanced hydrophilic–hydrophobic interactions between wall materials. The findings are expected to provide a scientific basis for improving phycocyanin stability and broadening its applicability in functional food and pharmaceutical formulations.

2. Methods

2.1. Extraction and Purification of Phycocyanin

Phycocyanin was extracted from Spirulina platensis following the modified method of Bennett and Bogorad (1973). A total of 45 g of dry biomass was mixed with 900 mL phosphate buffer (pH 6.7; 1:20 w/v), prepared from solution A (7 g $Na_2HPO_4 \cdot H_2O$ in 250 mL distilled water) and solution B (9 g $Na_2HPO_4 \cdot 2H_2O$ in 250 mL distilled water), then diluted to 1 L. The mixture was sonicated using a probe sonicator (Qsonica Q500, USA) at 42 kHz and 50% amplitude for 23 min, with 10 s pauses between cycles to prevent overheating [12].

The homogenate was centrifuged using a refrigerated centrifuge (Eppendorf 5810R, Germany) at 3,220 × g (4000 rpm, rotor radius 10.8 cm) for 15 min at 4°C. The supernatant was treated with activated charcoal (2.5 g per 500 mL), stirred at 1000 rpm for 5 min, and centrifuged again under the same conditions for 10 min. The purified extract was filtered, and phycocyanin concentration and purity index were determined using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan) at 280, 620, and 652 nm. Calibration curves ($R^2 > 0.999$) were prepared by serial dilution, and the LOD and LOQ were established using signal-to-noise ratios of 3:1 and 10:1, respectively.

2.2. Preparation of Phycocyanin Microcapsules

Phycocyanin microcapsules were prepared using the freeze-drying technique (Labconco FreeZone 6, USA). The total concentration of wall materials was fixed at 10% (w/v) relative to the solvent volume. Maltodextrin (MD) and soy protein isolate (SPI) served as wall materials in five ratios: F1 (10:0 MD:SPI), F2 (9:1), F3 (8:2), F4 (7:3), and F5 (6:4). Phycocyanin solution was homogenized with wall materials using a high-speed homogenizer (IKA T25 Ultra-Turrax, Germany) at 10,000 rpm for 10 min. The mixture was centrifuged at 3,220 × g for 10 min to remove air bubbles, frozen at –80°C for 24 h, and subsequently freeze-dried through three sequential phases: Freezing phase at –40°C for 6 h, Primary drying (sublimation) at –50°C and 0.040 mbar for 72 h, and Secondary drying at 25°C and 0.010 mbar for 24 h. The resulting microcapsules were stored in airtight amber glass bottles at 4°C until further analysis [13].

2.3. Quantification of Phycocyanin Content and Purity

For liquid extracts, 0.2 mL of purified phycocyanin was diluted to 25 mL with phosphate buffer (pH 6.7), further diluted 50-fold, and analyzed at 280, 620, and 652 nm using the Bennett and Bogorad (1973) equations:

$$C = \frac{A_{620} - 0.7\,A_{652}}{\varepsilon}$$

Where A_{620} and A_{652} are the absorbance values at 620 nm and 652 nm, respectively, and ε specific extention coefficient of phycocyanin. The purity index (PI) of phycocyanin was determined using the ratio:

$$PI = \frac{A_{620}}{A_{280}}$$

Where A_{280} represents the absorbance of aromatic amino acids and proteins. A higher PI value indicates higher phycocyanin purity. The encapsulation yield (%) was calculated as:

For microcapsules, 40 mg of sample was dissolved in 10 mL phosphate buffer, vortexed, diluted 25-fold, and measured similarly. All measurements were performed in triplicate (n = 3) and expressed as mean ± standard deviation (SD).

2.4. Moisture Content Determination

Approximately 1.0 g of phycocyanin microcapsules was placed in a pre-dried porcelain crucible and dried in an oven (Memmert ULE400, Germany) at 105°C for 5 h. The sample was weighed every hour until a constant weight was achieved. Moisture content (%) was calculated based on the difference in mass before and after drying. Each analysis was performed in triplicate (n = 3).

