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Freeze-Dried Phycocyanin Microcapsules: Effect of Maltodextrin-Soy Protein Ratios on Encapsulation Efficiency and Particle Properties

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Abstract. Natural pigments such as phycocyanin are highly sensitive to environmental conditions and therefore require stabilization to broaden their applications in functional products. This study aimed to evaluate the effect of varying ratios of maltodextrin and soy protein isolate (SPI) on the physical properties and encapsulation performance of freeze-dried phycocyanin microcapsules. Phycocyanin was extracted using phosphate buffer and encapsulated in three formulations with different maltodextrin–SPI proportions. The obtained microcapsules were characterized using UV–Vis spectrophotometry and particle size analysis (PSA) to determine encapsulation efficiency (EE), yield, moisture content, purity index, and particle size distribution. All experiments were performed in triplicate ($n = 3$), and data were analyzed using one-way ANOVA ($p < 0.05$). The formulation with a higher proportion of maltodextrin exhibited the best performance, achieving high EE (≈96%), low moisture content, and uniform particle size. These findings highlight that optimizing biopolymer combinations can enhance the quality and stability of phycocyanin microcapsules, demonstrating potential applications in functional food and pharmaceutical formulations.

Keywords: microencapsulation, phycocyanin, freeze-drying, maltodextrin, soy protein isolate, biopolymers

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1. Introduction

Spirulina platensis is a microalga widely recognized for its nutritional and functional potential in food, pharmaceutical, and cosmetic industries due to its rich composition of proteins, vitamins, minerals, and the blue pigment phycocyanin [1]. Phycocyanin exhibits various biological activities including antioxidant, anticancer, antidiabetic, and immunostimulant effects[2]. However, despite its broad bioactivity, its industrial application remains limited because of its poor stability under environmental stress such as light, temperature, and pH fluctuations, leading to rapid degradation and loss of activity [3].

Microencapsulation is a promising strategy to overcome this instability by coating active compounds with a biopolymeric matrix, thereby protecting them from degradation, improving stability, and enhancing bioavailability [4]. Compared with advanced delivery systems such as liposomes or nanoencapsulation, microencapsulation offers simplicity, scalability, and cost-effectiveness for industrial applications [5]. Among available techniques, freeze-drying is particularly suitable for thermolabile compounds such as phycocyanin, as it avoids high-temperature degradation while maintaining functional integrity [6].

Selecting an appropriate wall material is a critical factor for successful encapsulation. Maltodextrin is frequently used due to its low cost, neutral taste, and excellent solubility [7]. However, its limited emulsifying capacity may lead to inadequate protection of sensitive bioactives [8]. To address this limitation, combinations of maltodextrin with proteins or gums (e.g., casein, alginate, gum arabic) have shown improved encapsulation stability and retention of active compounds [9], [10].

Soy protein isolate (SPI) represents an attractive complementary wall material due to its good water solubility, film-forming and emulsifying properties, and its Generally Recognized as Safe (GRAS) status for human consumption [7]. Nevertheless, SPI may contain potential allergens related to soy-derived proteins such as glycinin and β-conglycinin, which should be considered in food applications requiring allergen labeling [1].

Up to 2024, only approximately five studies have reported the use of maltodextrin–SPI combinations for the encapsulation of phycocyanin or other natural pigments [10], [11].These works demonstrated that incorporating a protein component can enhance microcapsule integrity and encapsulation efficiency. However, limited research has systematically evaluated the optimization of maltodextrin, SPI ratios specifically for freeze-dried phycocyanin systems.

Therefore, this study focuses on optimizing the maltodextrin–SPI ratio in the freeze-drying encapsulation of phycocyanin to enhance encapsulation efficiency, particle properties, and pigment stability. The findings are expected to contribute to the development of stable, functional, and safe natural colorant formulations for use in food, nutraceutical, and pharmaceutical applications.

