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Fast centrifugal partition chromatography for C-Phycocyanin purification from *Limnospira platensis* using eutectic solvent-based biphasic systems

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ABSTRACT

The development of sustainable separation strategies for high-value bioproducts from microalgae is essential for advancing integrated biorefinery approaches. This study investigates the use of eutectic solvent (ES)-based biphasic systems for the selective separation of C-phycocyanin (C-PC), a water-soluble pigment-protein complex, from the crude extract of *Limnospira platensis* (*L. platensis*). Two ESs combinations were selected based on phase stability and partitioning behavior: (i) a hydrophilic ES composed of glucose:glycerol:H_2O (1:2:2.5) diluted with deionized water and paired with the hydrophobic ES decanoic acid:menthol (2:1), and (ii) the same hydrophilic ES diluted with phosphate buffer solution (14 *wt%*) and combined with a hydrophobic ES comprising octanoic acid:decanoic acid:dodecanoic acid (3:2:1). These systems were evaluated using ultra-Fast Centrifugal Partition Chromatography (FCPC) under optimized conditions to enhance phase retention and resolution. The first system demonstrated effective separation with partition coefficients of 1.53 for C-PC and 0.50 for chlorophyll, achieving high purity at low pigment concentrations (15.9 $mg_{C\text{-}PC}{\cdot}L^{-1}$ purity 5 times higher towards total proteins and chlorophylls). In contrast, the second system yielded more concentrated fractions (up to 615.9 $mg_{C\text{-}PC}{\cdot}L^{-1}$ purity 5 and 0.87 times higher towards total proteins and chlorophylls, respectively) but exhibited low selectivity, with both pigments presenting similar partition behavior ($K \approx 0.03$). Overall, the results feature the potential of ES-based biphasic systems for pigment purification and highlight the trade-offs between resolution, purity, and concentration.

1. Introduction

Limnospira platensis (*L. platensis*), known as a superfood, is rich in bioactive compounds such as polysaccharides, phenolic compounds, lipids, proteins, enzymes, and natural pigments, making it valuable for nutrition, pharmaceuticals, and biotechnology [1–3]. Proteins constitute 60–70 % of the dry biomass, with C-phycocyanin (C-PC) being the most important due to its distinctive blue color and potent antioxidant and anti-inflammatory properties [4]. C-PC is a hydrophilic blue natural colorant applied in food and beverages, in nutraceuticals, cosmetics, dietary supplements and wellness products, and pharmaceuticals. [2,5,6]. Its market value is strongly dependent on purity, a parameter assessed by the A_{620}/A_{280} absorbance ratio [7,8]. Food-grade C-PC (purity $\geq$0.7) is primarily used as a natural colorant in the food and beverage industry, maintaining its vibrant blue color despite containing residual proteins and pigments, with prices ranging from $0.13 to $0.35 *per* mg [8,9]. Analytical-grade C-PC (purity $\geq$3.0) required for biotechnological and research applications, reaches prices up to $15.00 *per* mg [10], while pharmaceutical-grade C-PC (purity $\geq$4.0) represents even higher prices due to the additional processing and safety requirements [11]. Achieving higher purity expands applications and boosts economic value, [12] but producing pharmaceutical-grade C-PC is challenging, requiring the removal of impurities without loss of bioactivity [13].

Downstream processing of *L. platensis* has focused on cost-effective strategies for highly pure C-PC recovery. Efficient purification strategies are critical not only to ensure product quality but also to enhance the economic feasibility of large-scale applications [8,14]. Ultrafiltration is widely regarded as a scalable and selective alternative to ultracentrifugation, particularly for protein enrichment and impurity

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reduction. However, the high energy demand associated with this operation limits its suitability as a sustainable downstream operation [15,16]. Diafiltration is used to remove residual contaminants, followed by precipitation to concentrate the C-PC [17,18]. While effective, these methods are labor-intensive and time-consuming. Additionally, these membrane-based techniques rely primarily on size-based separation, which becomes inefficient when target compounds, such as C-PC, and co-extracted impurities (other proteins) exhibit similar molecular dimensions. This lack of selectivity can compromise purification efficiency and contribute to product loss during processing [19]. One of the primary challenges in C-PC purification is to maintain its structural integrity and functional bioactivity, as the pigment is highly susceptible to temperature fluctuations and pH variations [4,19]. Extended processing times and inappropriate process conditions can lead to protein denaturation, loss of color, and reduced bioactivity. Developing optimized and application-specific purification strategies is therefore imperative for economically viable, large-scale production of highly pure C-PC, simultaneously maintaining its main properties [8].

Ultra-Fast Centrifugal Partition Chromatography (FCPC) is an advanced liquid–liquid chromatography technique that achieves high-resolution separations via countercurrent partitioning between two immiscible liquid phases. It uses a rotating cylinder set up as a column of interconnected chambers to generate a uniform centrifugal force, [20,21] immobilizing one liquid phase as stationary [22] while the other acts as the mobile phase. Its ability to hold large stationary volumes makes it suitable for industrial-scale applications [23,24]. The system can operate in ascending or descending mode, depending on whether the lighter or heavier phase is mobile phase [25]. The liquid–liquid design eliminates the need for solid chromatographic supports, ensuring near 100 % compound recovery, facilitating solvent recyclability, and reducing maintenance costs [22], and sustainable large-scale operations [20,21,24]. Nevertheless, careful selection of the two immiscible phases is essential, considering phase stability, density difference, volume ratio, retention capacity, and separation efficiency [18,21,23,24]. Separation efficiency depends on the stationary phase retention factor (*Sf*%), with higher *Sf*% improving solute partitioning, peak resolution, and minimizing band broadening. *Sf*% optimization requires controlling the biphasic system composition (solvent polarity, interfacial tension, viscosity), rotational speed (centrifugal force and phase retention) and flow rate to balance mass transfer with phase stability. Column shape, volume, and material further influence hydrodynamics. However, optimal solvent selection and hydrodynamic tuning are critical to maintain phase stability and retention.

The liquid–liquid nature of the process eliminates the need for solid chromatographic supports, ensuring almost 100 % recovery of the target compounds, facilitating solvent recyclability, and avoiding the cost and maintenance of solid columns [25]. Traditionally, these systems have first consisted of water-organic solvent liquid–liquid extraction systems, [26,27] and then polymer-polymer or polymer-salt biphasic systems, but recently eutectic solvents (ESs)-based biphasic systems have emerged as a more sustainable and selective alternative [21,28]. Due to their tunability, ESs-based biphasic systems are particularly suited for the extraction and purification of biomolecules [21,28,29]. ESs are formed by combining hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), resulting in a eutectic mixture with a melting point significantly lower than that of its individual components. Hydrophilic ESs are particularly suitable for the extraction and stabilization of polar biomolecules such as C-PC, providing a compatible aqueous-like environment that reduces protein denaturation while enhancing selective partitioning. Hydrophobic ESs, on the other hand, are ideal for the purification of nonpolar compounds, including lipids, terpenoids, and chlorophylls, and improving separation efficiency while maintaining solvent immiscibility [28,30–32].

