

REVIEWS

CONVENTIONAL AND GREEN METHODS FOR THE EXTRACTION OF PHYCOCYANIN FROM ARTHROSPIRA PLATENSIS (SPIRULINA) AND FACTORS INFLUENCING ITS STABILITY. A LITERATURE REVIEW

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ABSTRACT

INTRODUCTION: Phycocyanin, a water-soluble phycobiliprotein and blue pigment primarily extracted from *Arthrospira platensis* (spirulina), has attracted growing scientific interest due to its well-established and potential future applications in the food industry, cosmetics, and pharmaceuticals.

AIM: This review examines and compares conventional and green methods for phycocyanin extraction and analyzes the factors affecting its stability under various environmental conditions, including pH, temperature, light, and others.

MATERIALS AND METHODS: A thorough review of scientific databases such as Scopus, Web of Science, PubMed, and ResearchGate was conducted.

RESULTS AND DISCUSSION: The reviewed literature reveals a lack of sufficient studies evaluating the applicability of green extraction methods for phycocyanin at an industrial scale. Data regarding the interactions of phycocyanin with various food matrices, which affect its stability and bioactivity, remain limited. Furthermore, there is a scarcity of comprehensive investigations into the long-term stability of phycocyanin in different formulations, hindering the optimization of technological processes for its application in the food and other industries.

CONCLUSION: The extraction and stability of phycocyanin are critical factors determining its potential for broad application across various industries. Green extraction methods offer promising alternatives to conventional techniques, providing higher yields and purity with reduced environmental impact. However, challenges related to the stability of phycocyanin under diverse conditions remain, necessitating further research, particularly focused on the development and scale-up of stable formulations.

Keywords: *phycocyanin, superfood, spirulina, conventional extraction, green extraction, stability*

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INTRODUCTION

Phycocyanin is a pigment-protein complex belonging to the phycobiliprotein family, primarily extracted from cyanobacteria such as *Arthrospira platensis* (spirulina) (1). It is utilized across various industries due to its bioactivity, including antioxidant, anti-inflammatory, antiviral, antihyperlipemic, antihypertensive, and immunostimulatory properties (2,3,4,5) as well as neuroprotective effects, cognitive enhancement, and wound healing potential (6). Effective extraction and stabilization of phycocyanin are essential for its incorporation into stable formulations.

AIM

This review aims to examine and compare conventional and green methods for phycocyanin extraction and analyze the factors affecting its stability under various environmental conditions. The present work also focuses on better understanding how physical and chemical parameters affect C-phycocyanin structure, including a discussion about stabilizing agents applied in C-phycocyanin extracts. The literature that was reviewed was published in the last 10 years, with some research results going even further back.

METHODS FOR PHYCOCYANIN EXTRACTION

The extraction of biologically active compounds (BACs) of plant origin is of critical importance, as these compounds find widespread application in the pharmaceutical and cosmetic industries, as well as in the development of foods and dietary supplements (7). Due to factors such as varying harvest times of the plant materials, the multiple processing stages they undergo, and other environmental influences, differences in the qualitative and quantitative composition of the target BACs are inevitable. These factors necessitate appropriate standardization of plant substances to ensure they meet the requirements specified in the relevant pharmacopoeial monographs. Furthermore, the European Pharmacopoeia (Ph. Eur.) mandates specific tests listed under the Herbal Drugs section, which include assessments of identity, impurities (such as dust, soil, mold, etc.), loss on drying, residual moisture, pesticide residues, heavy metals, total ash content, micro-

biological contamination, content of extractable substances, quantitative content of BACs, among others (8).

Once the plant substances are standardized according to pharmacopoeial specifications, their suitability as starting materials for the production of extracts or other phytoproducts is determined. Subsequently, with an appropriate extraction method selected, the raw material must undergo necessary preparation processes. These typically involve comminution (using various mills, grinding, crushing, or grating of the plant material), sieving the ground material to achieve an optimal particle size (using a suitable set of sieves with different pore sizes), and swelling of the plant material for a specified time in an appropriate amount of extractant before proceeding with the actual extraction (8).

The primary objectives in selecting an extraction method are achieving high yield and purity of the BACs. The choice of an extractant with high selectivity in an optimal quantity, process duration, medium pH, temperature, particle size of the plant substance, and the plant material-to-extractant ratio, known as the hydromodule, are all essential factors for conducting an effective extraction process.

Conventional (Classical) Extraction Methods

Classical extraction methods include maceration, percolation, Soxhlet extraction, steam distillation, hydrodistillation, cold pressing, and others. These methods employ mechanical and physical processes such as homogenization, freeze-thaw cycles, and maceration. Such techniques disrupt the cell membranes of plant cells and release phycocyanin, but they often lead to its degradation at higher temperatures or with prolonged treatment (9,10,11).