2.5. Encapsulation Efficiency (EE)

Encapsulation efficiency (%) was calculated by comparing the phycocyanin content after encapsulation ($C_{encapsulated}$) to the initial phycocyanin content before encapsulation ($C_{initial}$), using the following formula:

$$EE(\%) = \frac{C_{encapsulated}}{C_{initial}} \times 100\%$$

An EE value ≥80% indicates efficient encapsulation (almiahsari et al2019

2.6. Antioxidant Activity (DPPH Radical Scavenging Assay)

Antioxidant activity was determined using the DPPH radical scavenging method described by Rahmawati et al. (2017) with slight modifications. A stock solution (250 ppm) was prepared by dissolving 25 mg of microcapsules in 10 mL phosphate buffer (pH 6.7) and diluting to 100 mL with methanol (1:9 buffer:methanol). Serial dilutions (25–125 ppm) were prepared and mixed with an equal volume of 0.4 mM DPPH in methanol. Samples and the vitamin C standard were incubated in the dark at 25°C for 30 min, and absorbance was measured at 514 nm [14]. The percentage inhibition was calculated using:

$$\%\ Inhibition = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined by linear regression analysis Statistical Analysis

Encapsulation efficiency shows the percentage of fycocyanin successfully trapped in the microcapsule matrix. This value is calculated based on the ratio between the phycocyanin content after the encapsulation process and the phycocyanin content before the encapsulation process, then multiplied by 100%. According to Almiahsari et al. (2019), an efficiency value of ≥ 80% indicates good encapsulation efficiency.

2.7. Statistical Analysis

All experiments were conducted in triplicate (n = 3). Results were expressed as mean ± standard deviation (SD). Data were analyzed using SPSS v20.0. Normality and homogeneity were assessed using the Shapiro–Wilk and Levene's tests, respectively. One-way ANOVA followed by Tukey's post-hoc test was used to determine significant differences among formulations. The F-value, degrees of freedom, and exact p-values were reported, with significance accepted at $p < 0.05$.

3. Results and Discussion

Phycocyanin, a polar pigment, was extracted from Spirulina platensis using phosphate buffer (pH 6.7). The extracted phycocyanin concentration was 0.80 ± 0.002 mg/mL, with a purity index of 0.26 ± 0.002, which is below the food-grade standard (≥0.4). The purity index, calculated from the A_{620}/A_{280} ratio, reflects the proportion of phycocyanin relative to other proteins. This result was higher than that obtained using distilled water [15], but lower than those achieved by ultrasonication or freeze–thaw methods [16]. The A_{620}/A_{280} ratio also reflects extract stability and clarity, as overlapping pigments such as allophycocyanin absorb near 652 nm.

The relatively low purity is likely due to partial adsorption of phycocyanin by activated charcoal during purification. It may also be attributed to the structural fragility of phycocyanin under pH variations and ionic interactions, which could cause partial denaturation during extraction.

Optimization of purification strategies, such as membrane ultrafiltration, could improve pigment recovery and purity.

Table 1. Impact of maltodextrin – soy protein ratios on microcapsule caracteristics

Maltodextrin : SPI (%)	Phycocyanin content (mg/mL)	Yield (mg/g)	Moisture content (%)	Encapsulation efficiency (%)	Size distribution (Span)	Particle size (μm)
10 : 0	0.602 ± 0.01^{c}	14.160 ± 0.12^{b}	7.8 ± 0.05^{a}	75.1 ± 0.08^{b}	3.043 ± 0.09^{b}	61.4 ± 0.06^{d}
9 : 1	0.624 ± 0.02^{c}	14.607 ± 0.10^{b}	6.0 ± 0.03^{ab}	77.8 ± 0.07^{b}	3.245 ± 0.11^{b}	64.4 ± 0.04^{c}
8 : 2	0.710 ± 0.01^{a}	16.822 ± 0.09^{a}	6.0 ± 0.02^{ab}	88.5 ± 0.06^{a}	2.615 ± 0.08^{a}	70.2 ± 0.03^{b}
7 : 3	0.666 ± 0.02^{b}	12.429 ± 0.11^{c}	5.7 ± 0.03^{b}	83.1 ± 0.05^{ab}	2.428 ± 0.10^{a}	106.7 ± 0.05^{a}
6 : 4	0.610 ± 0.01^{c}	12.066 ± 0.12^{c}	3.6 ± 0.04^{c}	76.1 ± 0.08^{b}	3.823 ± 0.12^{c}	93.6 ± 0.04^{ab}

Values are mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences ($p < 0.05$, ANOVA followed by Duncan's multiple range test).

The impact of maltodextrin–soy protein isolate (SPI) ratios on microcapsule characteristics is summarized in Table 1. Significant differences ($p < 0.05$) were observed across formulations for most parameters, confirming that wall composition strongly influences encapsulation performance.