This study is based on a previous work [29] in which C-PC was successfully extracted and stabilized using a hydrophilic ES composed of glucose:glycerol:H_2O (1:2:2.5). Although this solvent effectively enriched the extract in C-PC, it remained contaminated with other biomolecules. To overcome this limitation, the present work focuses on the design of biphasic systems incorporating hydrophobic ESs able to form stable, immiscible phases while achieving a partition coefficient (K) within the optimal range of FCPC, which is around $0.5 < K < 3$. In this context, the main objectives of the present study are to i) develop a system that selectively partitions C-PC, enhancing its separation from other biomolecules while maintaining its stability; ii) formulate and evaluate biphasic systems based on their ability to generate well-defined interphases, enabling efficient and selective separation; iii) apply the most promising biphasic systems in FCPC, optimizing the key process parameters, such as stationary phase retention, flow rate, and rotational speed, to maximize separation performance. This setup enables the fractionation of the crude C-PC extract into multiple streams with different compositions, facilitating the isolation of a more refined and purified C-PC fraction.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich, and the purity and molecular formula are in brackets. Reagents for ESs preparation: menthol (purity >95 %, $C_{10}H_{20}O$), glycerol (purity = 95 %, $C_3H_8O_3$), glucose (99.5 %, $C_6H_{12}O_6$), octanoic acid (purity ≥99 %, $C_8H_{16}O_2$), decanoic acid (purity >99.5 %, $CH_3(CH_2)_8COOH$), dodecanoic acid (purity = 98 %, $CH_3(CH_2)_{10}COOH$). All reagents were directly used for the synthesis of ESs without further purification. The inorganic salts potassium phosphate dibasic (purity >98 %, K_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were used to prepare the phosphate buffer solution (PBS) with a mass ratio of 0.44 (K_2HPO_4) to 0.56 (NaH_2PO_4) to obtain a pH of 6. C-PC extracted from *L. platensis* was purchased at Sigma-Aldrich with purity $A_{620}/A_{280} > 3.5$.

2.2. ESs preparation

The ESs were prepared as described in the literature [28] with minor modifications. Briefly, a fixed ratio of HBD and HBA was stirred and heated at 100 °C for hydrophilic ESs and at 50 °C for hydrophobic ESs until a colorless solvent was obtained. The list of all ESs used in this study is present in Table 1.

2.3. Biomass source

In this study, a single source of *L. platensis* biomass was used throughout all stages, from the initial extraction of C-PC to final purification of FCPC to ensure consistency in biomass characteristics. The biomass was acquired as a food supplement to *Alma & Valor* (Setúbal, Portugal).

2.4. Extraction process

The extraction process was performed according to [29]. A fixed quantity of *L. platensis* biomass was weighed directly into a falcon tube, and hydrophilic ES composed of 60 *wt%* Glu:Gly:H_2O (1:2:2.5) combined either with 40 *wt%* of H_2O or PBS (14 *wt%*), was added to attain the specified solid-liquid ratio (SLR) of 0.02 g·mL^{-1}. The mixture was then vortexed and stirred using a Rotator Loopster digital from IKA-Werke GmbH & Co. KG at 80 rpm for 30 min at room temperature, under dark conditions to prevent degradation of C-PC.

2.4.1. Determination of the partition coefficients and phases composition

The biphasic systems were prepared in 2 mL Eppendorf tubes, following the method reported in [28] with minor modifications. A total of 1 g of 60 *wt%* hydrophilic ESs combined either with 40 *wt%* H_2O or PBS (14 *wt%*) was mixed with the extract obtained from *L. platensis*

Table 1
Identification of the ESs investigated in this work regarding composition, molar ratio and starting materials.

ESs		Component A	Component B	Component C	Reference
Code	Molar ratio				
Glu:Gly:H_2O	1:2:2.5	Glucose	Glycerol	H_2O	[29]
Suc:ChCl:H_2O	2:5:5	Sucrose	Cholinium chloride	H_2O	[29]
Bet:Sor:H_2O	1.5:1:2	Betaine	Sorbitol	H_2O	[29]
AA:Thy	1:4	Acetic acid	Thymol	–	[28]
LA:Thy	1:4	Lactic acid	Thymol	–	[28]
LevA:Thy	2:1; 2:3	Levulinic acid	Thymol	–	[28]
OctA:DecA	1:2; 3:1	Octanoic acid	Decanoic acid	–	[33]
DecA:Thy	3:2; 1:2; 2:1	Decanoic acid	Thymol	–	[28]
OctA:Ment	1:5	Octanoic acid	Menthol	–	[12]
DecA:Ment	2:1	Decanoic acid	Menthol	–	[12]
DodA:Ment	2:1	Dodecanoic acid	Menthol	–	[12]
1,3-prop:Thy	2:1	1,3-Propanediol	Thymol	–	This study
1,3-prop:Oct	2:1	1,3-Propanediol	Octanoic acid	–	This study
OctA:DecA:DodA	3:2:1	Octanoic acid	Decanoic acid	Dodecanoic acid	[34]

biomass. This mixture was then brought into contact with 1 g of hydrophobic ESs and vortexed for 1 min to promote phase contact before equilibration. Phase contact was promoted under controlled temperature at 25.0 ± 0.1 °C to ensure the thermostability of the resulting ES-based biphasic systems. The specific ESs combinations tested in this work are presented in Table 2.

The ES-based biphasic systems were screened through macroscopic observation, selecting only those that exhibited clear phase separation without C-PC precipitation. The choice of the optimal hydrophilic ESs for C-PC was based on two key criteria: the partition coefficient (K) within the system and the protein apparent stability, ensuring minimal denaturation during the process.

After equilibration (25.0 ± 0.1 °C, for 24 h), the two phases were visually distinct and presented a clear interphase. The volume of the top (V_T) and bottom (V_B) phases was determined gravimetrically by carefully separating each phase with micropipettes into pre-weighed Eppendorf tubes and measuring the collected mass, while using the experimentally determined densities (Table S3) to convert mass to volume. Although partial miscibility of the phase-forming components cannot be excluded, the systems selected in this study showed stable and reproducible phase separation, without turbidity or precipitation (see Supplementary Tables S1 and S2), allowing reliable quantification of V_T and V_B, and subsequent calculation of partition coefficients and extraction efficiency.

The phase volume ratio is defined as the volume ratio of the V_T and V_B after thermodynamic equilibrium (eq. 1).

$$Volume\ ratio = \frac{V_T}{V_B} \tag{1}$$

The K and the extraction efficiency (EE) of the protein in the biphasic systems were calculated according to eq. 2 and eq. 3, (respectively).

$$K = \frac{C_T}{C_B} \tag{2}$$

$$EE(\%) = \frac{C_T \times V_T}{C_T \times V_T + C_B \times V_B} \times 100 \tag{3}$$

C_T and C_B represent the concentration of C-PC in both the top- and bottom-phases, after the system reaches the thermodynamic equilibrium.

