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Methods for Extraction, Isolation and Purification of C-phycocyanin: 50 years of Research in Review

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Abstract

Context: *Spirulina (Arthrospira)* exerts a wide spectrum of pharmacological activities that are largely attributed to its phycobiliprotein content, mainly to C-phycocyanin. The extraction, isolation and purification of C-phycocyanin have been studied for many years, resulting in diverse methodologies with a range of yields and grades of purity.

Objective: We performed a systematic review of the literature, consulting all the available years in TOXNET, PubMed/MEDLINE and Science Direct-Scopus. Search criteria included the separation, isolation, and purification methods for C-phycocyanin from different microorganisms. Search words were: *extraction, separation, isolation and purification of C-phycocyanin.*

Results: The combination of aqueous two-phase systems for extraction and ultrafiltration for purification results in the best yields and highest purity of the desired nutraceuticals. It is also essential to consider the freshness and species of the primary biomass, as these factors heavily influence the concentration and viability of the phycobiliproteins and therefore affect the yield and purity.

Conclusion: In order to preserve the valuable properties and health benefits of nutraceuticals, such as C-phycocyanin, it is essential to seek innovative methods for isolating and purifying these bioactive substances from natural sources. The information herein gathered indicates the best methods currently available.

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Keywords: C-phycocyanin; Extraction; Isolation; Phycobiliproteins; Purification; *Spirulina*

Introduction

Spirulina spp, or *Arthrospira*, is a microscopic and filamentous cyanobacteria with a wide variety of applications including its use as a food source; in fact, Spirulina has been used as food in Mexico since pre-Hispanic times. (Dillon, Phuc, & Dubacq,[1]; Venkataraman,[2]).

Nutritional and functional properties of Spirulina

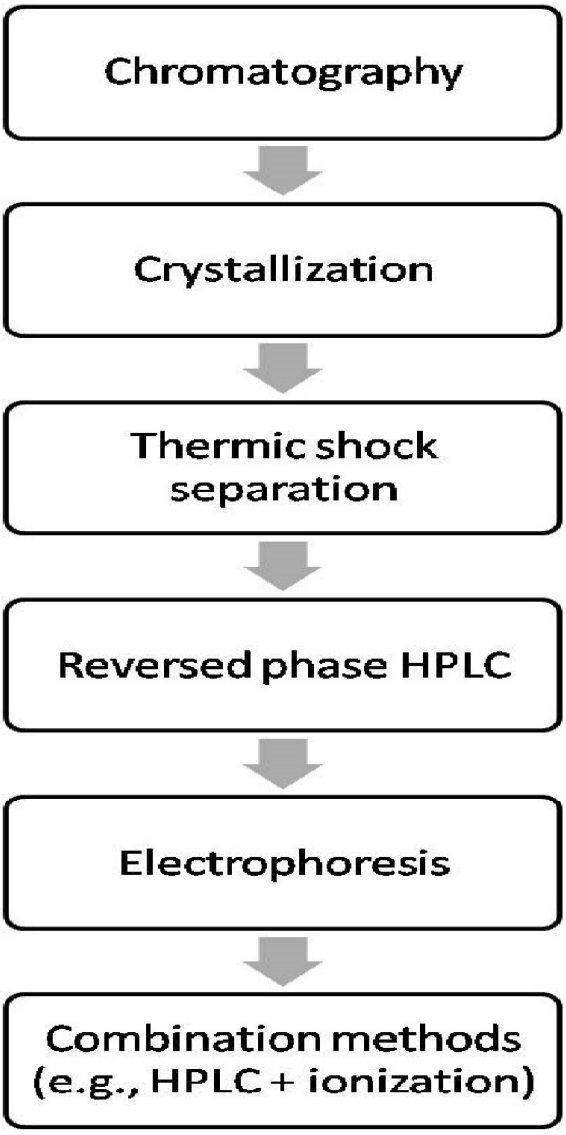
The popularity of *Spirulina* as a food supplement is due to its high protein content (about 70% of its dry weight) and high

biological value (containing essential amino acids like phenylalanine and methionine). *Spirulina* also contains vitamins: B_{12} (cyanocobalamin), B_6 (pyridoxine), B_1 (thiamine) and B_2 (riboflavin), as well as beta carotenes (precursors of vitamin A); minerals (e.g., iron, zinc, selenium, calcium and magnesium), phytochemicals (phenolic acids and tocopherols), and essential fatty acids (such as gamma linoleic acid) (Belay,[3]; Dillon et al.,[1]; Habib et al., 2008).