The phycocyanin content in the microcapsules ranged from 0.602 to 0.710 mg/mL, with the highest value observed at the 8:2 ratio and the lowest at 10:0. ANOVA analysis confirmed that wall material composition had a significant effect ($p < 0.05$). Compared with previous studies, the phycocyanin content obtained in this study was lower than that reported by Purnamayati et al. (2016) [17], who achieved 1.729 mg/mL using maltodextrin–carrageenan via spray drying, and Kurniasih et al. (2018) [7], who reported 2.05–2.42 mg/mL using maltodextrin–alginate. This difference is likely due to pigment loss during sublimation in freeze-drying. However, the absence of heat degradation makes freeze-drying more suitable for thermolabile pigments, as it better preserves phycocyanin bioactivity for functional food and pharmaceutical applications.

Although the differences were not substantial, the use of dual wall materials (maltodextrin–SPI) proved more effective in maintaining phycocyanin stability than using a single component. Furthermore, the use of phosphate buffer during extraction improved pigment recovery by maintaining pH stability and promoting ionic interactions, enhancing solubility and pigment integrity compared with extraction using distilled water.

Moisture content is a critical parameter determining the stability and shelf life of microcapsules. According to SNI 01-4320-1996, the optimal moisture content for powdered products ranges between 3–5% [18]. As shown in Table 1, the moisture content of the microcapsules ranged from 2.70% to 9.60%, with the lowest value observed in the 6:4 formula and the highest in 10:0. Among all formulations, only the 6:4 sample met the SNI standard. ANOVA indicated that variations in the maltodextrin–SPI ratio had no significant effect on moisture content ($p = 0.236$), so no further Duncan's test was conducted.

Differences in moisture content may arise from the hygroscopic nature of the wall materials and minor inconsistencies during freeze-drying. SPI possesses hydrophilic groups capable of binding water, effectively reducing residual moisture [19]. Lower moisture content is advantageous as it enhances storage stability and prevents oxidative degradation of phycocyanin during shelf life [20].

Encapsulation efficiency (EE) indicates the proportion of active compound successfully entrapped within the wall matrix [10]. As shown in Table 1, the EE of phycocyanin microcapsules ranged from 72.3% to 89.8%, with the highest at the 8:2 ratio and the lowest at 10:0. These values demonstrate relatively high encapsulation performance, exceeding those reported [7], who found 29.74–40.74% using maltodextrin–alginate. ANOVA results confirmed a significant effect of wall material composition on EE ($p = 0.012$).

The improvement in EE with SPI addition can be attributed to its surface-active properties, which enhance pigment entrapment within the capsule matrix. The interaction between SPI and maltodextrin creates a denser, more protective shell that minimizes pigment leakage during freeze-drying. Additionally, low-temperature processing (–67 °C) helps maintain phycocyanin stability, minimizing degradation and improving encapsulation efficiency [21,22].

Particle size and distribution are key parameters influencing the stability and performance of microcapsules. In this study, particle sizes ranged from 61.4 to 106.7 μm, within the microparticle scale. The 7:3 formula produced the largest particles (106.7 μm), whereas the 10:0 formula produced the smallest (61.4 μm). Increasing SPI content generally led to larger particles due to the formation of a thicker and denser wall structure [23].

Span values ranged from 2.116 to 3.823, indicating generally heterogeneous size distributions, except for 7:3 (span = 2.428), which showed greater uniformity. According to Caetano et al. (2016), span values ≤ 2.5 indicate narrow distributions, whereas > 2.5 indicate heterogeneity. ANOVA confirmed a significant influence of wall material ratio on particle size distribution ($p < 0.05$). Variations may result from aggregation, uneven mixing, or instability during freeze-drying.

Smaller and more uniform particles are typically preferred for controlled-release applications, while larger particles provide better protection for sensitive bioactives. Considering both size and uniformity, the 8:2 formula represented the most balanced formulation, combining optimal particle size (≈ 70.2 μm), moderate homogeneity, and enhanced encapsulation stability.