The purity of C-PC towards the total proteins and chlorophylls was calculated using eqs. 4 and 5, (respectively).

$$\text{Purity}_{\text{C-PC,proteins}} = \frac{A_{620}}{A_{280}} \tag{4}$$

$$\text{Purity}_{\text{C-PC,chlorophylls}} = \frac{A_{620}}{A_{470}} \tag{5}$$

Table 2
Biphasic systems used in this study. Combinations of hydrophilic and hydrophobic ESs used to formulate the biphasic systems for C-PC extraction. Hydrophilic ESs were combined with H_2O or PBS (14 *wt%*).

Hydrophilic phase (ES + H_2O or PBS 14 *wt%*)	Hydrophobic ESs
Suc:ChCl:H_2O (2:5:5)	AA:Thy (1:4)
Glu:Gly:H_2O (1:2:2.5)	LA:Thy (1:4)
	LevA:Thy (2:1; 2:3)
	DecA:Thy (2:1)
Bet:Sor:H_2O (1.5:1:2)	DecA:Ment (2:1)
	1,3-prop:Thy (2:1)
	1,3-prop:OctA (2:1)
	OctA:DeA:DodA (3:2:1)

2.4.2. Spectroscopic measurement

The absorption spectra were measured between 260 and 800 nm. The concentration of C-PC was determined with the absorbance peak at 620 nm. C-PC standards were prepared to establish a linear relationship between absorbance and concentration, ranging from 0 to 0.75 $g{\cdot}L^{-1}$. Samples were analyzed against blanks without C-PC, with the blank composition corresponding exactly to the phase under analysis.

2.4.3. Viscosity, density and volume ratio measurements

The stable ESs produced were characterized in terms of density (± 0.0005 $g{\cdot}cm^{-3}$) and dynamic viscosity (± 0.35 %) measured with an automated SVM 3000 Anton Paar rotational Stabinger viscometer–densimeter (Graz, Austria) according to literature procedures [35]. These measurements were performed in a range of temperatures from 25 to 45 ± 0.03 °C, at atmospheric pressure.

2.5. Purification of C-PC from the main contaminants using FCPC

An FCPC system, model FCPC–C from Kromaton Rousselet-Robatel (Annonay, France) was used for the C-PC separation from the other bioactive components co-extracted with the blue protein, by applying the most performant ES-based biphasic systems. The rotor comprises 13 stainless-steel disks, each containing 64 twin cells, totaling 832 PTFE-coated twin cells. The column has a total volume of 50 mL and can withstand pressure drops of up to 70 bar. The system operates at a maximum rotational speed of 3000 rpm, regenerating a centrifugal field of approximately 1500 *g*. The FCPC system is connected to an ECOM ECP2010 analytical HPLC pump, capable of delivering flow rates from 0.02 to 10.00 $mL{\cdot}min^{-1}$ with a maximum pressure of 40 MPa (400 bar). Detection is performed using an ECOM Flash14 diode array detector

from ECOM Spol. S.r.o. (Czech Republic). Samples are injected through a Rheodyne valve model 3055–023 from IDEX Health & Science (Wertheim, Germany), equipped with a 10 mL PEEK sample loop. Fractions are collected using an ADVANTEC Super Fraction Collector CHF122SC from Advantec Toyo Kaisha, Ltd. (Tokyo, Japan). Each ES was prepared individually in separate glass bottles, filtered through 0.45 μm HPLC-grade membranes, and degassed for 10 min in an ultrasonic bath. The biphasic systems used in the FCPC experiments are listed in Table 3. The hydrophilic phase consisted of Glu:Gly:H_2O (1:2:2.5), prepared with either H_2O or PBS (14 *wt%*). This phase was introduced into the FCPC equipment, where it was brought into contact with a hydrophobic ES. Two different compositions of hydrophobic ESs were tested, namely (i) a binary mixture of decanoic acid and menthol (DecA:Ment 2:1) and (ii) a ternary mixture comprising octanoic acid, decanoic acid, and dodecanoic acid (OctA:DecA:DodA 3:2:1).

As illustrated in Fig. 1, the rotor is composed of interconnected chambers where both phases coexist under centrifugal force. The schematic highlights the distribution of the stationary (blue) and mobile (green) phases under ascending and descending modes, showing how C-PC and chlorophylls partition differently among phases, which enables their selective separation.

To purify natural compounds using FCPC, approximately 80 mL of the stationary-phase, equivalent to twice the column volume, is first pumped into the column at a controlled flow rate to ensure complete and uniform fill of the stationary phase. Subsequently, the mobile phase is introduced at a different predefined flow rate while operating the system at a designated rotational speed. After the system reaches equilibrium, the stationary phase retention factor (S_f) is determined. This factor represents the percentage of the column volume occupied by the stationary phase and is calculated by measuring the volume of stationary phase retained in the column after the mobile phase elution (eq. 6):

$$S_f(\%) = \frac{V_S}{V_{column}} \times 100 = \frac{V_{column} - V_e}{V_{column}} \times 100 \quad (6)$$

V_S is the volume of the stationary phase in the column, V_{column} is the total column volume, and V_e is the volume of the stationary phase eluted. The detector was configured to measure absorbance at 280, 340, 470, and 620 nm, covering the primary absorption ranges for *L. platensis* extract components, particularly C-PC (A_{620}), mixed with 2.5 mL of the top phase and 2.5 mL of the bottom phase. The column was operated in ascending mode and at a specific flow rate, with the top and bottom phases serving, respectively, as the mobile and stationary phases. Operational parameters (flow rate and rotation speed) were initially optimized in this work to ensure maximum stationary phase retention and resolution. Once phase equilibrium was established, a 5 mL mixture of both phases containing the C-PC rich extract was injected. Elution continued until there was no change in the volume of permeate or until no further absorbance was observed at the monitored wavelengths (280, 340, 470, 620 nm). Elution–extrusion, a modified approach used for recovering strongly retained compounds [39], was carried out in ascending mode, with the top phase serving as the mobile phase and the bottom phase as the stationary phase. The initial flow rate was set to 1.5 $mL{\cdot}min^{-1}$. Once equilibrium was established, the C-PC-rich extract was injected, and elution proceeded for 50 min. Subsequently, the bottom phase was introduced into the column to extrude the original stationary phase. To accommodate the higher viscosity of the bottom phase and avoid excessive pressure buildup, the flow rate was decreased to 0.7 $mL{\cdot}min^{-1}$. Extrusion continued for 70 min, until no additional absorbance was detected at the four monitored wavelengths.

Table 3
Description of the biphasic systems used for C-PC purification in FCPC.

Biphasic systems composition		
Hydrophilic ES	**Co-solvent**	**Hydrophobic ESs**
Glu:Gly:H_2O (1:2:2.5)	H_2O	DecA:Ment (2:1)
	PBS (14 *wt%*)	OctA:DecA:DodA (3:2:1)

2.5.1. FCPC fractions analysis

To evaluate separation efficiency and quantify the compound's recovery, collected fractions were analyzed using a SpectraMax® iD3 multi-mode microplate reader (Molecular Devices, San José, CA, USA). Absorbance spectra were recorded from 260 nm to 800 nm, enabling the detection of both C-PC and chlorophylls as the main co-extracted compounds. Standard solutions of purified C-PC and chlorophylls were prepared to establish calibration curves, from 0 to 0.75 $g{\cdot}L^{-1}$. For accuracy, all measurements were performed against appropriate blank controls matching the composition of each phase analyzed. Spectrophotometric measurements were performed in triplicate.