Spirulina is considered a functional food because of its broad spectrum of biologic effects, which have been demonstrated *in vitro* and *in vivo*. Among these, Spirulina has been reported to exhibit anti-inflammatory (Remirez, Ledón, & González,[4]), anti-hyperlipidemic (Torres-Duran, Ferreira-Hermosillo, & Juarez-Oropeza,[5]), hypoglycemic (Lima, Facchinetti, & Santos,[6]), antihypertensive (Torres-Duran et al.,[5]), antineoplastic (Mittal, Suresh Kumar, Banerjee, Rao, & Kumar,[7]), antiviral (Lee et al.,[8]), antianemic (Simsek, Karadeniz, Kalkan, Keles, & Unal,[9]), and antioxidant activity (Karkos, Leong, Karkos, Sivaji, & Assimakopoulos,[10]). All of these nutraceutical benefits are attributed to substances in *Spirulina* known as phycobiliproteins (C-phycocyanin, allophycocyanin, phycoerythrin and phycoerythrocyanin), which constitute their own protein complex in association with their linker polypeptides, called phycobilisome (Gantt, Lipschultz, Grabowski, & Zimmerman,[11]; Hoseini, Khosravi-Darani, & Mozafari,[12]). Isolation and testing of these phycobiliproteins has shown that they possess same beneficial effects as the whole microalgae. Hence, they are considered as the actual bioactive agents in this functional food (Eriksen,[13]; Hoseini et al.,[12]; Khan et al.,[14]).

Obtention of C-phycocyanin: brief historical perspective

Different methods of extraction, isolation and purification (summarized in Figure 1) have been assayed in order to obtain these phycobiliproteins (especially C-phycocyanin) from *Spirulina spp* (Glazer, Lundell, Yamanaka, & Williams,[15]; Khan et al.,[14]). The first attempts involved simple chromatography by using precipitations previously obtained with ammonium sulphate. Subsequently, phycobiliproteins were isolated by crystallization (Carra,[16]). However, these methods lacked specificity as they extracted the whole phycobilisome without separating each pigment.



Years later, a thermal shock-based separation technique allowed pigment separation via density-gradient centrifugation with sucrose (Bekasova, Muslimov, & Krasnovskii,[17]). Although these processes represented an advance, the overall purity and yield of the process was still low.

With the use of reversed phase high-performance liquid chromatography (RP-HPLC), it was possible to achieve an isolation of up to 85% of C-phycocyanin and allophycocyanin. (Swanson & Glazer,[18]) Nevertheless, other compounds apart from the phycobilisome were present in the final products due to the sample pre-treatments needed to perform HPLC. In order to optimize phycobiliprotein isolation, the chromatographic method was modified by adapting resins used in the solid phase, varying the polarity and pH of the eluent solution (Moreno et al.,[19]), and using magnesium chloride precipitation with further diffusion in polyethylene glycol gel. By enhancing overall specificity, the isolation of pure C-phycocyanin and allophycocyanin was obtained, but the yielding mass was significantly decreased.

Towards the end of the 20th century, electrophoresis-based techniques were tested with the addition of laser-induced fluorescence (LIF) detectors. This novel achieved separation with a fairly good yield (≈90 - 100%). Nevertheless, such extractions corresponded to a mixture of both C-phycocyanin and allophycocyanin (Viskari & Colyer,[20]). Yet another type of electrophoresis was used with polyacrylamide/dodecyl sulphate gel, pre-treating the samples by precipitation with ammonium sulphate followed by further separation in chromatographic columns by Sephadex (*Minkova et al., 2003*). This technique achieved an isolation of pure C-phycocyanin with a yield of ≈ 45%.

Afterwards, different combinations of methods were tested in order to increase the ease of separation and isolation as well as the grade of purity and final yield. One such method, HPLC coupled with a flame ionization detector (Zolla & Bianchetti,[21]), was able to separate C-phycocyanin from allophycocyanin, yet it destroyed the original sample. In order to resolve this problem, an integral procedure was devised that extracted the phycobiliproteins with sodium phosphate neutral buffer, and then further purified them via dialysis and gel filtration chromatography (Bhaskar, Gopalaswamy, & Raghu,[22]). This procedure yielded C-phycocyanin with a purity of 4.98. Another method, pre-treated the sample in the same manner, but the purification process consisted of ion-exchange chromatography (Patel, Mishra, Pawar, & Ghosh,[23]), yielding C-phycocyanin with a purity of 4.42.

