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Original Article

Sustainable production and encapsulation of antioxidant-rich phycocyanin from novel cyanobacterium *Leptolyngbya* sp. CCAH036/1 for functional food innovation

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Abstract

Phycocyanin is a blue pigment–protein with antioxidant properties, but its use in foods is limited by poor stability. A cyanobacterium, *Leptolyngbya* sp. CCAH036/1, was isolated from the Can Gio Mangrove Biosphere Reserve, Vietnam, and identified by morphology and 16S rRNA sequencing (96.5% similarity to the closest reference). C-phycocyanin was extracted by freeze–thaw and lysozyme treatment and then encapsulated with maltodextrin using spray drying. The optimized powder contained 20.53 mg/g phycocyanin, 5.14% moisture, 79.43% encapsulation efficiency, and 56.66% antioxidant retention. After three weeks of storage at 4 °C, both pigment content and antioxidant activity remained above 80%. Heating at 50–70 °C preserved about half of the activity, but stronger heat caused rapid decline. Stability was also greatest at pH 5–7. The powder was added to sticky rice mooncakes at 5–20%. At 15% supplementation, the cakes contained 1.874 mg/g phycocyanin and 43.78% antioxidant activity, with no loss of texture or sensory quality. The results indicate that spray drying with maltodextrin is recommended as an effective approach to stabilize phycocyanin from the local *Leptolyngbya* strain for incorporation into functional foods processed at moderate temperatures and near-neutral pH.

Keywords: Phycocyanin, *Leptolyngbya* sp., Microencapsulation, Spray-drying, Antioxidant, Mooncake

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Introduction

Phycocyanin (C-phycocyanin, C-PC) is a blue phycobiliprotein widely studied for food, nutraceutical, and cosmetic uses because of its antioxidant and anti-inflammatory activities (Wu et al., 2016b; Kannaujiya and Sinha, 2016), yet its poor thermal and pH stability still constrains adoption in processed foods. Currently, commercial supply relies primarily on *Arthrospira platensis* (*Spirulina*), whose large-scale cultivation in ponds or photobioreactors demands substantial water and nutrients, and whose pigment degrades rapidly under heat, acidic pH, or prolonged storage (Pradeep and Nayak, 2019; Hadiyanto et al., 2019).

Cyanobacteria of the genus *Leptolyngbya* have been reported as alternative producers of phycocyanin (Schipper et al., 2020; Mahanil et al., 2021). They occur on soils and subaerial surfaces and tolerate broad variation in temperature, moisture, and nutrient availability, enabling lower-input cultivation than *Arthrospira* (Singh et al., 2014; Gongi et al., 2022). Some strains also accumulate high pigment levels (for example, *Leptolyngbya* sp. QUCCCM 56 reached ~8.6% of dry weight, whereas *Arthrospira* typically ranges ~5–17%), yet food applications remain limited (Gongi et al., 2022; Gallina et al., 2024).

Enhancing the physicochemical stability of CPC remains a major challenge. Recent reviews summarize encapsulation strategies (e.g., spray-drying, extrusion) and wall materials (maltodextrin, carrageenan, chitosan, whey protein, sodium alginate) that enhance resistance to heat, pH, and storage (Hadiyanto et al., 2019; Agustina et al., 2019; Iqbal and Hadiyanto, 2020; İlter et al., 2021; Qiao et al., 2022). More recently, advanced approaches have focused on engineering hydrogen bonding, electrostatic attraction, and hydrophobic interactions between C-PC and carbohydrate/protein walls to limit denaturation and color loss under stress (Mao et al., 2024).

The Culture Collection of Algae at Ho Chi Minh City (CCAH) was established to collect and maintain native cyanobacteria from mangrove and wetland habitats in Vietnam. As part of this program, *Leptolyngbya* sp. CCAH036/1 was isolated from moss-covered substrates in the Can Gio Mangrove Biosphere Reserve. Its full-length 16S rRNA sequence shared only 96.5% identity with the closest reference (below the 98.7–99% species-level threshold for cyanobacteria) supporting placement as a distinct lineage (Komárek, 2014; Dvořák et al., 2017; see Figure 1 and Figure 2). Preliminary tests confirmed C-PC production with antioxidant activity and stability under moderate heat (≤ 70 °C) and near-neutral pH.

This study addresses the instability of cyanobacterial C-PC in processed foods by coupling low-input cultivation of a native subaerial *Leptolyngbya* sp. CCAH036/1 with maltodextrin-based spray-drying to produce a storage-stable powder. Unlike prior work that emphasized yield or color (often in *Arthrospira* systems), we evaluate both antioxidant and coloring performance in a solid food matrix (sticky-rice mooncake prepared at moderate heat and near-neutral pH), thereby providing a clean-label alternative to synthetic colorants. We hypothesized that maltodextrin microencapsulation would preserve C-PC activity at 50–70 °C and pH 5–7 and demonstrate practical functionality in a model confectionery product.

Material and Methods

This work developed an extraction and spray drying process for phycocyanin from *Leptolyngbya* sp. CCAH036/1. The procedure included strain isolation, pigment extraction, microencapsulation with maltodextrin, and evaluation of stability and functionality. The powder was also tested in sticky rice mooncakes as a natural colorant and antioxidant (Figure 1).