3. Results and discussion

3.1. Partitioning behavior of C-PC and chlorophylls in ES-based biphasic systems

In this study, ESs-based biphasic systems were prepared with the goal of evaluating their ability to selectively separate C-PC (a hydrophilic macromolecule) from chlorophylls (hydrophobic compounds) co-extracted from *L. platensis*. To evaluate the extraction efficiency of the systems under study, various combinations of hydrophilic and hydrophobic ESs were tested. The initial screening involved a macroscopic evaluation of phase behavior, including turbidity and visible C-PC precipitation, to identify systems with sufficient stability and compatibility with the target compounds. Among the ESs listed in Table 1, some systems exhibited instability at low temperatures and were therefore excluded from further tests. A detailed list of these unstable combinations is provided in Supplementary Materials, Table S1. Furthermore, systems resulting in turbid mixtures or causing visible C-PC precipitation were discarded (Supplementary Materials, Table S2). In contrast, biphasic systems that exhibited clear and stable phase separation were considered suitable for subsequent extraction and partitioning studies. Representative examples of these systems are illustrated in Figs. S1, S2 and S3 in Supplementary Materials, depicting successful immiscibility and effective phase formation. The ES-based biphasic systems that successfully formed two distinct phases, without turbidity or pigment precipitation, are summarized in Table 4. These systems were then evaluated for their partitioning behavior, using the partition coefficient of C-PC and chlorophylls as key selection criteria. The addition of H_2O or PBS (14 *wt%*) as co-solvents played a critical role in reducing the viscosity of the hydrophilic phase. High viscosity is a well-known limitation of sugar- and polyol-based ESs, as it impairs mass transfer and reduces solute partitioning efficiency. Dilution with H_2O or PBS significantly decreased viscosity, thereby improving both extraction kinetics and phase equilibrium dynamics, consistent with previous studies findings on ES viscosity and protein partitioning [28,36]. In contrast, the hydrophobic ESs used in this study generally exhibited low viscosity, posing no significant barrier to mass transfer. A complete list of viscosity values for all the tested ESs is available in Table S3 from Supplementary Material.

At the molecular level, the preferential partitioning of C-PC into the hydrophilic phase can be attributed to its strong hydrogen-bonding capacity and polar surface residues, which are stabilized within the Glu:Gly:H_2O network [32]. In turn, chlorophylls and other lipophilic impurities are more compatible with the hydrophobic ESs (DecA:Ment, OctA:DecA:DodA), where van der Waals and π–π interactions favor their solubilization [12]. This polarity contrast was consistent with the observed partition coefficients ($K_{C\text{-}PC}$ of 1.53 and K_{Chlo} of 0.50), highlighting the selective stabilization of C-PC in the polar phase. In PBS-containing systems, phosphate ions further reinforced hydrogen bonding and electrostatic interactions around C-PC, explaining its higher retention. Thus, selective extraction arises from the interplay of hydrogen bonding, hydrophobic exclusion, viscosity, and electrostatic

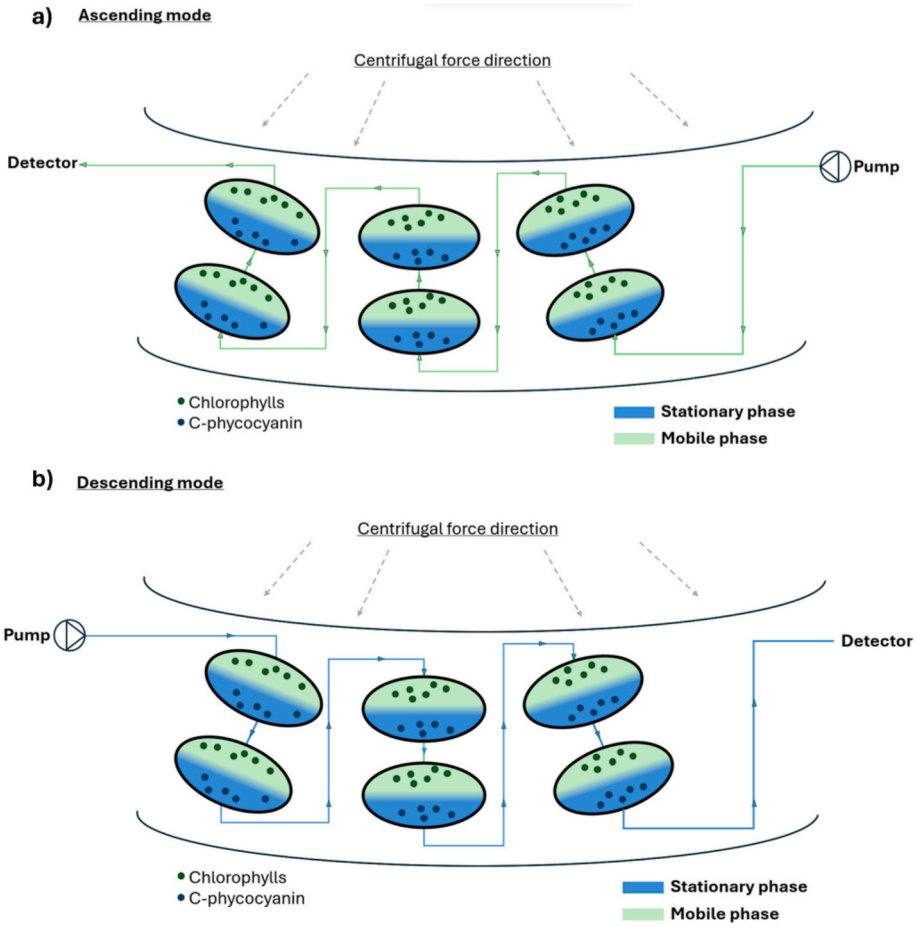

Fig. 1. Representation of FCPC operation under (a) ascending and (b) descending modes with ES-based biphasic systems. Blue (stationary, hydrophilic ES) and green (mobile, hydrophobic ES) phases represent the two immiscible ES phases distributed within the rotor compartments, where C-PC and chlorophylls partition differently among phases, enabling their selective separation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Partition coefficients of C-PC ($K_{C\text{-}PC}$) and chlorophylls (K_{Chlo}) in various ES-based biphasic systems. The dash (–) indicates that partition coefficients could not be determined.