Another widely studied method for isolating C-phycocyanin from *Spirulina platensis* combined chromatography with expanded bed adsorption, anion interchange, and hydroxyapatite columns (Niu, Wang, Lin, & Zhou,[24]). These techniques yielded 4.45 mg of C-phycocyanin per gram of dried *S. platensis* with a purity of 3.2. This method offers several advantages, such as the possibility of using different resins with special charge characteristics (i.e., anionic or cationic). For instance, by using Q-Sepharose (*Silveira, de Menezes Quines, Burkert, & Kalil, 2008)* it was possible to isolate C-phycocyanin from *Spirulina platensis* with ~75% yield and a purity of 3.4. The problem with these kinds of techniques is that they are often strongly dependent on the pH and temperature of the eluent solutions.

On the other hand, hydrophobic interaction chromatography with ammonium sulphate and liquid nitrogen precipitation pretreatments (Soni, Trivedi, & Madamwar,[25]) are capable of isolating C-phycocyanin with a purity of 4.5 but with poor yields. This may be due to the original cyanobacterium (*Phormidium fragile*) from which the phycobilin was isolated. To improve the yield, C-phycocyanin was extracted from *Spirulina platensis* with high-speed counter-current chromatography (HSCCC) (Yin et al.,[26]), obtaining 78.7 mg per 200 mg of crude extract with a purity of 4.25. Nowadays, one of the most widely used methods is ionic exchange chromatography, which involves pre treating vegetable samples of *Spirulina platensis* with two aqueous phases(Patil, Chethana, Sridevi, & Raghavarao,[27]), leading to a purity of 6.69.

The aim of the present review was to describe different methods for C-phycocyanin extraction and purification and compare the results in order to determine the method with the best cost-benefit ratio.

Methods

We performed an exhaustive search (using Scopus and PubMed databases) to find methods for the separation, isolation and purification of C-phycocyanin from different microorganisms. Search words were: *extraction, separation, isolation* and *purification of C-phycocyanin*.

Results

Summaries and general characteristics are herein presented (see Table) for the 86 reports found.

Extraction by cell disruption	Purification method	Observations	Reference
Thermal treatment	Density gradient by centrifugation	The phycobilisomes of *N. muscorum* were separated into two subunits containing C-phycocyanin and allophycocyanin. However, they had traces of phycoerythrocyanin, thus presenting low purity and low yields (data not shown).	Bekasova et al.[17]
	Reverse phase chromatography using dicarboxylic acids and methanol-butanol washes	Traces of C-phycocyanin and phycoerythrocyanin were identified by mass spectrometry chromatography.	Fu, Friedman, and Siegelman[28]