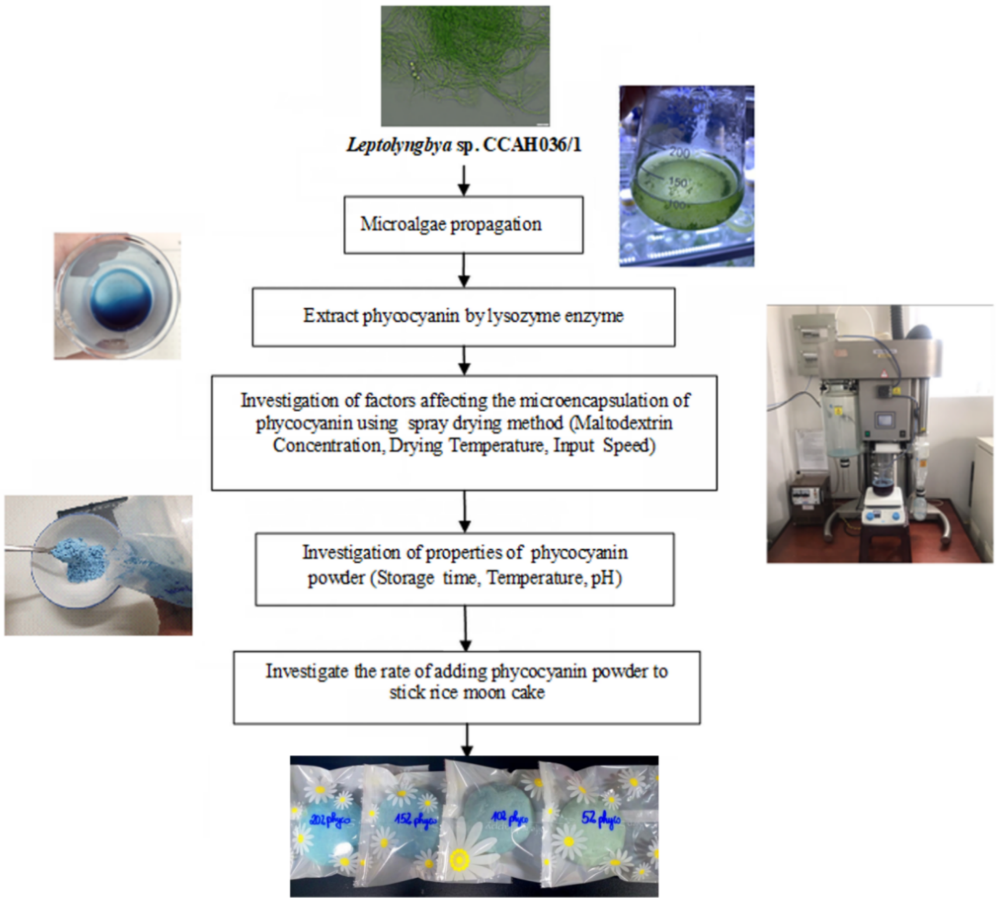

Figure-1. Overview of the experimental workflow for phycocyanin extraction from *Leptolyngbya* sp. CCAH036/1, microencapsulation by spray-drying, and application in sticky rice mooncakes.

Sampling and isolation

On 24 August 2024, ten environmental samples were taken from moss-covered soil, rocks, and tree bark in the brackish–freshwater ecotone of the Can Gio Mangrove Biosphere Reserve (10.50160°N, 106.86880°E). Samples were kept at 4 °C and transferred to the laboratory within 6–10 h. Filamentous cyanobacteria were enriched in Watanabe–Melkonian medium (W-Mel) and purified on BBM agar plates (1.5% agar) under continuous light (40–60 µmol photons m^{-2} s^{-1}) at 28 ± 2 °C. Distinct colonies were picked and propagated in liquid BBM.

Morphological and molecular identification

Morphology was examined under light microscopy following Komárek (2014). Filaments were thin (0.8–2.5 µm in diameter, mean ≈1.4 µm), flexible, and unbranched, with isodiametric cells, no heterocysts or akinetes, and surrounded by transparent sheaths. Genomic DNA was extracted using a modified CTAB method (Doyle and Doyle, 1987). The 16S rRNA gene was amplified with primers 27F/1492R and sequenced (Macrogen, Korea). BLASTn analysis and phylogenetic reconstruction (MEGA11, Tamura–Nei model, 1000 bootstraps) showed 96.5% identity with *Leptolyngbya* sp. ACT689. This supports its classification as a distinct lineage. The sequence has been submitted to GenBank; accession number is pending.

Culture and biomass collection

Cultures were initiated in 100 mL BBM with 10% inoculum (OD_{750} ≈ 0.5) and grown in 250 mL Erlenmeyer flasks under continuous light (40–60 µmol photons m^{-2} s^{-1}, Philips TL-D 18W/840) at 28 ± 2 °C. Flasks were hand-shaken two to three times daily. After 14 days, biomass was harvested by centrifugation (5000 rpm, 15 min, Hermle Z326), pellets were washed to neutral pH, and stored at −30 °C. Three biological replicates were prepared under identical conditions, and each culture was harvested and processed independently for pigment extraction. For maintenance, slants on BBM were kept at 4 °C and subcultured every 1–2 months.

Phycocyanin extraction

Wet biomass (1 g) was suspended in 3 mL phosphate buffer (0.1 M, pH 7.0) and subjected to two freeze–thaw cycles (–30 °C, 24 h; thaw at room temperature). Lysozyme (0.25 mg mL^{-1}; ≥20,000 U mg^{-1}, Bio Basic Inc.) was added and incubated at 37 °C for 4 h. Extracts were centrifuged (11,000 rpm, 10 min, 4 °C) to obtain a phycocyanin-rich supernatant. Phycocyanin concentration was measured (Shimadzu UV-1800) using Bennett and Bogorad (1973):

$$\text{(PC) (mg mL}^{-1}\text{)} = \frac{[A_{615} - 0{,}474\,(A_{652})]}{5{,}34} \quad (1)$$

where A615 and A652 represent absorbances at respective wavelengths.

Spray drying microencapsulation

C-PC extracts were spray-dried (EYELA SD-1000). Emulsions were prepared from 30 mL extract, 30 mL water, and maltodextrin (5–20 g), homogenized at 6000 rpm for 5 min. Drying was performed at inlet temperatures 120–160 °C and feed rates 240–420 mL h^{-1}. Parameter ranges were selected from preliminary trials to ensure workable viscosity, stable droplet formation, drying efficiency, and pigment stability, and align with prior maltodextrin–C-PC studies (Agustina et al., 2019; Iqbal and Hadiyanto, 2020; Jafari et al., 2008). Powder was tested for moisture, pigment content, microencapsulation efficiency (ME%), and antioxidant activity (I%). Total pigment was measured from aqueous extracts; surface pigment from ethanol–water (1:1) extracts. ME% was calculated as (TP–SP)/TP × 100 (İlter et al., 2021).

$$\mathrm{ME}(\%) = \frac{\mathrm{TP}-\mathrm{SP}}{\mathrm{TP}} \times 100 \quad (2)$$

Phycocyanin content in the powder (mg/g) was determined by the equation:

$$\mathrm{C-PC(mg/g)} = \frac{\text{The total phycocyanin(mg/mL)} \times \text{Solvent volume(60mL)}}{\text{Phycocyanin powder (1,5g)}} \quad (3)$$

Spray-drying was performed on three independently prepared extracts obtained from three separate algal cultivation batches. Each extract was spray-dried once under identical operating conditions, and the resulting powder batch was considered one biological replicate. Analytical measurements (pigment concentration, moisture content, ME%, I%) were conducted in triplicate as technical replicates for each batch.