Biphasic systems					
Hydrophilic phase		Hydrophobic ESs			Partition coefficient
Co-solvent	ESs	DecA:Thy (2:1)	OctA:DecA:DodA (3:2:1)	DecA:Ment (2:1)	
H_2O	Suc:ChCl:H_2O (2:5:5)	0.46	0.36	–	$K_{C\text{-}PC}$
		0.66	0.54	–	K_{Chlo}
	Glu:Gly:H_2O (1:2:2.5)	0.14	0.01	1.53	$K_{C\text{-}PC}$
		0.21	0.01	0.50	K_{Chlo}
	Bet:Sor:H_2O (1.5:1:2)	0.05	0.06	0.07	$K_{C\text{-}PC}$
		0.05	0.05	0.08	K_{Chlo}
PBS (14 *wt%*)	Suc:ChCl:H_2O (2:5:5)	–	–	0.11	$K_{C\text{-}PC}$
		–	–	0.23	K_{Chlo}
	Glu:Gly:H_2O (1:2:2.5)	–	0.03	0.21	$K_{C\text{-}PC}$
		–	0.03	0.45	K_{Chlo}
	Bet:Sor:H_2O (1.5:1:2)	–	–	–	$K_{C\text{-}PC}$
		–	–	–	K_{Chlo}

stabilization [37].

An unexpected result was observed with the inclusion of PBS 14 *wt%* in the hydrophilic phase: it did not promote a significant salting-out effect, contrary to what is typically seen in aqueous two-phase systems, where salts reduce protein solubility by competing with the solute for H_2O molecules, leading to reduced hydration of the solute and thus its precipitation or phase separation. Instead of promoting the migration of biomolecules from the aqueous-rich bottom phase to the organic-rich top phase, PBS appears stabilizing the interaction of C-PC in the bottom phase. This behavior is consistent with previous reports in ES-based systems, where phosphate ions in PBS can influence hydrogen bonding and electrostatic interactions, thereby enhancing solute retention in the polar phase [37,38]. In contrast, systems diluted exclusively with deionized water exhibited a more pronounced migration of components towards the top phase. This may be attributed to the lower ionic strength in the absence of PBS, which can weaken the stability of solute–solvent

interactions, thus facilitating solute transfer to the less polar phase. Such trends have been previously observed in aqueous biphasic systems and ES-biphasic systems [38]. The aim of this study was to identify ESs-based biphasic systems capable of selectively separating C-PC from chlorophylls based on their differing solubilities. Biphasic systems with K within the range of $0.5 < K < 3$ were targeted, as this range enables a balanced distribution of solutes between the two phases and facilitates the separation. Achieving this balance is critical to maximize purification potential while avoiding complete retention or transfer of the target molecules. Among all systems tested, the combination of Glu:Gly:H_2O (1:2:2.5) as the hydrophilic ES, diluted with deionized water, and DecA:Ment (2:1) as the hydrophobic ES yielded the most favorable results. This system provided a $K_{C\text{-}PC}$ of 1.53 and K_{Chlo} of 0.50, both within the desired range, and demonstrated clear selectivity towards C-PC. The remaining systems tested showed very low and nearly identical K values for both pigments, making selective separation impractical. This may be explained by insufficient polarity contrast, limited solubility differences, or unfavorable interactions within the phase-forming components. Despite the poor separation efficiency, one additional system comprising Glu:Gly:H_2O (1:2:2.5) with PBS as co-solvent and OctA:DecA:DodA (3:2:1) as the hydrophobic ES, was selected for comparative purposes. The inclusion of this system enabled the evaluation of PBS influence relative to deionized water, particularly regarding solute behavior, phase affinity, and potential purification outcomes.

3.2. Performance evaluation of FCPC to separate C-PC and chlorophylls

To obtain a more refined product with higher C-PC purity, FCPC was employed. In this study, the separation efficiency was optimized by adjusting three key operational parameters, the rotation speed, flow rate, and flow mode (ascending or descending), depending on the selection of mobile and stationary phases. These variables were tuned to achieve a *Sf* close to 50 %, a typical target value in FCPC to ensure balanced retention and elution of compounds. A value close to 50 % indicates that the stationary phase is adequately retained while allowing the mobile phase to flow through efficiently, maximizing resolution and system stability. The parameter combinations tested, and their corresponding *Sf* are listed in Table 5.

The efficiency of FCPC separations is strongly influenced by the operational parameters: rotation speed, flow rate, and mobile phase selection. Rotation speed directly controls the centrifugal force acting on the biphasic system: higher speeds increase stationary phase retention and reduce remixing but may also raise backpressure and prolong separation. In this work, 800 rpm proved to be optimal for the Glu:Gly:H_2O/DecA:Ment system (*Sf* = 60.8 %), while 1500 rpm was more suitable for the Glu:Gly:H_2O/OctA:DecA:DodA system (*Sf* = 68.6 %). Flow rate governs the mass transfer between phases: lower rates enhance resolution by allowing longer solute–phase interactions, whereas higher rates reduce separation efficiency due to insufficient equilibration. For instance, at 1.75 mL·min^{-1} with 800 rpm, selective resolution of C-PC was achieved, while at 3.0 mL·min^{-1} separation became incomplete. Finally, the mobile phase selection defines the ascending or descending mode. In ascending mode (lighter phase), the Glu:Gly:H_2O/DecA:Ment system promoted selective partitioning of C-PC. Conversely, in descending mode (denser phase), the Glu:Gly:H_2O/OctA:DecA:DodA system enabled faster elution and higher recovery of C-PC, although with reduced selectivity due to chlorophyll co-elution. These results confirm the critical role of hydrodynamic parameters in balancing stationary phase retention, resolution, and performance of ES-based FCPC separations.

Phase stability was monitored using pressure readings and UV absorbance, which served as indicators of system equilibrium. Following the injection of approximately two column volumes (81.6 mL, based on a single column volume of 40.8 mL) of the stationary phase, it was confirmed that the column cavities were filled before introducing the mobile phase. Equilibrium was confirmed when the eluent ceased dripping, indicating full phase distribution. Several conditions led to either excessively high backpressure, preventing the continuation of the run, or excessively long separation times (>3 h). These conditions were excluded from further testing.

For the biphasic system composed of Glu:Gly:H_2O (1:2:2.5) diluted with H_2O and DecA:Ment (2:1), the best operational condition for sample injection was identified as 800 rpm of rotation speed, 1.75 mL·min^{-1} of flow rate, and ascending mode, yielding a *Sf* of 60.8 %. Based on the favorable partition coefficients presented in Table 4, this system was expected to achieve effective pigment separation. The separation run under these conditions lasted 1 h and 37 min, demonstrating the practicality of FCPC for pigment purification with a short processing time. This supports FCPC as a rapid and straightforward method to achieve pigment purification, avoiding the need for solid materials that can clog or wear out over time. Moreover, FCPC systems are easily scalable and commonly used both in laboratory and industrial settings, aligning with biorefinery principles by supporting solvent-free fractionation, minimizing waste, and enabling mild and selective recovery of sensitive biomolecules.

Fig. 2 shows the fractionation profile across 100 collected samples. UV absorbance analysis revealed that protein-rich fractions were primarily eluted within the first 40 samples, as indicated by the absorbance

Table 5
Summary of operational conditions tested and corresponding *Sf* values obtained in FCPC.