Pressure homogenization	Aqueous two-phase system (ATPS)	Ionic exchange chromatography	C-phycocyanin was obtained at a6.69 grade of purity from the aqueous extract of *Spirulina platensis.*	Patil et al.[29]
		Polyethylene glycol 4000 and potassium phosphate saturation	C-phycocyaninwas obtained from *Spirulina platensis* in a single extraction step. With multiple extractions, the purity of the isolates increases from 3.23 to 4.02.	Patil, Chethana, Madhusudhan, and Raghavarao[29]
		Ultra filtration / Salting out (precipitation crystallization)	C-phycocyaninwas obtained from *Spirulina maxima* with a purity of 3.8%.	Rito-Palomares, Nuñez, and Amador[30]
	Hexane extraction	SDS-PAGE electrophoresis	C-phycocyanin was obtained from *Spirulina spp* at a yield of 10.2% and a purity of 1.	Seo et al.[31]
	Stirring-centrifugation	Expanded bed anion exchange with 80% ammonium sulfate	25.7 mg g^{-1}dm of C-phycocyanin was obtained from fresh *Spirulina platensis* at a purity of 4.8.	Moraes, Mazutti, Maugeri, and Kalil [32]
		Precipitation with ammonium sulfate Fast flow chromatography DEAE-Sepharose and hydroxyapatite columns	C-phycocyanin was isolated from *Spirulina platensis* with a yield of 30 mg g^{-1}dm and a purity of 3.94.	Ou, Lin, Yang, Pan, and Cheng[33]
Freeze-unfreeze agitation	Aqueous two-phase system (ATPS)	Gel filtration chromatography	C-phycocyaninwas obtained from *Spirulina maxima* with a yield of 46.5 % and a purity of 3.4.	Cruz de Jesús[34]
		Ion exchange chromatography	C-phycocyanin was obtained from *Spirulina maxima* with a yield of 37.5% and a purity of 3.5 (determined by DEAE-Cellulose).	
		Ultra filtration	In this last stage, C-phycocyanin was isolated from fresh *Spirulina maxima* with a yield of 57% and a purity of 3.9.	
		Ultracentrifugation	C–phycocyaninwas isolated from cyanobacteria (*Spirulina maxima* and *Porphyridium cruentum*) with a yield of 98%, but with low purity (2.1).	Benavides and Rito-Palomares[35]
	Organic solvents and buffer extraction	Centrifugation and filtration	C–phycocyanin obtained from *Spirulina platensis* had low yield and low purity (0.46).	Silveira, Burkert, Costa, Burkert, and Kalil[36]
		Polyethylene glycol systems 1500, 4000 and 6000/aqueous two-phase system (ATPS)	2.67 mg/g of C-phycocyanin was isolated from *Spirulina platensis* with a purity of 0.79.	Antelo, Anschau, Costa, and Kalil[37]
Freeze-unfreeze cycles	Agitation-centrifugation		C-phycocyanin was extracted and isolated from some cyanobacteria (*Synechocystisspp, Glueucopsaspp, Anabaena spp* and *Lyngbyaspp*) with a yield of 100 µg/g dm and a purity of 3.1.	Maurya, Maurya, and Pandey[38]
	Ultra filtration	Ion exchange chromatography	C-phycocyanin was isolated from *P. ceylanicum* with a yield of 63.50% anda purity of 4.15.	Singh, Parmar, and Madamwar (2009)
			A phycobiliprotein was obtained fromthe fresh biomass of *Spirulina spp* witha yield of 82.9 to 88.6% and a purity of 1.0.	Chaiklahan, Chirasuwan, Loha, Tia, and Bunnag [39]
		Tri chloro acetic precipitation (TCA)/centrifugation Electrophoresis SDS-PAGE	C-phycocyanin was isolated from cyano bacteria *Phorphyra columbina* with a yield of 19.9 mg/g dm and a purity of 0.08.	Cian, López-Posadas, Drago, Medina, and Martínez-Augustin[40]
	Cell disruption by pressure / agitation and centrifugation	Purification by hydroxyapatite column chromatography and anion exchange / ultra filtration / electrophoresis SDS-PAGE	C-phycocyanin was extracted from the cyano bacteria *Anabaena spp* with a yield of 10% and a purity of 2.7.	Ducret, Sidler, Wehrli, Frank, and Zuber[41]
			C-phycocyaninwas isolated from *Spirulina spp* and purified, with a yield of 85 % and a purity of 3.66.	Yoshida, Takagaki, and Nishimune[42]