Evaluation of phycocyanin powder properties

Encapsulated C-phycocyanin (C-PC) powder from *Leptolyngbya* sp. CCAH036/1 was tested for storage, heat, and pH stability. Samples were stored at 4 °C in the dark for three weeks and checked weekly. Thermal stability was examined at 50–110 °C for 60 min, and pH stability was assessed in phosphate–citrate buffer ranging from pH 3 to 8 (24 h, room temperature, dark). Powders were reconstituted in distilled water (1:40 w/v), homogenized (IKA T25 ULTRA-TURRAX®, 10,000 rpm, 15 min), and centrifuged (Hermle Z326, 5000 rpm, 15 min) to collect the supernatant. Phycocyanin was quantified at 615 and 652 nm, and antioxidant activity was measured by the DPPH method (1 mL sample and 3 mL DPPH 0.1 mM in methanol, 30 min incubation, absorbance at 517 nm). The formula was:

$$\text{DPPH Radical Scavenging Activity(\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

where Acontrol is the absorbance without sample. Spectral scans (400–700 nm) showed no major interference from chlorophyll or carotenoids.

Morphological and chemical characterization (SEM and FTIR)

SEM was used to observe particle size, shape, and surface features. Samples were mounted on carbon-coated stubs and examined under high vacuum at different magnifications. Samples were mounted on carbon-coated stubs and imaged under high vacuum at 2000×, 6000×, and 7000×; scale bars were 10 µm (2000×) and 1 µm (6000×, 7000×). FTIR spectra (4000–400 cm^{-1}) were recorded to identify functional groups and confirm interactions between phycocyanin and maltodextrin that contribute to pigment stability.

Application in sticky rice mooncakes

The powder was added to sticky rice mooncakes after preliminary trials with other foods (data not shown).

Each batch contained 50 g glutinous rice flour and 70 mL sugar syrup (>50° Brix), with C-PC added at 5, 10, 15, or 20% (w/w). Sensory evaluation by ten trained assessors (balanced gender, aged 25–40) set 20% as the upper acceptable limit and 5% as the minimum effective level. Ten trained assessors (balanced gender, 25–40 y) evaluated color, appearance, and overall acceptability on a 9-point hedonic scale; all provided informed consent under an exempted sensory protocol. Each formulation was evaluated individually by all assessors for color, appearance, and overall acceptability using a 9-point hedonic scale. Scores represent the mean ± SD of ten panel responses (n = 10). Statistical analysis was performed on the panel mean scores to determine significant differences ($p < 0.05$). Dough was kneaded until uniform. Pigment distribution was checked by digital microscopy (Olympus CX31, DP20) and ImageJ analysis, showing 2–10 µm particles evenly dispersed. Mooncakes were wrapped in plastic to prevent drying. C-PC content was determined at 615 and 652 nm (Bennett and Bogorad, 1973). Recovery of spiked standards (0.1–1.0 mg g^{-1} , n=3) was >95%. Antioxidant activity was measured by the DPPH assay validated for this matrix (Wu *et al.*, 2016a), with RSD <5%, R^2 >0.99, and matrix interference <3%. The optimal level was chosen by combining pigment retention, antioxidant activity, sensory acceptance, and texture quality.

Statistical analysis

All tests were done in triplicate. Results are expressed as mean ± standard deviation (SD) of three biological replicates (n = 3). Each biological replicate was analyzed in triplicate (technical replicates), and statistical analyses were performed on the biological replicate means. One-way ANOVA with Duncan's multiple range test was applied after confirming normality (Shapiro–Wilk) and variance homogeneity (Levene's test). Outliers were checked with Grubbs' test. Analyses were performed with Statgraphics Centurion XV (v15.2.05) at $p < 0.05$.

Results and Discussion

Isolation and identification of cyanobacterial strain

Morphological identification

Light microscopic observations revealed that strain CCAH036/1 had long, straight, flexible trichomes characteristic of the genus *Leptolyngbya* (Figure 2). Trichomes often reached a length of over 100 µm and had a width of 0.8–2.5 µm, measuring a mean value of 1.4 ± 0.3 µm (n = 50) (Figure 2a). Cells were cylindrical to isodiametric with a mean length of 1.5 ± 0.4 µm, which approximated the width of the filament (Figure 2b). No heterocysts or akinetes could be found. Cell walls were smooth and uninterrupted, lacking any marked constrictions. Each trichome bore a thin, translucent layer of mucilage, which stood out particularly well around the margins of the filaments and in regions of curvature (Figure 3). Round terminals often occurred, as in previous descriptions of *Leptolyngbya* (Komárek, 2007; Kim et al., 2015). Cytoplasmic pigmentation was even and of blue-green hue. Under starvation conditions, the pigmentation often faded to pale yellow-green, a phenomenon observed in previous studies of *Leptolyngbya*. In liquid culture, the filaments had been only partially motile, often forming a loose layer on the surface of the culture medium. All of these characteristics (thin flexible trichomes; isodiametric cells; sheaths; terminals) collectively can be used as a distinguishing feature of *Leptolyngbya*.