Trial	Mode	Rotation speed (rpm)	Flow (mL·min^{-1})	*Sf* (%)	Mobile phase	Stationary phase
1	Ascending	1500	2.0	74.3	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
2	Ascending	1500	1.5	–	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
3	Ascending	2000	1.5	–	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
4	Ascending	2000	3.0	–	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
5	Ascending	800	1.5	60.8	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
6	Ascending	800	1.25	62.0	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
7	Ascending	800	1.75	60.8	DecA:Ment (2:1)	Glu:Gly:H_2O (1:2:2.5)
8	Descending	1500	2.0	–	Glu:Gly:H_2O (1:2:2.5)	DecA:Ment (2:1)
9	Descending	600	3.0	–	Glu:Gly:H_2O (1:2:2.5)	DecA:Ment (2:1)
10	Descending	800	2.0	–	Glu:Gly:H_2O (1:2:2.5)	DecA:Ment (2:1)
11	Descending	2000	1.0	–	Glu:Gly:H_2O (1:2:2.5)	DecA:Ment (2:1)
12	Ascending	1500	1.5	66.7	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
13	Ascending	1500	2.5	–	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
14	Ascending	1500	2.0	74.3	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
15	Ascending	2000	1.5	–	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
16	Ascending	2500	2.5	–	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
17	Ascending	800	1.5	65.9	OctA:DecA:DodA (3:2:1)	Glu:Gly:H_2O (1:2:2.5)
18	Descending	800	2.0	–	Glu:Gly:H_2O (1:2:2.5)	OctA:DecA:DodA (3:2:1)
19	Descending	2000	1.0	–	Glu:Gly:H_2O (1:2:2.5)	OctA:DecA:DodA (3:2:1)
20	Descending	1500	1.0	68.6	Glu:Gly:H_2O (1:2:2.5)	OctA:DecA:DodA (3:2:1)

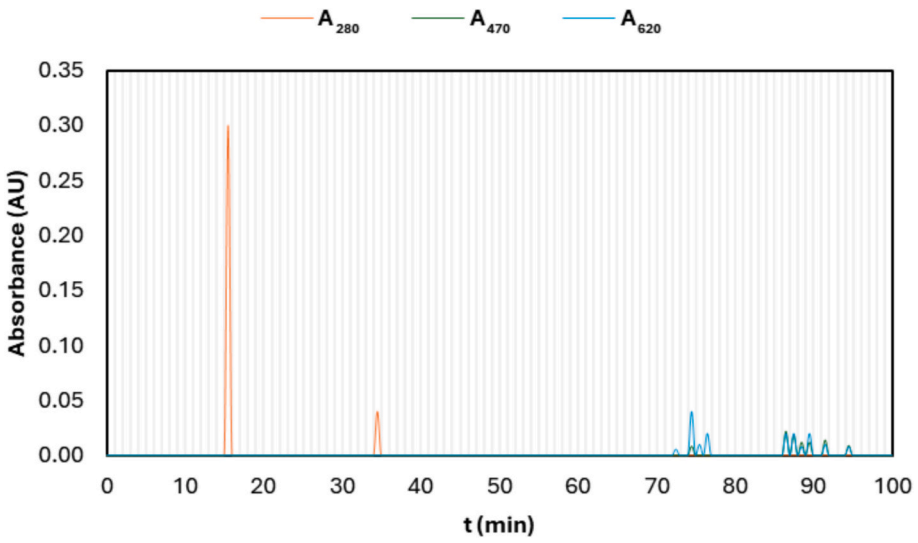

Fig. 2. FCPC separation of *L. platensis* crude extract using the system 60 *wt%* Glu:Gly:H_2O (1:2:2.5) ES and 40 *wt%* H_2O), and DecA:Ment (2:1). Experimental conditions: rotation speed of 800 rpm; flow-rate of 1.75 mL·min^{-1}; *Sf* = 60.8 %; P ≈ 3 MPa; detection wavelengths: 280 nm, 470 nm, 620 nm; initial concentration of C-PC in the crude extract: 1780 mg·L^{-1}.

peak at 280 nm. In contrast, fraction 73 exhibited a single band at 620 nm, consistent with the presence of isolated C-PC. However, subsequent fractions contained overlapping absorbance signals at both 620 nm and in the chlorophylls' absorption region at 470 nm, suggesting partial co-elution. This co-separation could be attributed to several factors. First, incomplete phase equilibrium or small *K* differences may result in overlapping elution profiles. Second, the centrifugal force applied might not have been sufficient to fully resolve components with marginal partitioning differences. Third, the potential interaction of chlorophylls with protein complexes or micellar aggregates could lead to co-elution. In addition, the low overall absorbance values (maximum ~0.30) suggest either dilution during elution or low initial concentration of pigments in the injected crude extract. Specifically, C-PC concentration in fraction 77 was estimated at 15.9 mg·L^{-1}, which may reflect limited pigment content or partial retention in the stationary phase. However, despite the low concentration, this fraction exhibited high purity, as no overlap with total protein was observed.

In the second case, the biphasic system composed of Glu:Gly:H_2O (1:2:2.5) with PBS (14 *wt%*) as the co-solvent conjugated with the hydrophobic ES OctA:DecA:DodA (3:2:1) was evaluated. This system differs from the previous one in two key aspects: the use of PBS, which maintains the system at approximately pH 6, a pH reported as optimal for stabilizing C-PC and preserving its structural integrity and chromophore stability, and the nature of the hydrophobic ES [28,39]. The buffered pH helps protect C-PC from denaturation and oxidative degradation, supporting its bioactivity and characteristic blue color [39]. Additionally, the hydrophobic ES in this system exhibits slightly lower viscosity than DecA:Ment (2:1), which can enhance mass transfer, make phase equilibrium faster, and support better solute partitioning. During experimental optimization in the FCPC equipment, it was observed that the *Sf* across different conditions were relatively similar, indicating consistent stationary phase behavior. To explore a distinct flow dynamic and improve comparative analysis, the descending mode was selected in contrast to the previously tested ascending configuration. The selected operational conditions, a rotation speed of 1500 rpm and a flow rate of 1.0 mL·min^{-1}, were specifically chosen to optimize the stationary phase retention while reducing phase boundary disruption. A higher rotation speed enhances centrifugal force, which improves the trapping of the stationary phase within the rotor and minimizes phase leakage. Additionally, the lower flow rate helps maintain phase equilibrium and prevents remixing. This configuration resulted in a *Sf* of 68.6 %, supporting both operational stability and efficient phase retention. As shown in Fig. 3, this run exhibited substantially higher absorbance values compared to the first case, with a maximum absorbance peak of approximately 0.87. The protein peak appeared early in the elution, as observed previously; however, tailing was noted between the first and second fractions, indicating inefficient resolution of components during the initial elution stage. Regarding pigment separation, the expected challenge of co-elution between C-PC and chlorophylls was confirmed, consistent with the similar partition coefficients ($K_{C\text{-}PC}$ and K_{Chlo} of 0.03) obtained for both pigments in this system. This low selectivity hindered effective resolution, despite PBS potentially enhancing C-PC stability. Fraction 12 exhibited the highest relative co-elution of C-PC, with an estimated concentration of 466.9 mg·L^{-1}. The subsequent fraction showed higher total pigment content but was predominantly chlorophylls, indicating co-migration of both pigments.