	Precipitation with ammonium sulfate (25%)	Column elution hydroxyapatite / Sephadex -DEAE ion exchange / Bio-Gel electrophoresis P	C-phycocyanin was extracted from lyophilized *Spirulina platensis* with a purity of 4.0.	Bermejo-Bescós, Piñero-Estrada, and Villar del Fresno[43]
			C-phycocyanin was isolated from *Synechococcus spp* and *Aphanocapsa cyano bacteria* with a yield of 50 % and a purity of 6.1.	Glazer and Cohen-Bazire[44]
			C-phycocyaninwas extracted and purified from *Porphyrayezoensis cyano bacterium* with a yield of 20 % and a purity of 0.9	He, Hu, and Jiang[45]
			C-phycocyanin was isolated and purified from the fresh biomass of *Spirulina platensis* with a yield of 13.1 % and a purity of 4.71.	Li, Zhang, Gao, and Chu [46]
			C-phycocyanin was extracted and isolated from *Spp Chroomonas* cyano bacterium with a yield of 59 % and a purity of 0.92.	MacColl, Habig, and Berns[47]
			C -phycocyanin was isolated from fresh *Spirulina platensis* with a yield of 95 µg/g dmand a purity of 3.9.	Piñero Estrada, Bermejo Bescós, and Villar del Fresno[48]
		Step chromatography with DEAE cellulose-11	The extract isolated from *Spirulina platensis* was identified as C–phycocyaninby SDS-PAGE, with a yield of 80% and a purity of 4.5.	Kumar, Dhar, Pabbi, Kumar, and Walia[49]
			C-phycocyanin was extracted and isolated from *Anabaena variabilis cyano bacterium* with a yield of 36 % and a purity of 2.75.	Chakdar, Saha, and Pabbi[50]
		Precipitation with ammonium sulfate (50 %)	C-phycocyanin was obtained from *Galdieria sulphuraria cyano bacteria* with a yield of 80 % and a purity of 4.	Moon et al.[51]
			C-phycocyanin was isolated and purified from *Spirulina maxima* with yield of 24% and a purity of 2.25.	Abd El-Baky and El-Baroty[52]
			C-phycocyaninwas extracted and isolated from cyanobacterium of the *Nostoc spp* genus, with a yield of 59% and a purity of 2.8.	Gray, Lipschultz, and Gantt[53]
			C-phycocyaninwas isolated and purified from *Spirulina fusiformis* with a yield of 60% and purity of 3.8.	Madhyastha, Radha, Sugiki, Omura, and Maruyama[54]
		Ultracentrifugation	C-phycocyanin was isolated from *Cyanidium caldarium* cyanobacterium with a yield of 15 mg g-1dmand a purity of 7.	Stec, Troxler, and Teeter[55]
		Activated carbon and chitosan , flow filtration	High purity C- phycocyanin was obtained from *Limnotrix spp* with low ammonium sulfate concentrations.	Gantar, Simović, Djilas, Gonzalez, and Miksovska[56]
	Ionic Exchange chromatography (DEAE-Sephadex)		A phycobiliproteinwas obtained from *Spirulina platensis* under a three-step procedure, increasing its purity to 4.3 (identified by SDS–PAGE).	Liao, Zhang, Wang, Yan, and Zhang[57]
	Anion exchange chromatography by hydrophobic interactions (butyl- Sepharose column)	Ion exchange chromatography (Q-Sepharose column) / filtration SDS-PAGE gel	C-phycocyaninwas obtained from *Synechococcus spp* with high purity and good yield.	Abalde, Berancour, Torres, Cid, and Barwell[58]
	Tricalcium phosphate gel chromatography		A set of phycobiliproteins was extracted from *Smithoranaiadum* microalga. Allophycocyanin, phycoerythrocyanin and C- phycocyanin were obtained after centrifugation, the latter at a low yield.	ÓhEocha and Haxo[59]