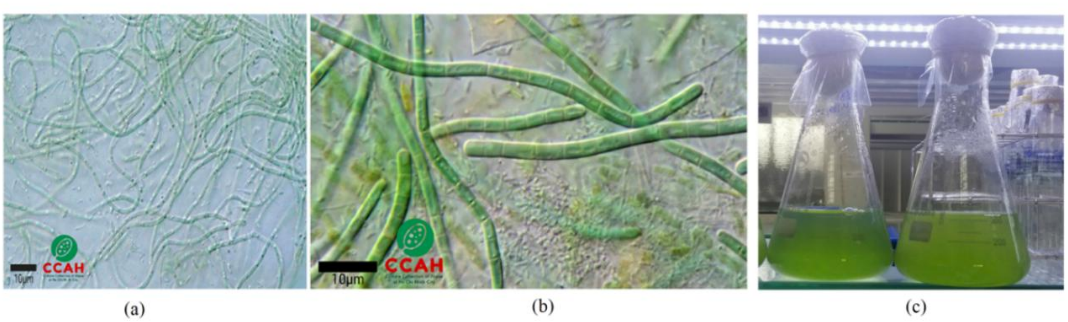

Figure-2. Morphological characteristics of *Leptolyngbya* sp. CCAH036/1 under light microscopy and in laboratory culture: (a) Long, thin, unbranched filaments with transparent sheaths (scale bar: 10 µm); (b) Cylindrical, isodiametric cells in flexible trichomes, without heterocysts or akinetes (scale bar: 10 µm), and (c) Culture of *Leptolyngbya* sp. CCAH036/1 in BBM medium.

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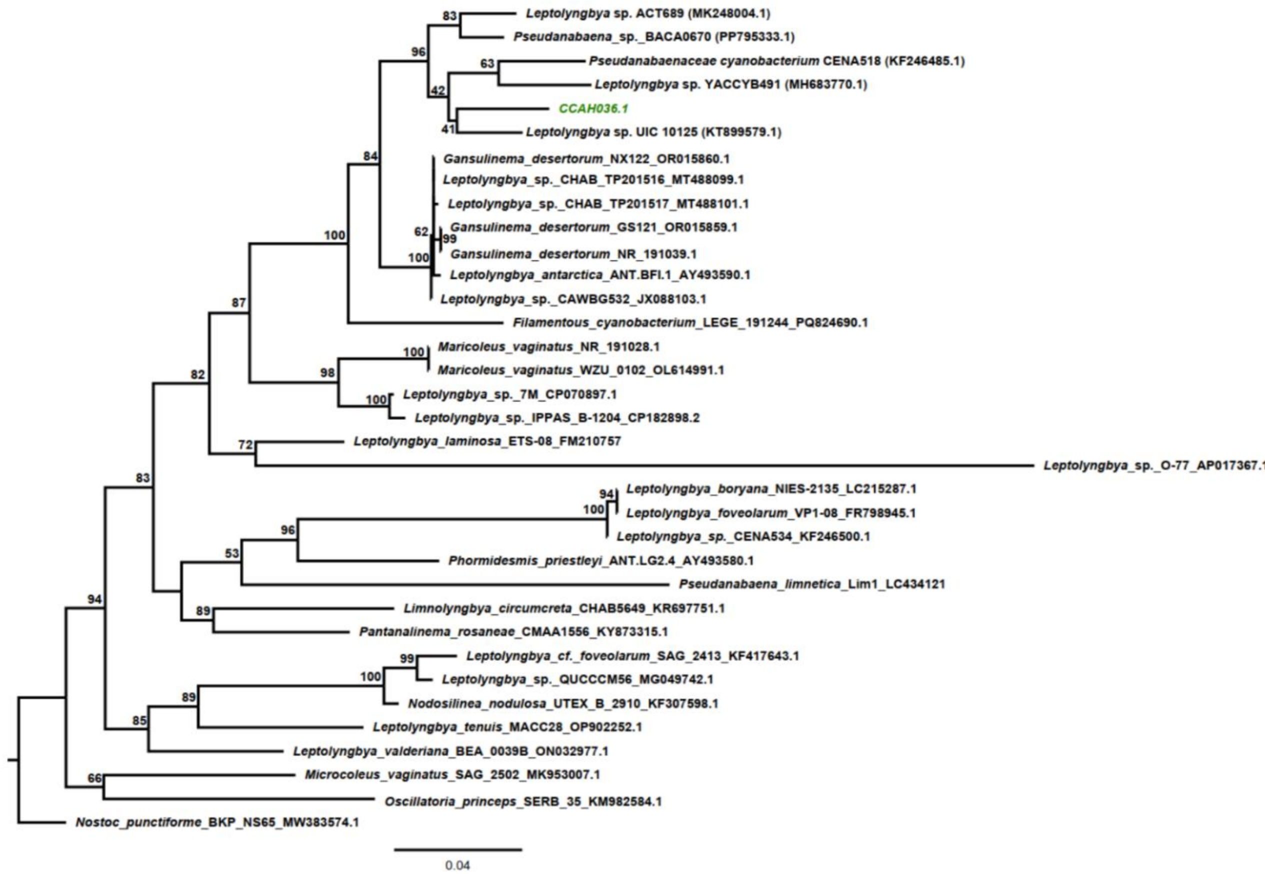

Figure-3. Maximum Likelihood phylogenetic tree based on complete 16S rRNA gene sequences (1479 nucleotide positions) showing the phylogenetic placement of *Leptolyngbya* sp. CCAH036/1.

Phylogenetic analysis

A phylogenetic analysis carried out using a full-length sequence of the 16S rRNA gene with a Maximum Likelihood method enabled the determination of the-taxonomic rank of *Leptolyngbya* sp. CCAH036/1 (Figure 3). A tree generated, based on a data set of 35 full-length sequences of the 16S rRNA gene of strains of free-living cyanobacterial species, is presented in Figure 3. Bootstrap values of 1000 iterations are presented on the nodes of the tree. As evident from the tree, a clade (with a high confidence level of 96%) of the strain CCAH036/1 is included in a separate distinct group. This group consists of *Leptolyngbya* sp. ACT689 (MK248004.1), *Leptolyngbya* sp. YACCYB491 (MH683770.1), and *Leptolyngbya* sp. UIC10125 (KT899579.1), forming a monophyletic group that is sufficiently distinct from the already existing *Leptolyngbya* strains as well as from any of the already established Cyanobacterial genera. Despite the high tree branch support, The highest sequence similarity (96.5% with *Leptolyngbya* sp. ACT689) falls below the 98.7–99% species-level threshold, confirming that strain CCAH036/1 represents a distinct lineage. Consequently, this again supports that CCAH036/1 is a distinct group within *Leptolyngbya*.