Some classical extraction methods require operation within a specific temperature range, while others can be carried out at room temperature. They allow the use of various solvents—water, ethanol, aqueous-ethanol mixtures, among others—some of which can achieve exhaustive extraction of the plant material. Although classical extraction methods are accessible and straightforward to implement, they are time-consuming and labor-intensive processes that require large volumes of extractant. Additionally, they are not sufficiently effective or efficient, typ-

ically yielding relatively lower recovery and purity compared to modern extraction methods (11).

Green Extraction Methods

Modern extraction approaches reduce environmental impact through limited solvent use and lower energy consumption. Among these are ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), all of which result in higher yields and greater purity (12).

Studies comparing conventional and green methods have shown that green extraction methods outperform conventional techniques in terms of yield, purity, and product sustainability. For example, Tavanandi et al. (2020) reported a significant increase in yield and purity using enzyme-assisted ultrasound extraction (13).

STABILITY OF PHYCOCYANIN

The industrial utilization of phycocyanins is still constrained by limitations associated with extraction techniques and the poor stability of the obtained products (14). In recent years, various alternative extraction approaches combined with stabilizing agents under different experimental conditions have been investigated to address these challenges (10,15,16,17,18,19,20,21,22,23,24). Efficient extraction of phycocyanin requires consideration of several critical factors, including cell structure, the nature of the biomass (fresh or dried), pH, temperature, light intensity, solvent selection, biomass-to-solvent ratio, and the incorporation of preservatives (10).

The membrane structure of spirulina is composed of four distinct layers: an outer layer coated with protein fibrils, a peptidoglycan layer responsible for maintaining cellular rigidity, and an inner fibrillar layer (25). An optimal extraction strategy should selectively release the target compound while minimizing energy consumption (10).

Type of Plant Material

Dried biomass is generally preferred for phycocyanin extraction; however, conventional drying methods at temperatures reaching 60°C are not advisable because they may promote phycocyanin degradation (15). Only a limited number of studies have examined phycocyanin extraction from fresh biomass (26,27,28), and current evidence does not con-

clusively demonstrate superior extraction yields. Nevertheless, one investigation reported that rehydration of spirulina powder in extraction buffer for two hours enhanced phycocyanin recovery (13).

pH Stability

Maintaining an appropriate pH environment is crucial for preserving phycocyanin stability, as well as its spectral characteristics and distinctive blue coloration. Maximum stability is typically observed at neutral pH values (~7.0), whereas acidic environments (pH < 5.0) lead to loss of color, disruption of structural integrity, and reduced bioactivity (29,30). Investigations employing ultrafine fibers of polylactic acid or polyethylene oxide as pH indicators demonstrated more extensive phycocyanin degradation at pH 3.0–4.0 (28,31). Multiple studies further confirmed that pH values between 5.5 and 7.0 provide optimal conditions for maintaining phycocyanin stability (16,31,32,33).

Thermal Stability

Elevated extraction temperatures are generally suitable for thermally stable bioactive compounds. However, phycocyanin has been shown to exhibit instability at temperatures exceeding 45°C, under acidic conditions, and following exposure to light (16,32,34,35,36,37,38,39,40). Similar observations were reported by Wu et al. (2016) (31), who demonstrated that phycocyanin remains stable only up to 45°C. Degradation begins above 47°C and becomes more pronounced at temperatures exceeding 70°C (16,31,35,36). Overall, available evidence suggests that temperatures below 45°C and pH 6 constitute favorable conditions for preserving phycocyanin stability (16,32). Furthermore, stabilizing agents and encapsulation strategies have been shown to reduce thermally induced degradation (31,41).

Light Intensity and Solvent Type

Light quality and intensity significantly influence algal growth, photosynthetic activity, and consequently phycocyanin biosynthesis (37). Each algal species exhibits optimal growth under specific light spectra and irradiation conditions. Regarding spirulina cultivation, Walter et al. (2011) (38) demonstrated that photobioreactors operating under red light achieved superior photosynthetic efficiency and higher phycocyanin yields compared to blue light systems. Similarly, Wicaksono et al. (2019) (37) report-

ed that red light exposure resulted in the highest phycocyanin extraction efficiency. Interactions between light exposure and temperature (39) also contribute to phycocyanin degradation. In one study conducted at 25°C with photosynthetic photon flux densities (PPFD) of 50 and 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, phycocyanin concentration decreased progressively over time in a dose-dependent manner (40). Moreover, continuous exposure to 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD for 36 hours reduced protein concentration by approximately 20%, with greater degradation observed compared to exposure at 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (40). Consequently, storage under dark conditions is recommended to preserve phycocyanin stability (31).