Cellular disruption	Purification method		Observations	References
Liquid nitrogen	Precipitation/ crystallization	Ammonium sulfate	A mixture of C-phycocyanin and R- phycocyanin-phycoblasts were obtained from *N. muscorum*.	Carra[16]
		Ammonium-sulfate / hydrophobic interaction chromatography	C-phycocyanin was isolated from *Phormidium fragile*, with low yield and a purity of 4.52.	Soni et al.[25]
		SDS-PAGE and mercaptoethanol / chromatography (column sulfonated polystyrene)	Allophycocyanin and C–phycocyanin were isolated among *Phormidiumluridum* phycobili proteins, with low yield and low purity.	Kobayashi, Siegelman, and Hirs[60]
		Stirring and precipitation with ammonium sulfate / SDS-PAGE electrophoresis	C-phycocyanin was isolated from *Oscillatona cyanobacterial* agardhii with 70% yield and a purity of 4.35.	Torjesen and Sletten[61]
	High performance liquid chromatography (HPLC)	Reversed phase	C-phycocyanin and allophycocyan in were isolated with a yield of 85 %.The impurities were traces of compounds outside the phycobilisome.	Swanson and Glazer[18]
		Flame ionization	C-phycocyanin was separated from allophycocyanin(derived from *Synechocystis*). However, in the identification process the sample was lost.	Zolla and Bianchetti 2001
	SDS-PAGE-electrophoresis	Laser induced fluorescence (LIF)	A mixture of C-phycocyanin and allophycocyanin was extracted, with yields of about 93-105 %.	Viskari and Colyer[20]
		Polyacrylamide gel dodecyl sulfate / Sephadex column	C-phycocyanin was extracted from *Spirulina fusiformis* with a 46% yield.	Minkova et al. (2003)
		Freezing and thawing cycles / dialysis / centrifugation / ammonium sulfate (20%) precipitation, and chromatography by filtration on DEAE - Sepharose gel	C-phycocyanin was obtained from cyano bacterium *Oscillatoria tenuis* with a yield of 61.8 % and a purity of 4.88.	Thangam et al.[62]
	Chromatography	Magnesium chloride / polyethylene glycol 6000	A mixture of C-phycocyanin and allophycocyanin was obtained from *Spirulina platensis* with low yield and high purity, analyzed by X-ray diffraction.	Moreno et al.[19]
		Gel filtration / ammonium sulfate / dialysis	C-phycocyanin was obtained from *Spirulina platensis* at a purity of 4.98.	Bhaskar et al.[22]
		Ionic exchange	C-phycocyanin was extracted from *Spirulina* with a purity of 4.42.	Patel et al.[23]
			C–phycocyanin was isolated from *S. platensis*-using Q-Sepharose, with a yield of 77.3 % and a purity of 3.4.	Silveira et al. (2008)
		Hydrophobic interaction chromatography / ion exchange chromatography	C–phycocyanin was extracted from *Calothrixsp* with apurity of 3.3.	Santiago-Santos, Ponce-Noyola, Olvera-Ramírez, Ortega-López, and Cañizares-Villanueva[63]
		Bed adsorption / anion exchange / hydroxyapatite column	4.45mg g-1 of C–phycocyanin was isolated from dry *S. platensis* with a purity of 3.2.	Niu et al.[24]
			C-phycocyanin was isolated from cyanobacterium *Aphanizomenonflos-aquae* with a purity of4.78.	Benedetti et al.[64]
			C-phycocyanin was purified from *Porphyrayezoensisby* electrophoresis, with a good yield and high grade of purity.	Cai et al.[65]
			C-phycocyanin was isolated from *Anabaena marine* with a yield of 62%and a purity of 4.	Ramos, Acién, Fernández-Sevilla, González, and Bermejo[66]
		Liquid phaseisoelectric focusing	C-phycocyanin was isolated from *Spirulina platensis* with a yield of 39.2 % and a purity of 4.0.	Huang, Yang, Zheng, and Guo[67]
		High-speed counter current chromatography (HSCCC) / reversed phase	79 mg of C-phycocyanin was extracted from *Spirulina platensis* with a purity of 4.25, and was identified by SDS-PAGE.	Yin et al[26]