2. Methods

2.1. Extraction and Purification of Phycocyanin

A total of 450 g dried Spirulina platensis powder was extracted using 0.1 M phosphate buffer (pH 6.7) with a biomass-to-solvent ratio of 1:20 (w/v). Extraction was conducted with an ultrasonic sonicator (Biobase UCD-250) at 42 kHz frequency, 50% amplitude (~150 W) for 22.5 min, with 10 s on/off pulses to enhance cell disruption. The temperature was maintained below 30 °C using an ice bath during sonication. The extract was centrifuged using a centrifuge (Oregon LS-04S) at 4000 × g (radius 12.4 cm) for 15 min at 4 °C, and the supernatant was collected. Purification was carried out by adding 2.5 g of activated charcoal per 500 mL of extract, stirred at 1000 rpm for 5 min, followed by a second centrifugation (4000 × g, 10 min). The supernatant was filtered through Whatman No. 1 filter paper to obtain purified phycocyanin. The concentration and purity of phycocyanin were determined using a UV–Vis spectrophotometer (Shimadzu UV-1800) at 280, 620, and 652 nm, following the method of Bennett and Bogorad (1973) [12].

2.2. Preparation of Phycocyanin Microcapsules

Phycocyanin microcapsules were prepared using maltodextrin and soy protein isolate (SPI) as encapsulating agents at three different ratios (w/w): F1 (100% maltodextrin), F2 (75% maltodextrin + 25% SPI), and F3 (50% maltodextrin + 50% SPI). The encapsulant powders were dissolved in 100 mL of purified phycocyanin solution and homogenized using a rotor–stator homogenizer (IKA T25 Ultra-Turrax, Germany) at 10,000 rpm for 5 min at room temperature. The resulting emulsions were pre-frozen at –80 °C for 24 h, followed by freeze-drying using a BenchTop Pro with Omnitronics (USA) at a shelf temperature of –67 °C and chamber pressure of 0.01 mbar for 96 h. The obtained dry microcapsule powders were collected, sealed in amber glass vials, and stored at 4 °C until further analysis.

2.3. Determination of Phycocyanin Content, Purity, and Yield

Phycocyanin content, purity, and yield were quantified using a UV–Vis spectrophotometer. For the purified extracts, 0.2 mL of phycocyanin extract was diluted to 25 mL using phosphate buffer (pH 6.7), thoroughly mixed, and its absorbance measured. For the microcapsules, 40 mg of phycocyanin microcapsules were dissolved in 10 mL of phosphate buffer, vortexed until completely homogenized, and analyzed. The absorbance of all samples was measured at wavelengths of 280 nm, 620 nm, and 652 nm. Phycocianin concentration (C, mg/mL) was calculated using following equation:

$$C = \frac{A_{620} - 0.7\,A_{652}}{\varepsilon} \quad (1)$$

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:

$$PI = \frac{A_{620}}{A_{280}} \quad (2)$$

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:

$$Yield(\%) = \frac{C_m}{C_e} x100 \quad (3)$$

Where C_m is the phycocyanin content in the microcapsules and C_e is the initial phycocyanin content in the extract.

2.4. Moisture Content Analysisis

Moisture content was determined gravimetrically. Approximately 1 g of microcapsules was placed in a pre-weighed porcelain crucible and dried at 105 °C for 5 h. The crucible was weighed every hour until a constant weight was achieved. Moisture content (%) was calculated as:

$$Moisture(\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} x100 \quad (4)$$

2.5. Particle Size and Distribution Analysis

Particle size distribution was determined using a Particle Size Analyzer (PSA) (Mastersizer 3000, Malvern Instruments, UK), based on the principle of laser diffraction (10). Samples (3 g) were dispersed with compressed air (0.1–4 bar) and measured across a size range of 0.01–3500 µm using a 633 nm red laser. The span value, indicating distribution width, was calculated using:

$$Span = \frac{D_{90} - D_{10}}{D_{50}} \quad (5)$$

Where D_{90}, D_{50}, and D_{10} represent particle diameters at 90%, 50%, and 10% cumulative volume, respectibely.