The purity of the collected fractions was further assessed using the A_{620}/A_{280} and A_{620}/A_{470} absorbance ratios (Table 6), which respectively indicate the relative protein purity and pigment selectivity towards C-PC over chlorophylls. Fractions labelled as pure by A_{620}/A_{280} and A_{620}/A_{470} correspond to negligible absorbance from proteins and chlorophylls, reinforcing the high purity of C-PC in those fractions. For the Glu:Gly:H_2O/DecA:Ment biphasic systems, three fractions (73, 76, and 77) were classified as pure. The highest A_{620}/A_{470} ratio (4.76) was found in fraction 75, indicating strong C-PC. Other fractions displayed pigment selectivity values ≤1.67, indicating that co-elution with chlorophylls predominated in these samples. In contrast, Glu:Gly:H_2O/OctA:DecA:DodA biphasic systems achieved its highest A_{620}/A_{470} ratio (1.62) in fraction 8 (466.9 mg·L^{-1}C-PC), whereas the highest C-PC concentration (615.9 mg·L^{-1}) was found in fraction 9, with a lower selectivity (0.87). Although the maximum ratio was lower than that of the Glu:Gly:H_2O/DecA:Ment biphasic systems, in this case the higher selectivity was directly associated with the fraction containing the highest C-PC concentration. Overall, while the DecA:Ment system produced isolated fractions of high purity but low yield, the OctA:DecA:DodA system provided a better balance between purity and recovery in the target fraction, which is crucial for process scalability.

A comparative analysis of both FCPC separations highlights the critical impact of the system composition, namely the choice of co-solvent, ES, and operational mode, on the separation performance, product purity, and process dynamics. Both systems employed biphasic mixtures composed of hydrophilic and hydrophobic ESs; however, their distinct physicochemical profiles led to significantly different outcomes in terms of pigment separation and product quality. In the first system,

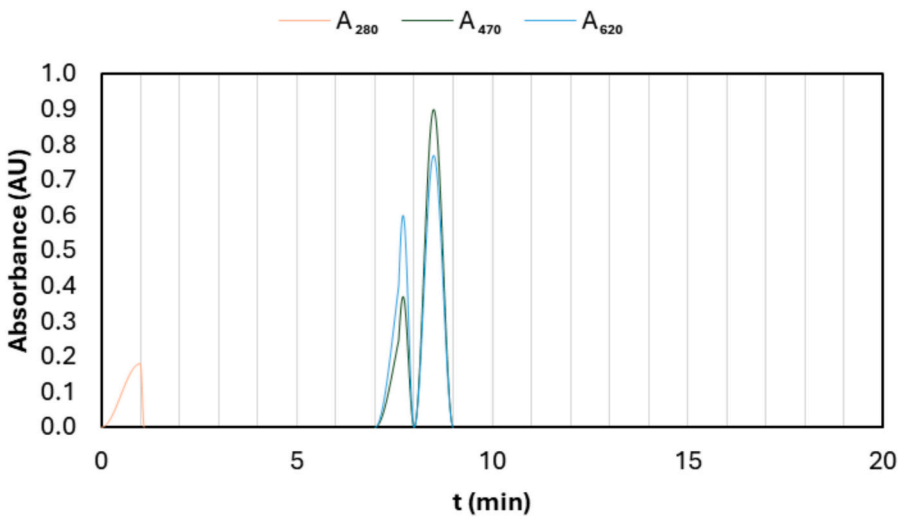

Fig. 3. FCPC separation of *L. platensis* crude extract using the system 60 *wt%* Glu:Gly:H_2O (1:2:2.5) ES and 40 *wt%* PBS (14 *wt%* salts), and OctA:DecA:DodA (3:2:1). Experimental conditions: rotation speed of 1500 rpm; flow-rate of 1.0 mL·min^{-1}; *Sf* = 68.6 %; P ≈ 6.2 MPa; detection wavelengths: 280 nm, 470 nm, 620 nm; initial concentration of C-PC in the crude extract: 1780 mg·L^{-1}.

Table 6
Purity (A_{620}/A_{280} and A_{620}/A_{470}) of C-PC-rich fractions towards the presence of other proteins and chlorophylls obtained by FCPC using Glu:Gly:H_2O/DecA:Ment and Glu:Gly:H_2O/OctA:DecA:DodA biphasic systems.

ES-based biphasic systems							
Glu:Gly:H_2O/DecA:Ment				Glu:Gly:H_2O/OctA:DecA:DodA			
	Purity				Purity		
Fractions	C-PC, proteins	C-PC, chlorophylls	[C-PC] mg·L^{-1}	Fractions	C-PC, proteins	C-PC, chlorophylls	[C-PC] mg·L^{-1}
73	Pure (> 5)		4.5	8	–	1.62	466.9
75	–	4.76	31.8	9	–	0.87	615.9
76	Pure (> 5)		8.0				
77	Pure (> 5)		15.9				

the use of deionized water as co-solvent (i.e., unbuffered and free of ions) in the hydrophilic phase resulted in greater differentiation between the partition coefficients of C-PC and chlorophylls. This polarity contrast favored a selective partitioning, enabling more effective separation of the target pigment from the interfering compounds. Consequently, this configuration yielded fractions with relatively low pigment concentrations but very high purity, in some cases approaching the analytical-grade threshold ($A_{620}/A_{280} \geq 3.0$) required for biotechnological and research applications, or even the pharmaceutical-grade threshold ($A_{620}/A_{280} \geq 4.0$) where purity is critical. Such high-purity fractions are particularly relevant for pharmaceutical and analytical-grade applications. In contrast, the second system incorporated PBS (14 *wt%*) as a co-solvent, introducing a buffered environment at pH ~ 6 known to stabilize C-PC by preserving its tertiary structure and chromophore integrity. However, the presence of ionic species and enhanced hydrogen-bonding capacity may have reduced the polarity contrast between the phases, thereby diminishing the partition efficiency of the solute. This effect was reflected in similar *K* values for both C-PC and chlorophylls, which limited the resolution between pigments. Nevertheless, this configuration produced more concentrated pigment fractions, but with lower purity (below analytical-grade thresholds), making it more suitable for applications where volume minimization is prioritized over absolute purity, namely as bio-based colorant or bulk extract use, compatible with food-grade requirements (≥ 0.7). This differentiation in yield–purity profiles indicates that each system can be strategically applied to distinct market sectors: the first system targeting niche, high-value applications that demand exceptional purity, and the second favoring higher-volume, lower-cost markets where purity requirements are less stringent.

The operational mode of the FCPC runs also played a key role. The ascending mode (system (i)) required a longer run (~97 min) but facilitated a more complete resolution of C-PC from chlorophylls, particularly when coupled with systems showing favorable *K* differences. In contrast, the descending mode (system (ii)) enabled a significantly shorter run time (~20 min to collect main fractions), due to faster elution dynamics of denser mobile phases. Although this configuration may lead to earlier overlap between compounds, it offers substantial gains in throughput and productivity.