Culture, productivity of biomass, and phycocyan

Leptolyngbya sp. CCAH036/1, under optimal culture conditions, formed fine filaments that tended to accumulate loosely in a way that facilitates harvesting. At day 14, the biomass of 0.544 g/100 mL of solution with a moisture content of 85.3% (≈ 5.44 g L^{-1} wet weight), which translates to approximately 0.80 g L^{-1} of dried weight, is in range with previous studies of strains of a similar kind that ranged between 0.8–2.2 g L^{-1} (Schipper et al., 2020; Singh et al., 2014; Gongi et al., 2022). Extraction of phycocyanin involved a freeze/thaw cycle assisted by lysozyme treatment. The concentration of the obtained solution stood at 2.53 mg mL^{-1}, which corresponds to either 10.13 mg of phycocyanin per gram of wet biomass or approximately 68.9 mg g^{-1} of dried biomass. The purity factor (A620/A280) ratio of approximately 2.32 met food-grade standards. This compares well with those of most terrestrial cyanobacteria, having a range of either 25.3–72 mg g^{-1} of dried weight (Saad et al., 2024).

Table-1. Influence of spray-drying parameters on phycocyanin microencapsulation and powder properties.

Parameter	Level	C-PC content (mg g^{-1})	ME (%)	I (%)(DPPH)	Powder weight (g)	Moisture content (%)
Maltodextrin (g)	5	13.63 ± 1.05^{b}	60.39 ± 2.56^{b}	46.24 ± 0.51^{b}	2.78 ± 0.01^{a}	5.00 ± 0.01^{a}
	10	15.02 ± 0.62^{b}	72.78 ± 2.17^{c}	53.57 ± 3.33^{c}	4.43 ± 0.70^{ab}	$5.19 \pm 0,01^{a}$
	15	6.25 ± 0.34^{a}	50.71 ± 2.21^{a}	35.74 ± 1.28^{a}	5.76 ± 0.42^{b}	4.98 ± 0.09^{a}
	20	4.77 ± 0.71^{a}	48.08 ± 2.81^{a}	32.08 ± 3.52^{a}	7.78 ± 0.90^{c}	$4.54 \pm 0,14^{b}$
Temperature (ºC)	120	10.02 ± 0.51^{a}	37.57 ± 2.78^{a}	44.71 ± 1.36^{a}	3.96 ± 0.20^{a}	7.70 ± 0.49^{d}
	130	$9.53 \pm 0,34^{a}$	41.62 ± 1.08^{a}	48.44 ± 0.86^{b}	4.06 ± 0.26^{a}	6.67 ± 0.20^{c}
	140	$15.39 \pm 0,15^{c}$	71.37 ± 1.73^{c}	52.28 ± 0.33^{b}	4.22 ± 0.15^{b}	5.86 ± 0.13^{b}
	150	20.65 ± 0.44^{d}	79.77 ± 3.32^{d}	57.07 ± 0.42^{c}	4.75 ± 0.14^{b}	5.15 ± 0.26^{ab}
	160	11.67 ± 0.37^{b}	55.61 ± 2.01^{b}	45.78 ± 0.54^{a}	4.89 ± 0.15^{b}	4.49 ± 0.25^{a}
Input speed (mL h^{-1})	240	6.73 ± 0.16^{a}	38.42 ± 1.37^{a}	33.22 ± 2.67^{a}	1.89 ± 0.06^{b}	4.18 ± 0.14^{a}
	300	20.53 ± 0.59^{c}	79.43 ± 2.74^{c}	56.66 ± 2.38^{c}	4.68 ± 0.14^{c}	5.14 ± 0.22^{b}
	360	9.73 ± 1.66^{b}	46.02 ± 1.84^{b}	44.31 ± 2.15^{b}	1.45 ± 0.14^{a}	7.23 ± 0.33^{c}
	420	8.93 ± 0.11^{b}	39.24 ± 0.98^{a}	39.74 ± 0.86^{b}	1.78 ± 0.16^{b}	8.26 ± 0.22^{d}

Values are mean ± SD (n = 3 biological replicates; each analyzed in technical triplicate). Different superscripts within a column indicate significant differences (one-way ANOVA, Duncan, $p < 0.05$).

Microencapsulation of phycocyanin by spray drying

Effect of maltodextrin concentration

As shown in Table 1, raising the maltodextrin (MD) concentration from 5 to 10 g markedly improved encapsulation efficiency and antioxidant activity, indicating better phycocyanin protection. However, further increases in MD led to reduced phycocyanin content despite higher powder yield. For this factor, a one-factor-at-a-time design was used while other parameters were held at their selected settings. At low MD levels, the carrier matrix was insufficient to shield phycocyanin from thermal degradation, whereas excessive MD (15–20 g) increased solution viscosity and total solids (21–25 °Bx) and apparent viscosity, resulting in uneven phycocyanin dispersion and lower encapsulation efficiency. Mechanistically, 10 g MD likely maximized rapid crust formation and hydrogen-bonding/physical entrapment within the maltodextrin network, improving retention while avoiding viscosity-driven maldistribution, consistent with previous reports that excessive carrier levels reduce phycocyanin protection (Agustina et al., 2019; Iqbal and Hadiyanto, 2020).

Effect of inlet temperature

The temperature of spray drying had a significant effect on phycocyanin yields and microencapsulation efficiency (Table 1). Raising the inlet-air temperature from 120 °C to 150 °C encouraged high microencapsulation efficiency as well as high phycocyanin yields. This result revealed that moderate temperatures increased evaporation rate, leading to rapid formation of a microcapsule wall layer around phycocyanin particles. Nevertheless, at a higher temperature of 160 °C, phycocyanin yields and antioxidant activity decreased, suggesting that higher temperatures accelerated excessive moisture evaporation and caused wall surface cracks, resulting in a loss of phycocyanin protection despite efficient water removal. This behavior reflects maltodextrin's crust-formation process controlled by its glass-transition temperature (Tg): around 150 °C promotes fast shell formation and pigment entrapment, whereas 160 °C causes protein denaturation and micro-cracks, increasing pigment leakage. Hence, 150 °C represented an optimum compromise between effective dehydration and phycocyanin stability, consistent with previous reports that excessive spray-drying temperatures trigger pigment denaturation (Purnamayati et al., 2018; Hadiyanto et al., 2019).