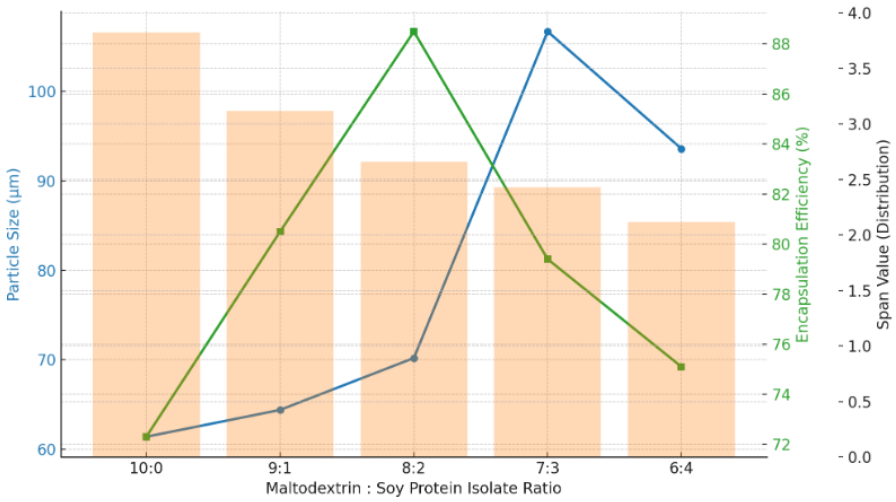

Figure 1. Relationship between particle size, encapsulation efficiency (EE), and span values of phycocyanin microcapsules at different maltodextrin-to-soy protein isolate ratios.

A non-linear relationship was observed between particle size and EE (Figure 1). Moderate particle sizes (≈ 70 μm) at an 8:2 maltodextrin-to-SPI ratio yielded the highest EE (88.5%). Excessive SPI content (> 30%) resulted in larger particles (> 90 μm) but lower EE, likely due to structural imperfections and wall rupture during freeze-drying. Conversely, insufficient SPI content (< 65 μm) produced smaller particles with weaker protective capacity. Thus, the 8:2 formulation offered the optimal balance between particle size and encapsulation efficiency, ensuring improved pigment protection

Following comprehensive evaluation of physicochemical properties, the 8:2 formulation exhibited superior performance and was selected for antioxidant testing using the DPPH radical scavenging assay.

Table 2. IC_{50} Value of Vitamin C and Phycocyanin Microcapsules (8:2)

Sample	Concentration (ppm)	Abs	Inhibition (%)	IC_{50} (ppm)	Category
Vitamin C	2	0.534	37.98	6.91 ± 0.07	very strong
	4	0.490	43.09		
	6	0.450	47.74		
	8	0.409	52.50		
	10	0.365	57.61		
Phycocyanian microcapsule (8:2)	25	0.586	31.94	102.29 ± 0.08	moderate
	50	0.532	38.21		
	75	0.479	44.37		
	100	0.436	49.36		
	125	0.388	54.94		

The DPPH method was used to evaluate the antioxidant potential of the samples at a wavelength of 514 nm. Phycocyanin microcapsules demonstrated 31.94–54.94% inhibition, while vitamin C showed 37.98–57.61%. The IC_{50} value for phycocyanin microcapsules was 102.29 ± 0.08 ppm (moderate activity), whereas vitamin C exhibited 6.91 ± 0.07 ppm (very strong activity).

Encapsulation significantly enhanced antioxidant activity compared with unencapsulated phycocyanin reported by Nurzanah et al. (2017) (IC_{50} = 209.30 ppm) [24]. This suggests that microencapsulation protects phycocyanin's chromophore groups from oxidative damage, thereby maintaining its radical-scavenging capacity. Lower IC_{50} values indicate stronger antioxidant potential [14]. Furthermore, the preservation of antioxidant activity confirms that freeze-drying combined with soy protein isolate effectively stabilizes phycocyanin. These findings demonstrate that the maltodextrin–SPI matrix offers a promising strategy for stabilizing phycocyanin for applications in functional foods, pharmaceuticals, and cosmetics. Future optimization should focus on improving pigment retention while achieving moisture levels compliant with industrial standards.

4. Conclusion

This study demonstrated that the microencapsulation of phycocyanin extracted from Spirulina platensis using a maltodextrin–soy protein isolate (SPI) matrix through the freeze-drying method effectively enhanced pigment stability and antioxidant activity. The formulation with a maltodextrin-to-SPI ratio of 8:2 exhibited the best performance, characterized by the highest encapsulation efficiency (88.5%), optimal moisture content (6%), and particle size of approximately 70 μm, contributing to improved antioxidant activity with an IC_{50} value of 102.29 ppm. The combination of maltodextrin and SPI formed a compact and protective microcapsule wall, minimizing pigment degradation and maintaining the stability of heat-sensitive bioactive compounds. For future research, it is recommended to conduct morphological analysis (SEM), molecular interaction studies (FTIR), and long-term stability testing to further elucidate the mechanism and enhance the industrial applicability of this encapsulation system.

Declaration of AI and AI assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT (OpenAI) in order to assist in language editing and structuring the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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