3.3. An economic and environmental perspective of the processes developed using FCPC

The environmental and economic sustainability of the FCPC process was assessed by combining cost analysis of the biphasic systems (Supplementary Materials, Table S4), operational performance, and the targeted market segment for each configuration. Based on conservative bulk prices of the reagents at industrial ton-scale preparation, the hydrophilic phase consisted of a 60 *wt%* Glu:Gly:H_2O ES and 40 *wt%* PBS (14 *wt%* salts) and hydrophobic phase DecA:Ment (2:1) used in system (i) cost 0.86 €·L^{-1} and 12.17 €·L^{-1}, respectively. Given that each FCPC run requires 50 mL of each phase, the solvent cost per run is 0.65 €. System (i) yielded C-PC fractions (73, 76, and 77) with concentrations up to 15.9 mg·L^{-1} and high purity ($A_{620}/A_{280} \geq 4.0$), meeting the analytical/pharmaceutical-grade threshold. However, the recovery of high-purity fractions was relatively low (0.64 %, equivalent to 0.06 mg of C-PC from 8.9 mg loaded), reflecting the trade-off between selectivity

and yield. Despite this, market prices for C-PC of this grade are $15.00 *per* mg [10], meaning that even very small amounts recovered can offset solvent costs and deliver high profit margins for niche, high-value applications such as diagnostic reagents and bioimaging probes. In system (ii), the hydrophilic phase consisted of a 60 *wt%* Glu:Gly:H_2O ES and 40 *wt%* PBS (14 *wt%* salts), with an estimated cost of 2.93 €·L^{-1}, and the hydrophobic phase OctA:DecA:DodA (3:2:1) costing 9.31 €·L^{-1}. The total solvent cost *per* run was 0.61 €. This system produced higher C-PC concentrations (up to 615.9 mg·L^{-1}) and an overall recovery of 24.3 % (2.2 mg from 8.90 mg loaded), albeit with moderate purity (A_{620}/A_{280} = 2.0–2.5), consistent with food-grade specifications. Market prices for C-PC of this grade are typically $0.13 to $0.35 *per* mg [8,9], enabling economic viability when operated at larger scale for the food and nutraceutical markets.

From a green chemistry standpoint, both systems employ bio-derived and biodegradable components (glucose, glycerol, fatty acids, menthol) which are sourced from renewable and natural feedstocks and exhibit low toxicity. The avoidance of synthetic polymers and high-salt aqueous phases common in polymer–salt ATPS reduces the environmental burden in downstream wastewater treatment [40]. Technologically, the integration of ES into FCPC leverages their tunable polarity and density to maintain phase stability under centrifugal fields, enabling short separation times (approximately 80 min for main fractions in system (i)) and eliminating the need for multiple sequential steps typical in ultrafiltration or chromatographic polishing. Economically, the dual-system strategy offers flexibility: system (i) addresses low-volume/high-value markets, while system (ii) supports high-volume/lower-value applications, optimizing return on investment and enhancing process adaptability to demand. Taken together, these findings emphasize the necessity of system-specific optimization depending on the target product end use. Systems operated under ascending mode and with high K contrast tend to yield lower quantities of C-PC but with higher purity, including fractions completely free of chlorophylls. Such fractions not only meet the requirements for high-value markets (e.g., pharmaceutical or analytical grade) but can also be used in applications where the presence of chlorophyll-derived pigments is not acceptable. On the other hand, descending mode and PBS-based systems produced higher C-PC quantities in shorter times but at lower purity, making them more suitable for bulk applications such as food and beverage coloring, where food-grade purity is sufficient.

Although not experimentally performed in this study, both the hydrophilic ES Glu:Gly:H_2O (1:2:2.5) and the hydrophobic ESs DecA:Ment (2:1) and OctA:DecA:DodA (3:2:1) can in principle be recovered and reused, as they are non-volatile and remain chemically stable. The liquid–liquid nature of FCPC further facilitates phase recovery without loss of separation performance. Recycling would rely on phase separation, filtration, adsorption, and vacuum evaporation of H_2O (for Glu:Gly:H_2O (1:2:2.5)), while mild distillation could be applied to volatile constituents such as menthol in DecA:Ment (2:1), but not to the non-volatile ES backbones. These purification steps would not only regenerate the solvent but simultaneously enable the recovery of residual biomolecules (e.g., C-PC, chlorophylls, proteins), thereby supporting the design of a true biorefinery process. The suitability of regenerated solvents can be verified through density and viscosity measurements and by performing control partition tests (*K*), ensuring their reuse in subsequent extraction cycles. This closed-loop strategy minimizes solvent consumption, enables recovery of valuable co-products, and aligns the process with zero-waste and circular economy principles. Moreover, the low cost of ES preparation at industrial scale (Table S4) further supports the feasibility of such recycling strategies.

4. Conclusions

This study investigated the use of ESs-based biphasic systems for the purification of C-PC from crude extracts of *L. platensis* using FCPC. Various combinations of hydrophilic and hydrophobic ESs were screened based on macroscopic stability and partitioning performance. Two contrasting systems were selected for FCPC: (i) Glu:Gly:H_2O (1:2:2.5) with H_2O paired with DecA:Ment (2:1), and (ii) the same hydrophilic ES diluted with PBS (14 *wt%*) combined with OctA:DecA:DodA (3:2:1). System (i) exhibited favorable selectivity with $K_{C\text{-}PC}$ of 1.53 and K_{Chlo} of 0.50, enabling clear separation of C-PC. In contrast, system (ii), yielded higher pigment concentrations but selectivity (identical *K* around 0.03), indicating co-elution. Leveraging its straightforward scalability, FCPC was employed to exploit these partitioning differences under varying operational conditions like flow rate, rotation speed, and flow direction, to maximize *Sf* and resolution. Optimal separation for system (i) occurred in ascending mode, at 800 rpm and 1.75 mL·min^{-1}, yielding a *Sf* of 60.8 %. Fractions 73, 76 and 77 contained up to 15.9 mg·L^{-1} of C-PC with no detectable chlorophylls, corresponding to high-purity analytical/pharmaceutical grade material ($A_{620}/A_{280} \geq 4.0$). System (ii), tested in descending mode, at 1500 rpm and 1.0 mL·min^{-1}, had higher C-PC recovery (absorbance up to 0.87, *Sf* of 68.6 %) but poor resolution due to overlapping *K* values. Nevertheless, fraction 12 had 466.9 mg·L^{-1} of C-PC but also contained chlorophylls, restricting purity to food-grade levels ($A_{620}/A_{280} \approx$ 2.0–2.5). The high yield and lower production make this system more suitable for large-scale, cost-sensitive markets such as food, nutraceuticals, and bulk pigment formulations.

From a green chemistry perspective, both systems rely on bio-derived, biodegradable, and low-toxicity solvents, eliminating synthetic polymers and high-salt aqueous phases that increase downstream waste-treatment burdens. Operationally, solvent consumption is low and further reduced through phase reuse, contributing to environmental and economic sustainability.

CRediT authorship contribution statement

Bruno A. Pereira: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Alexandra Conde:** Methodology, Formal analysis. **João Vasco Valente:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Mara G. Freire:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Sónia P.M. Ventura:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2025.135616.

Data availability

Data will be made available on request.

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