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Effect of feed rate

Feed rate had a significant influence on microencapsulation efficiency and phycocyanin yield (Table 1). A higher feed rate of 300 mL h^{-1}, as opposed to 240 mL h^{-1}, increased microencapsulation efficiency considerably. This improvement mainly reflects shorter residence time under heat at 300 mL h^{-1}, still sufficient for complete drying and shell formation. Nevertheless, beyond 360–420 mL h^{-1}, residence time became too short, causing incomplete moisture removal. Consequently, the spray-dried product showed higher residual moisture, more wall deposition, and lower powder recovery. At such high feed rates, rapid discharge of the feed solution produced particles with under-developed shells instead of well-formed microcapsules, reducing both phycocyanin yield and encapsulation efficiency. These "wet-core" particles (evident from the higher moisture values in Table 1) lower overall product quality. This phenomenon has also been reported in previous microencapsulation studies (İlter et al., 2021). Therefore, a feed rate of 300 mL h^{-1} was considered optimal under the applied thermal load.

Stability and functional properties of encapsulated phycocyanin

Storage stability of encapsulated phycocyanin under controlled conditions

Storage stability was evaluated for three weeks at 4 °C in the dark under oxygen-restricted conditions (Table 2). The encapsulated powder (20.53 mg g^{-1}; 56.66 % antioxidant activity at time zero) changed little over the first two weeks ($p > 0.05$). By week 3 it contained 17.26 ± 1.27 mg g^{-1} (about 84 % of the initial level) and showed 47.01 ± 0.96% antioxidant activity (≈ 83% of the initial value).

Table-2. Phycocyanin content and antioxidant activity (I%) of encapsulated powder and liquid extract over three weeks.

	Phyocyanin powder		Phycocyanin extract	
Storage time (week)	C-PC content (mg g^{-1})	I% (DPPH)	C-PC content (mg mL^{-1})	I%(DPPH)
1	20.03±0.18^{b}	54.73 ± 1.00^{b}	2.05 ± 0.01^{c}	57.28 ± 0.42^{c}
2	19.14± 0.30^{b}	54.16 ± 0.31^{b}	1.41 ± 0.02^{b}	45.86 ± 1.05^{b}
3	17.26 ± 1.27^{a}	47.01 ± 0.96^{a}	1.19 ± 0.02^{a}	30.99 ± 0.79^{a}

Values are mean ± SD (n = 3 biological replicates; each analyzed in technical triplicate). Different superscripts within a column indicate significant differences (one-way ANOVA, Duncan, $p < 0.05$).

In contrast, the non-encapsulated extract (2.53 mg mL^{-1}; 67.82% activity at time zero) deteriorated quickly: at week 3 it measured 1.19 ± 0.02 mg mL^{-1} (≈ 47 % of initial) with 30.99 ± 0.79% activity and visible spoilage. The strong retention in the microcapsules indicates that the maltodextrin shell effectively isolated phycocyanin from oxygen and moisture through hydrogen-bond interactions, minimizing oxidative degradation during storage**.** Encapsulation limited losses at 4 °C: week 3 powder retained ~84% pigment and ~83% activity, while the extract fell to ~47% and ~3 %.

Thermal stability of encapsulated phycocyanin under heat treatment

Encapsulated powder was heated for 60 min at 50–110 °C (Table 3). At 50–70 °C, losses were modest: 20.14 ± 0.21 to 19.67 ± 0.11 mg g^{-1}, with antioxidant activity of 54.23 ± 0.20% and 53.18 ± 0.33%, respectively. Above 90 °C, degradation accelerated. Values fell to 12.69 ± 0.08 mg g^{-1} and 48.30 ± 0.53% at 90 °C, and to 8.96 ± 0.26 mg g^{-1} and 44.18 ± 0.60% at 110 °C. Moderate temperatures likely preserved the pigment by maintaining the amorphous maltodextrin matrix below its glass-transition point (≈ 150 °C), allowing the hydrogen-bond network to remain intact; above 90 °C the matrix softens, leading to pigment diffusion and protein denaturation. These results indicate that the microcapsules protect the pigment under moderate heating, while higher temperatures still cause substantial loss, as also noted in earlier work on phycocyanin systems (e.g., Pradeep and Nayak, 2019).

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Table-3. Phycocyanin content (mg/g) and antioxidant retention (I%) of microencapsulated powder after 60 min heat treatment (50–110 °C); and after 24 h incubation at different pH values (3.0–8.0).

Parameter	Level	C-PC content (mg g^{-1})	I (%) (DPPH)
Temperature (°C)	50	20.14 ± 0.21^{d}	54.23 ±0.20^{d}
	70	19.67 ± 0.11^{c}	53.18 ± 0.33^{c}
	90	12.69 ± 0.08^{b}	48.30 ± 0.53^{b}
	110	8.96 ± 0.26^{a}	44.18 ± 0.60^{a}
pH	3	4.29 ±0.44^{a}	32.36 ± 0.52^{a}
	5	13.85 ± 0.21^{c}	50.29 ± 0.98^{c}
	7	19.94 ± 1.27^{d}	55.40 ± 0.42^{d}
	8	9.58 ± 0.39^{b}	42.15 ± 2.41^{b}

Values are mean ± SD (n = 3 biological replicates; each analyzed in technical triplicate). Different superscripts within a column indicate significant differences (one-way ANOVA, Duncan, $p < 0.05$).

pH stability of spray-dried encapsulated phycocyanin

Stability varied with pH (Table 3). At pH 3.0 the powder measured 4.29 ± 0.44 mg g^{-1} with 32.36 ± 0.52% activity. Performance improved at pH 5.0–7.0; the highest values were at pH 7.0 (19.94 ± 1.27 mg g^{-1}; 55.40 ± 0.42%). At pH 8.0, values declined to 9.58 ± 0.39 mg g^{-1} and 42.15 ± 2.41%. This pattern reflects the charge stability of the C-phycocyanin chromophore: near-neutral pH minimizes conformational changes in the protein and preserves its chromophore environment, whereas strong acidity disrupts ionic and hydrogen bonds, leading to color fading and reduced radical-scavenging capacity**.** This pattern matches prior reports that phycocyanin is most stable near neutral pH (Wu et al., 2016b). In practice, the preferred range for the encapsulated powder is pH 5.0–7.0; strong acidity leads to color loss and reduced antioxidant activity, so additional protection would be needed for acidic beverages.