Solvent selection also plays a critical role in phycocyanin extraction due to differences in ionic strength among solvents (10). The optimal solvent should maintain a pH range compatible with phycocyanin stability. Several studies have reported improved extraction yields using sodium phosphate buffer (pH 7.0) compared with distilled water (42,43,44,45). In contrast, other authors observed superior yields with distilled water (46,47), potassium phosphate buffer (pH 6.5) (48), or calcium dichloride solution (pH 7.4) (15). Conversely, acetate buffer at pH 5.0 produced lower phycocyanin yields (42).

Exposure to light and elevated ionic strength accelerates phycocyanin degradation. Encapsulation within biopolymers such as chitosan has been shown to improve resistance against these destabilizing factors (49,50).

Biomass-to-Solvent Ratio and Use of Preservatives

Increasing the biomass-to-solvent ratio is generally associated with enhanced extraction yields, although it may simultaneously reduce phycocyanin purity (10). Within ratios ranging from 1:6 to 1:10, studies employing sodium phosphate buffer (pH 6.8) demonstrated higher extraction yields at a 1:10 ratio and greater purity at a 1:6 ratio (10,13). Specifically, yields of 53.84 mg/g were reported at a 1:10 ratio, whereas a purity index of 0.6 with an extraction yield of 52.11 mg/g was obtained at a 1:6 ratio (51). In another study comparing different extraction methods and surfactants (Triton X-100, Tween 20, and Tween 80), higher purity values were likewise observed. At a 1:6 ratio, purity values ranged from 0.67 to 1.09, with

extraction yields between 52.92 and 92.73 mg/g dry biomass and extraction efficiencies of 50.35–77.92%, whereas at a 1:10 ratio, purity ranged from 0.68 to 0.9 with yields of 65.14–99.63 mg/g (13). This observation may be explained by the extraction of larger amounts of interfering proteins when higher solvent volumes are used (10).

Another investigation evaluating extraction ratios of 1:50, 1:25, and 3:50 using sodium phosphate buffer (pH 7.0) identified the 1:50 ratio as providing the highest phycocyanin yield and purity (purity 1.2; extraction yield ~80%) (43). Nevertheless, successful extraction has also been reported at ratios of 1:25 (purity 0.46; yield 3.68 mg/mL) (42), 1:20 (yield 41.90 mg/g) (52), and 1:15 (purity 0.8) (45). Therefore, optimization of the biomass-to-solvent ratio is essential to reduce extraction costs while maintaining satisfactory yield and purity.

Despite optimization of extraction conditions, phycocyanin remains highly susceptible to environmental degradation. Consequently, the incorporation of stabilizing agents is necessary to preserve both its stability and bioactivity (29). Sugars improve protein–solvent interactions, thereby contributing to stabilization of the protein structure (20). The stabilizing effect depends on both the type and concentration of the sugar employed. The addition of glucose (20–40% w/w), fructose (10–15% w/w), and sucrose (70% w/w) has been reported to prolong the half-life of phycocyanin (16,18,20). Chaiklahan et al. (2012) (16) demonstrated that supplementation with 20% w/w glucose or sucrose preserved more than 62% of phycocyanin after 15 minutes at 60°C, compared with only 47% retention in the absence of stabilizers. Wu et al. (2016) (31) further identified sodium chloride as an effective stabilizing agent; however, concentrations of 5% w/w resulted in solution turbidity (16).

Citric acid has also been investigated as a stabilizer for phycocyanin extracted from *Arthrospira platensis* to improve thermal stability during incubation at 80°C for 1 hour. In the presence of citric acid, phycocyanin concentration decreased from 65% to 19% over 1 hour, whereas in unstabilized solutions the residual concentration declined from 51% to 11% (53). Another study demonstrated that citric acid maintained up to 67% of the initial phycocyanin

concentration after 45 days, compared with only 3% retention in samples lacking stabilizer (17).

Crosslinking agents such as formaldehyde and glutaraldehyde form bonds between polymeric chains and proteins, thereby preventing phycocyanin degradation; however, their toxicity limits their applicability in the food industry (22). Natural polymers have also been investigated as stabilizing excipients. Although polysaccharides increase medium viscosity and may limit certain applications, whey protein, egg white protein, pea protein, and carrageenan have demonstrated protective effects on the chromophore structure, thereby preserving phycocyanin stability and maintaining its characteristic blue coloration (24,54).

Encapsulation approaches involving nanofibers, microparticles, and nanoparticles are likewise widely applied to protect phycocyanin against degradation under variable temperature and pH conditions (55,56,57,58,59,60). Nevertheless, the development of innovative methods for obtaining and preserving highly purified phycocyanin remains an important future challenge. Emerging technologies such as microfluidic systems and supercritical fluid extraction are currently regarded as promising alternatives for phycocyanin extraction (61). Top of Form Bottom of Form

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