2.6. Encapsulation efficiency (EE)

Encapsulation efficiency was determined spectrophotometrically as the ratio of phycocyanin retained after encapsulation to the initial phycocyanin before encapsulation. Approximately 40 mg of

microcapsule powder was dissolved in 10 mL of phosphate buffer (pH 6.7), vortexed for 1 min, and filtered prior to absorbance measurement. EE (%) was calculated as :

$$EE = \frac{Phycocyanin\ content\ after\ encapsulation}{Phycocyainin\ content\ before\ encapsulation}\ x\ 1 \quad (6)$$

This parameter reflects the protective ability of the encapsulant agains pigmnet degradation during freeze-drying.

2.7. Study Limitations

Due to facility constraints, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) analyses were not conducted. Instead, particle morphology and structural characteristics were inferred from particle size distribution data and freeze-drying process parameters. These advanced characterizations are planned as part of future follow-up studies to further elucidate the structural and chemical interactions between phycocyanin and biopolymer matrices.

2.8. Statistical Analysisi

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS v20 (IBM Corp., USA). Normality and homogeneity of variances were confirmed using Shapiro–Wilk and Levene's tests, respectively. Data were analyzed using one-way ANOVA, and significant differences among formulations were identified using Duncan's multiple range test at a 95% confidence level ($p < 0.05$).

3. Results and Discussion

The physicochemical characteristics of phycocyanin microcapsules produced using maltodextrin and soy protein isolate (SPI) at various ratios are summarized in Table 1. Statistical analysis using one-way ANOVA ($p < 0.05$) followed by Duncan's multiple range test indicated that the polymer ratio significantly affected several parameters, including phycocyanin content, yield, moisture, and encapsulation efficiency, while the effects on purity index, span, and particle size were not statistically significant.**Table 1. evaluation of phycocyanin microcapsule formula characteristics**

Maltodextrin : Soy Protein Isolate (%)	Purity index	Yield (mg/g)	Moisture content (%)	Encapsulation efficiency (%)	Size distribution (Span)	Particle size (µm)
10 : 0	0.26 ± 0.02[a]	16.29 ± 0.31[a]	5.77 ± 0.21[a]	93.59 ± 1.85[a]	3.007 ± 0.05[a]	93.33 ± 2.10[a]
7.5 : 2.5	0.20 ± 0.01[a]	16.82 ± 0.27[a]	4.73 ± 0.19[b]	96.65 ± 1.06[a]	2.663 ± 0.03[a]	77.93 ± 1.80[b]
5 : 5	0.17 ± 0.01[b]	14.78 ± 0.24[b]	2.78 ± 0.13[c]	85.89 ± 1.30[b]	2.566 ± 0.02[a]	83.80 ± 1.90[a]

Values within the same column followed by different superscript letters (a, b, c) differ significantly at $p < 0.05$ based on Duncan's multiple range test.

The phycocyanin content in the microcapsules ranged from 1.583 to 1.783 mg·mL⁻¹, with the highest concentration obtained from the 7.5:2.5 formulation (F2). This value is comparable to that reported (1.729 mg·mL⁻¹) using maltodextrin–carrageenan by spray drying, but lower than the findings of other [13] (2.05–2.42 mg·mL⁻¹; maltodextrin–alginate). The differences may arise from distinct wall materials and drying methods, as phycocyanin is highly sensitive to heat [14]. Freeze-drying, as used in this study, minimizes pigment degradation by preserving protein conformation, consistent with report [10]. ANOVA results confirmed that polymer ratio significantly influenced phycocyanin concentration ($p = 0.00$), while Duncan's test showed that F1 (10:0) and F2 (7.5:2.5) were not significantly different, but

both differed from F3 (5:5), which had a lower value. The decline in phycocyanin content at high SPI levels is attributed to increased solution viscosity, which interferes with droplet formation and pigment entrapment during homogenization.