Morphological and chemical characterization of spray-dried phycocyanin microcapsules by SEM and FTIR analysis

SEM images (Figure 4a–c) showed mainly spherical particles with diameters of 2–10 µm, a size range compatible with food and nutraceutical use. At higher magnification the surfaces appeared wrinkled with shallow indentations, a common outcome of rapid water loss and matrix shrinkage during spray drying. These features are consistent with maltodextrin forming a shell around a phycocyanin core. The increased surface roughness may aid rehydration and dissolution. Low-magnification views indicated a relatively uniform particle population. The morphology agrees with prior reports of maltodextrin-based phycocyanin microcapsules (Sritham and Gunasekaran, 2017; Zamani et al., 2020), confirming that the rapid crust formation typical of spray drying led to dense but porous particles that favor dispersion and color release.

FTIR spectra (Figure 4d) supported the presence of both protein and polysaccharide components and their interaction. The broad band near ~3396 cm^{-1} corresponds to N–H/O–H stretching of phycocyanin and maltodextrin; the ~2900 cm^{-1} band reflects C–H stretching of maltodextrin. Signals at ~1641 cm^{-1} (amide I region), ~1413 cm^{-1} (C–H bending), and ~1025 cm^{-1} (C–O stretching) indicate the coexistence of protein and carbohydrate. These peaks confirm hydrogen-bond formation between the hydroxyl groups of maltodextrin and the amide or chromophore residues of phycocyanin, providing molecular stabilization against denaturation and oxidation. These features point to hydrogen bonding and physical entrapment between phycocyanin and maltodextrin, as reported previously (Zamani et al., 2020; Sritham and Gunasekaran, 2017).

Together, the spherical 2–10 µm morphology with wrinkled shells and the FTIR bands characteristic of protein–polysaccharide interactions demonstrate effective encapsulation and explain the observed stability of C-PC under heat and storage. Longer storage tests (3–6 months) under commercial conditions would provide a fuller assessment of performance.

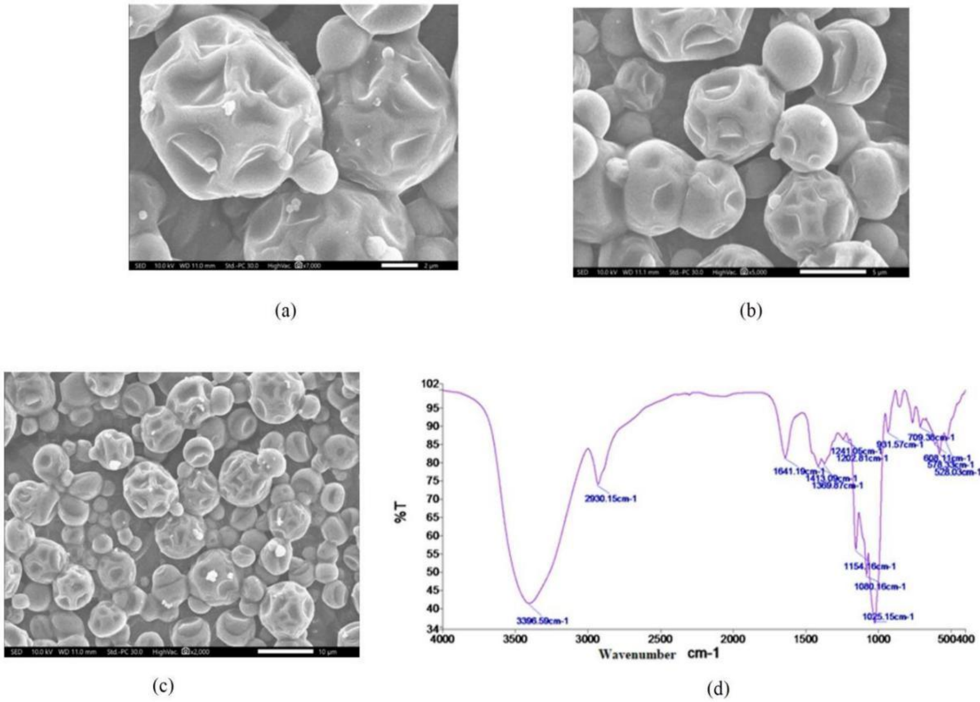

Figure-4. Structural and chemical characterization of spray-dried phycocyanin powder. (a–c) SEM images of phycocyanin microcapsules (2–10 µm) with wrinkled spherical morphology; (d) FTIR spectrum showing functional groups of phycocyanin and maltodextrin, indicating hydrogen bonding.

Table-4. Effect of the rate of adding phycocyanin powder to sticky rice mooncake.

The rate of adding phycocyanin powder %(w/w)	C-PC content (mg g^{-1}) sticky rice mooncake)	I (%) (DPPH)
5	0.284 ± 0.005^{a}	18.702 ± 0.400^{a}
10	0.748 ± 0.005^{b}	31.934 ± 1.891^{b}
15	1.874 ± 0.020^{c}	43.781 ± 0.312^{c}
20	2.549 ± 0.026^{d}	47.435 ± 1.243^{d}

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

Functional and sensory evaluation of spray-dried phycocyanin supplementation in sticky rice mooncake

Adding encapsulated C-PC increased both pigment content and antioxidant activity in a dose-dependent manner (Table 4). At 5% addition the cakes contained 0.284 mg g^{-1} phycocyanin and showed 18.70% antioxidant activity. At 20% these values rose to 2.549 mg g^{-1} and 47.44%. The control (0%) had negligible phycocyanin and 5.02% activity, attributable to native ingredients. Handling and sensory quality declined at 20% because of higher moisture and difficult dough. At 15%, the cakes contained 1.874 mg g^{-1} phycocyanin with 43.78% activity and retained acceptable dough consistency. This level produced a bright, natural blue color and the highest sensory scores, indicating the best balance between functionality and texture.

Earlier studies emphasized phycocyanin primarily as a colorant in foods (e.g., Mohammadi-Gouraji et al., 2019; Benchikh et al., 2021). Here, both the coloring function and antioxidant activity were evident in a confectionery matrix. Compared with non-encapsulated pigments, the maltodextrin microcapsules better preserved activity by limiting pigment oxidation and hygroscopicity during baking and storage. At 15% addition the cakes showed 1.874 mg g^{-1} pigment and 43.78% DPPH without texture loss, demonstrating a practical inclusion level for functional food development.