Osmotic shock	Bed adsorption chromatography (streamline - DEAE column)	Ion exchange chromatography (DEAE - cellulose)	C-phycocyaninwas isolated from *Spirulina platensis* with a purity of 4.6.	Moraes, da Costa Ores, Costa, and Kalil[68]
			C–phycocyanin was isolated from cyanobacterium *Synechocystisaquatilis* with a yield of 74% and a purity of 4.0.	Ramos, Acién, Fernández-Sevilla, González, and Bermejo[69]
			C–phycocyanin was isolated from *Spirulina platensis* with a yield of 59% and a high degree of purity, andwas identified by SDS-PAGE.	Ruperto Bermejo and Ramos[70]
			C-phycocyanin was isolated from *Spirulina platensis* with pharmaceutical purity (4), and was identified by SDS-PAGE.	R. Bermejo, Felipe, Talavera, and Alvarez-Pez[71]
	Enzymatic digestionwith lysozyme	Activated carbon and chitosan	C-phycocyanin was extracted from *Synechococcusspp* with a yield of 80 % and a purity of 4.27.	Gupta and Sainis[72]
		Dialysis / centrifugation/ precipitation with ammonium sulfate / ultrafiltration	C-phycocyanin was extracted and isolated from *Coccochloris cyanobacterial elabens* with a yield of 0.12 mg per gram of dry biomass and a purity of 2.5.	Kao, Berns, and Town[73]
		Agitation / centrifugation / precipitation with 35 % ammonium sulfate/ dialysis / fast flow column chromatography (DEAE-Sepharose)	C-phycocyanin was isolated from *Spirulina platensis* with a yield of 4.43 mg g^{-1} of dm and a purity of3.9.	Pleonsil, Soogarun, and Suwanwong[74]
		Agitation / centrifugation / precipitation with 35 % and 50% of ammonium sulfate / hydrophobic interaction Chromatography (DEAE–Sepharose column) / ion exchange column/ chromatographic gel	C-phycocyanin was isolated from fresh Spirulina platensis with a yield of 566.50 mg/g^{-1} and a purity of 5.32.	Song, Zhao, and Wang[75]
	Rivanol–ammonium sulfate (50%) precipitation	Activated carbon and chitosan / purification by Sephadex column	C-phycocyanin was isolated from two species of cyanobacteria (*Spirulina maximum* and *Spirulina fusiformis*) with a yield of 55% and a purity of 4.50 in both species, and was identified by SDS-PAGE.	Kaledona Minkova et al.[76]
		Sodium chloride precipitation/ Sephadex column purification	C-phycocyanin was obtained from cyanobacterium *Africanum Arthronema* with a yield of 55 % and a purity of 4.52, and was identified by SDS-PAGE.	K. Minkova et al.[77]
		Ion exchange chromatography (DEAE -Sepharose column) / filtration	C-phycocyanin was isolated from cyanobacterium *Spirulina platensis*, which was grown in medium enriched with selenium, achieving a purity of 5.12.	Chen, Wong, and Zheng[78]
			C-phycocyanin was isolated from *Spirulina platensis* after two purification processes with a yield of 67.04 % and a purity of 5.59.	Yan et al.[79]
			C-phycocyanin was isolated from *Spirulina platensis cyano bacterium* with a purity of 4.0.	Moraes and Kalil[80]
			C-phycocyanin was isolated from *Spirulina platensis* with pharmaceutical grade purity (5.06).	Zhang and Chen[81]
	Aqueous two-phase system (ATPS)	Ultrafiltration dialysis membrane / ion exchange chromatography (DEAE -Sepharose column)	C-phycocyanin was isolated from *Galdieria sulphuraria* with a yield of 42% and a purity of 4.5.	Sørensen, Hantke, and Eriksen[82]
	EDTA precipitation / filtration / agitation / centrifugation	SDS-PAGE electrophoresis	C-phycocyanin was extracted and isolated from *Acaryochloris* marine cyanobacterium with a yield of 15 % and a purity of 2.0.	Marquardt, Senger, Miyashita, Miyachi, and Mörschel[83]
	Ultrasonication with buffer		C-phycocyanin was isolated from *Spirulina platensis* with pharmaceutical grade purity.	Sun, Wang, and Qiao[84]

Discussion

Novel extraction, isolation, and purification processes for C-phycocyanin have been sought and developed since the 1980's. (Khan et al.,[73]) Throughout this process, it has been demonstrated that the original biomass is of critical importance in order to reach the best cost-benefit ratio when isolating phycobiliproteins. Another feature that must be considered is the freshness of the biomass and the subsequent pretreatment processes. In this series, C-phycocyanin was obtained from fresh biomass and dried at room temperature (as opposed to using lyophilized powders) (Chaiklahan et al.,[15] 2011; Niu et al.,[50]).

Regarding extraction and purification methods, previous studies have shown that multiple cycles improved the purity grade of the C-phycocyanin extract, although yields significantly decreased. For instance, an aqueous two-phase system with polyethylene glycol 4000 (Patil et al.,[4]) managed to increase purity, but yields were importantly reduced. Similarly, a multiple extraction process for obtaining C-phycocyanin by using a Sephadex column (Minkova et al., 2003) achieved a yield of 46% with acceptable purity (established by Rito-Palomares et al.,[5]).

Based on the information herein gathered, a protocol was proposed with a one-step extraction processin order to obtain both a good yield and a high grade of purity. This was accomplished by using an aqueous two-phase system with a posterior ultrafiltration, giving C-phycocyaninin a yield of 57% and a purity of 3.9, thus surpassing the results of previous methodologies. It can be clearly seen that the preferred method should not be based on adsorption or elucidation, thus ruling out chromatography, because these processes diminish the yield of the extract (Cruz de Jesus,[74]).

Conclusions

To maximize the health benefits that may be obtained from nutraceuticals, such as C-phycocyanin, it is essential to seek innovative methods for their isolation and purification, and thus preserve the valuable properties of these bioactive substances from natural sources. The current review makes it evident that to obtain nutraceuticals from extracts and achieve good yield and high purity; it is convenient to use aqueous two–phase systems for extraction together with ultrafiltration for purification. It is also essential to consider the freshness and species of the primary biomass, as these factors heavily influence the concentration and viability of the desired phycobiliproteins and therefore affect the yield and purity.

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