The yield of microcapsules ranged from 14.78 to 16.82 $mg \cdot g^{-1}$, increasing with decreasing SPI proportion up to 25%. The highest yield (16.82 $mg \cdot g^{-1}$) was obtained in F2, indicating efficient encapsulation and solid matrix formation. These results are higher than those reported [11] (11.37–16.14 $mg \cdot g^{-1}$; palm oil with maltodextrin–SPI) and [15] (5.4–13.6 $mg \cdot g^{-1}$). Higher yields are generally associated with the stronger film-forming and water-binding capacities of maltodextrin, which enhance drying efficiency [11]. ANOVA revealed a significant effect ($p = 0.02$), where F1 and F2 did not differ significantly, while F3 yielded the lowest value. This trend indicates that excessive SPI may reduce product recovery due to aggregation or incomplete solidification during freeze-dryin [16]

The purity index (A_{620}/A_{280}) values ranged between 0.17 and 0.26, decreasing slightly with increasing SPI ratio. Statistical analysis indicated no significant differences among formulations ($p > 0.05$), showing that the purification level of phycocyanin in all samples was relatively consistent. The obtained purity levels were lower than those reported [2] (0.57–0.80), [17] (1.61), and [18] (0.7). Industrially, food-grade phycocyanin typically requires a purity index ≥ 0.7, while analytical grade exceeds 4. The lower values found here suggest partial co-encapsulation of SPI protein, which absorbs at 280 nm, or minor pigment denaturation during drying [18].

The moisture content of the microcapsules ranged between 2.78% and 5.77%, indicating good drying efficiency and stability. The lowest moisture (2.78%) was observed in F3 (5:5), while F1 (10:0) showed the highest (5.77%). These values meet the SNI 0432:1996 standard for powder products (3–5%) [19] except for F1. ANOVA indicated a significant difference ($p = 0.05$), where F1 and F2 were similar, but F3 differed significantly. Who found that higher maltodextrin levels reduce moisture, the current findings suggest that the presence of SPI enhances water binding due to its hydrophilic amino acid residues [20]. The strong protein–water interactions create a more compact matrix and lower residual moisture. Moreover, the low processing temperature of freeze-drying effectively removes free water through sublimation [21].

The encapsulation efficiency (EE) was notably high, ranging from 85.89% to 96.65%, exceeding the minimal acceptable threshold of 80%. ANOVA confirmed a significant influence of the polymer ratio ($p = 0.00$), with Duncan's test showing that F1 and F2 were not significantly different, but both differed from F3, which recorded the lowest EE. The superior EE in F2 can be attributed to the synergistic combination of maltodextrin and SPI, where maltodextrin provides a dense carbohydrate matrix and SPI acts as an emulsifier and film-forming agent [7]. The efficiency achieved here surpasses those reported [13] (29.74–40.74%) and (2022) (37.79%) but aligns with other reported [22] (93–98.28%). The use of a freeze-drying temperature of –67 °C contributed to this high efficiency by preventing thermal degradation of phycocyanin protein [10].

The particle size of the resulting microcapsules ranged from 77.93 to 93.33 μm, consistent with the typical 20–5000 μm range for freeze-dried powders .[19]. ANOVA indicated no significant differences ($p > 0.05$), demonstrating that the encapsulation process produced relatively uniform particles. Although F1 (10:0) produced the largest particles (93.33 μm) and F2 the smallest (77.93 μm), the differences were minor. The observed trend suggests that increasing SPI concentration up to 25% improves emulsion stability and facilitates finer droplet formation, whereas higher protein levels (50%) may increase viscosity and cause droplet coalescence [23]. Compared with other report [24], who reported particle sizes between 181.4 and 688.1 μm using maltodextrin–alginate, the current results demonstrate finer and more homogeneous microcapsules, favorable for improved dispersibility and controlled release.