Overall Discussion and Implications

Strain *Leptolyngbya* sp. CCAH036/1 is sourced from the Mangrove Forest in the Can Gio Mangrove Reserve. The culture produced phycocyanin within the yield range reported for other *Leptolyngbya* strains**.** Spray-drying with maltodextrin yielded a stabilized pigment. The optimized product contained 20.53 mg g^{-1} phycocyanin with 5.14% moisture and high encapsulation efficiency (79.4%) coupled with 56.66 % antioxidant retention.

Storage tests revealed high stability. After three weeks at 4 °C, the powdered sample maintained 84% of its pigment content and 83% of antioxidant activity. By contrast, the liquid extract had lost over half of these in the same time span. The difference between the encapsulated and free extracts was statistically significant ($p < 0.05$, $n = 3$). Temperature studies revealed that activity had been sustained at 50–70 °C (approximately 53–56%), but decreased sharply above 90 °C, reaching 44% at 110 °C. Optimum stability had been found between pH 5–7, with activity of 55% at pH 7 as against only 32% at pH 3.

This is in agreement with the SEM analysis result since the protected phycocyanin formed spherical particles that ranged between 2–10 µm. The rough surface indicated that rapid evaporation of water occurred during the spray-drying process. It is evident that hydrogen bonds exist between maltodextrin molecules and protein molecules as revealed by the IR-spectral analysis. Upon spray-drying, rapid evaporation of water facilitates the formation of a continuous maltodextrin layer that physically shields phycocyanin from external factors. Moreover, hydrogen bonds are expected to form between the hydroxyl groups (-OH) of maltodextrin molecules and amino ($-NH_2$), carboxyl (-COOH), and carbonyl (-C=O) groups of phycocyanin. This increased the affinity between maltodextrin and phycocyanin molecules. Physical entrapment together with hydrogen-bond interactions between maltodextrin and phycocyanin reduces exposure to oxygen, light, and heat, thereby maintaining the structural integrity and bioactivity of phycocyanin compared with the crude extract. This is in agreement with İlter et al. (2021), who observed that various protecting agents operated in a similar way. Recent work has also proposed electrostatic and hydrogen-bond interactions between phycocyanin and carbohydrate matrices such as maltodextrin or sodium alginate (Qiao et al., 2022; Prasetyaningrum et al., 2025).

The food test further validated these findings. Sticky rice mooncakes were selected since they are processed under moderate temperatures and near-neutral pH. At a moderate level of addition of 15%, phycocyanin reached 1.874 mg g^{-1} with antioxidant activity of 43.78%. This had no negative effects on color or texture acceptability. Addition of a higher quantity of 20% increased pigment to 3.19 mg g^{-1}, but reduced dough handling and sensory acceptance**.** Addition of a small quantity of 5% gave only 0.284 mg g^{-1} pigment with activity of 18.7%. Thus, 15% represents a practical inclusion level, giving the best balance between pigment intensity, antioxidant activity, and sensory quality ($p < 0.05$).

Compared to yogurt and beverage systems, we found both color and antioxidant properties in a confectionery product. Mohammadi-Gouraji et al. (2019) utilized phycocyanin in yogurt, but only in refrigerated form. Benchikh et al. (2021) evaluated beverage systems, where only color effects were found. In this experiment, the compound acted as both antioxidant and coloring agent in a solid food system. This confirms that encapsulated phycocyanin can function beyond liquid matrices, broadening its potential for bakery and confectionery formulations**.** However, some issues need to be addressed. Freeze-thaw cycling with lysozyme is not cost-effective for large-scale extraction. Moreover, spray-drying at 150 °C produced stable pigment preparations, but scaling this process to ensure uniform particle formation needs further evaluation. Still, the pigment preparations remain sensitive to extreme high temperatures. Thermal treatment at 90 °C for one hour reduced pigment content to 12.69 mg g^{-1} and activity to 48.3%. This thermal tolerance is insufficient for UHT-type or high-temperature pasteurization processes**.** Future studies will involve co-encapsulation of the pigment with proteins or lipids to extend stability. A safety and regulatory assessment is also required: *Leptolyngbya* pigment preparations currently lack GRAS status, and toxin screening was not performed here. The next research phase will therefore include PCR assays (mcy for microcystin, sxt for saxitoxin, and nda for nodularin) and LC-MS/MS confirmation to verify safety for food applications. Overall, this study demonstrates that phycocyanin obtained from *Leptolyngbya* can be converted into a stable, food-grade powder via spray-drying with maltodextrin.

By far, most commercial phycocyanin is derived from *Arthrospira*, but diversity could be introduced by *Leptolyngbya* strains native to Vietnam. The global natural-colorant market is expected to exceed USD 3 billion by 2027; improving *Leptolyngbya* cultivation efficiency could reduce water and nutrient demand while diversifying pigment sources beyond *Arthrospira*. Native strains such as CCAH036/1 therefore hold promise for sustainable pigment production that supports both biodiversity conservation and food innovation in Vietnam.

Conclusion

Spray-drying with maltodextrin produced a stable powdered phycocyanin from *Leptolyngbya* sp. CCAH036/1 that preserved pigment integrity and antioxidant activity under moderate heat (50–70 °C) and near-neutral pH. At 15% inclusion, the powder served effectively as a natural colorant and antioxidant in sticky-rice mooncakes without affecting sensory quality, demonstrating its potential as a functional food ingredient. Further work should focus on industrial scale-up, toxin analysis and regulatory validation, and co-encapsulation strategies to enhance stability in acidic and high-temperature environments.

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Contribution of Authors

Huynh PPT: Data curation, investigation, formal analysis, methodology, visualization, validation, software, writing-original draft, and writing-review and editing.

Do TT, Nguyen TC, Bui MN & Le TL: Data curation, investigation and validation.

Pham TL: Conceptualization, investigation, writing review and editing.

Tran HD: Supervision, resources, funding acquisition, methodology, software, conceptualization, writing-original draft, and writing-review and editing.

All authors read and approved final draft of the manuscript.

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