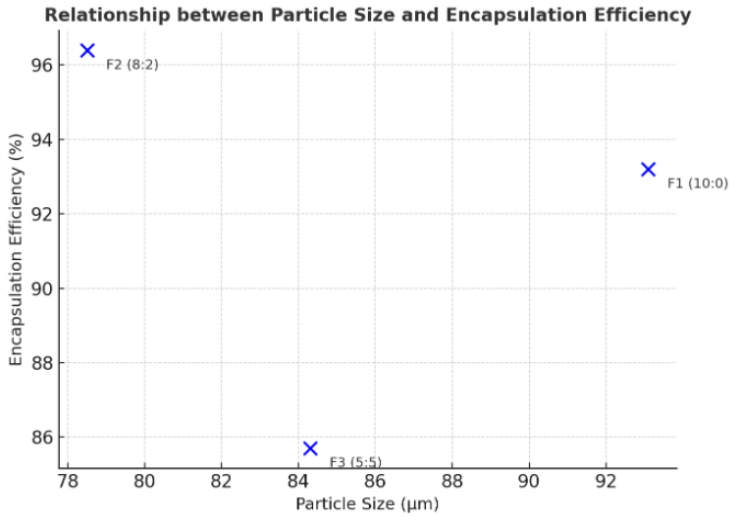

Figure 1. Relationship between Particle Size and Encapsulation Efficiency of Phycocyanin Microcapsule using maltodextrin and soy protein isolate with various concentration ratio.

The inverse relationship between particle size and EE is consistent with the findings of who reported that smaller and more uniform particles facilitate the formation of a compact matrix, leading to better encapsulation and protection of bioactive compounds. Increased SPI levels, however, raised the viscosity of the wall material, producing larger droplets during homogenization and less efficient pigment entrapment [25].

Although morphological and structural analyses such as scanning electron microscopy (SEM) or Fourier-transform infrared spectroscopy (FTIR) were not conducted due to facility limitations, prior studies indicate that freeze-dried maltodextrin–SPI systems form spherical, smooth microcapsules with compact walls, consistent with high encapsulation efficiency. Future studies will include these analyses to confirm surface characteristics and molecular interactions [25].

No accelerated stability or antioxidant activity tests were performed in this study. However, the high EE and low moisture content suggest that the microcapsules possess good potential stability. Future investigations will evaluate storage stability under varying temperatures (25 °C and 40 °C) and light exposure, along with antioxidant retention using DPPH and ABTS assays.

Overall, these findings confirm that the 7.5:2.5 formulation (F2) achieved the best balance between yield, moisture, and encapsulation efficiency while maintaining acceptable purity and particle uniformity. The synergistic interaction between maltodextrin and SPI enhanced structural integrity and pigment stability, supporting the effectiveness of this biopolymer combination in protecting phycocyanin during freeze-drying.

4. Conclusion

Phycocyanin was successfully encapsulated using maltodextrin and soy protein isolate through freeze-drying. The 75:25 formulation showed the best performance, achieving 96.65% encapsulation efficiency, 16.82 mg/g yield, 4.73% moisture, and 77.93 µm particle size. These findings confirm that optimizing the maltodextrin–SPI ratio improves the physicochemical quality and stability of phycocyanin microcapsules. These results apply only to the freeze-drying conditions and polymer ratios tested. Further characterization using SEM and FTIR is recommended to verify the surface morphology and molecular interactions within the matrix. Future studies should also evaluate storage stability, antioxidant retention, and process scale-up to strengthen the applicability of this encapsulation system for functional food and pharmaceutical use.

Declaration of AI and AI assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language improvement, academic writing refinement, organization of the manuscript, and grammar checking. The AI tool was also used to help summarize scientific information and improve the clarity of the text. After using this tool, the authors carefully reviewed, verified, and edited all generated content as necessary and take full responsibility for the accuracy, originality, and integrity of the final 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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