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Extraction and assessment of the colouring capacity of *Arthrospira platensis*-derived pigments

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ABSTRACT

This study presents a zero-waste biorefinery approach for the sequential extraction of phycocyanin and chlorophyll from *Arthrospira platensis*, followed by the valorisation of the remaining biomass as a plant biostimulant. Natural deep eutectic solvents were screened for phycocyanin recovery, with the mixture proline:glycerol:sorbitol:water (1:1:1:13 molar ratio) showing the highest potential (1.15 g·100 g^{-1}; $p < 0.05$). An initial ultrasound-assisted cell wall disruption step significantly enhanced phycocyanin yield by 400–450 % relative to the untreated control ($p < 0.05$). A response surface methodology optimised extraction achieved a recovery yield of 8.26 g·100 g^{-1} at 39.7 °C and 127.9 min. The phycocyanin-rich extract was used to mimic the blue colour of commercial blue gin, with a minimal colour difference (ΔE) of 4.53. Subsequent chlorophyll extraction from the phycocyanin leftovers yielded an extract that successfully coloured a commercial green alcohol-free apple liquor (ΔE = 3.93) and green gin (ΔE = 1.65). Finally, the residual biomass demonstrated a significant biostimulant capacity, increasing the germination index of various seeds by 80–150 % compared to water ($p < 0.05$). This work highlights the potential of *A. platensis* as a sustainable source for natural colourants and agricultural inputs.

1. Introduction

Since the 1800s, >2500 food additives have been intentionally added to food to preserve certain properties or extend shelf life. Approximately 40 of these additives are colourants, which are approved for consumption in the EU by the European Food Safety Authority (EFSA) (European Parliament and Council, 2008). Food colourants are additives used to restore, enhance or add colour to foods and beverages. The use of natural pigments is becoming increasingly popular among both, industries and consumers. This shift is driven by growing consumer awareness of the relationship between health, food and environmental sustainability (Novais et al., 2022).

Spirulina, and other microalgae, are gaining importance in different industrial sectors. For example, there are several plant biostimulants based on Spirulina currently available in the market (Morillas-España et al., 2022a). Spirulina is also being studied as a source of natural food colourants including chlorophylls and carotenoids. It is now being used in several commercial products. For example, the whole biomass of Spirulina was recently used to mimic the colour of commercial green macarons pigmented using E-150d and E-142 (Villaró et al., 2023). The chlorophyll content of Spirulina is in line with that of most cyanobacteria, containing exclusively chlorophyll a, which represents 0.1–1.5 % of its dry weight (Bortolini et al., 2022; Hynstova et al., 2018; Martins et al., 2021). Carotenoids are valued not only for their natural origin, but also for their potential health-promoting properties. A recent study indicated that an *Arthrospira sp.* extract rich in chlorophylls (658 mg·g^{-1} DW) and xanthophylls (5460 mg·g^{-1} DW) showed high antioxidant activity, with 68 % DPPH scavenging inhibition (Castro et al., 2025). Similarly, an extract rich in carotenoids (100 mg·g^{-1}) derived from *Spirulina* had high DPPH radical scavenging activity (IC_{50} 1.9 mg mL^{-1}) (Milovanovic et al., 2025).

Spirulina is also a source of high-quality protein. Phycobiliproteins

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such as phycocyanins and allophycocyanins are of particular interest because of their intense colour and bioactive properties. Phycocyanins, water-soluble pigments, represent around 10 % of the protein fraction in Spirulina, making them major compounds. Phycocyanins extracted from Spirulina are well known to exhibit several beneficial effects. For example, in a study carried out in 2019, a C-phycocyanin extract with a purity index of 0.67 showed strong DPPH radical scavenging activity, reaching a maximum value of 60.1 % at 0.5 mg mL^{-1} (Pan-utai and Iamtham, 2019). A different extract with a C-phycocyanin concentration of 0.5 mg mL^{-1} showed an ABTS radical scavenging activity of 91.49 % (Lin et al., 2025). In addition, an allophycocyanin extract (400 $\mu g \cdot mL^{-1}$) exhibited a strong anticancer effect, reducing HepG2 cell viability to 30 % compared to the control (Na et al., 2025).

Spirulina is primarily used in the food industry as a food colorant, a protein-rich ingredient, and for marketing purposes. Consequently, numerous products containing Spirulina or its derivative, mainly C-phycocyanin, are now commercially available. These include Blue Spirulina SuperAde® (Sol-ti, USA), Innocent Blue Break (Innocent, UK) and Rhin blue phycocyanin energy drink (Phycomania, Czech Republic). Few scientific publications exist that evaluate the colourant capacity of this pigment, as most of them focus on studying its bioactive properties. Nonetheless, in a prior investigation, the authors mimic the blue colour of some commercial beverages like tonic water, isotonic drinks, and wine (García et al., 2021). Moreover, another study evaluated the sensory perception of ice cream enriched with phycocyanin, revealing that 70.2 % of consumers liked its appearance (Bürck et al., 2024). There are some challenges associated with the extraction and use of natural pigments including low recovery yields, instability due to light exposure, oxidation, and sensitivity to extreme pH levels and temperatures. The objective of this study was to develop a method to recover phycocyanin from Spirulina using natural deep eutectic solvents (NADES) and following a zero-waste biorefinery approach, valorising the whole biomass into colourants and plant biostimulants. NADES are an appealing alternative to conventional organic solvents, given their natural origin, low toxicity, and excellent biodegradability. The biomass resulting from the phycocyanin extraction was then used as feedstock to produce chlorophylls and plant biostimulants, the latter assessed using different crops *in vitro*. Moreover, the pigmentation capacity of the different fractions was evaluated in different food matrices.

2. Materials and methods

2.1. Microalgae used

The microalga used was *Arthrospira platensis* BEA 005B purchased from the Spanish Bank of Algae (BEA; Las Palmas de Gran Canaria, Spain). The microalga was produced using a culture medium formulated using commercial fertilisers described in a previous study (Taragjini et al., 2022) in 80 m^2 raceway reactors as described elsewhere (Villaró et al., 2022). In this case, the biomass was harvested using 100 μm membranes, washed twice with tap water, frozen at −20 °C, and subsequently lyophilized prior to its use.

2.2. Preparation of eutectic solvents: solvent screening

Sixteen different NADES were prepared using analytical grade chemicals. The composition of the NADES is shown in Table 1. The different NADES were formulated by combining their constituents in the appropriate molar proportions, followed by heating at 40 °C under stirring at 350 rpm until a homogeneous and clear solvent resulted. This methodology was based on the procedure described in a previous work (Vladić et al., 2023). Solvent E, which was the one selected for optimisation, consisted of proline:glycerol:sorbitol:water at a molar ratio 1:1:1:13.

The different NADES were used to extract compounds from the freeze-dried biomass in a jacketed 0.2 L reactor, where the temperature was controlled at 60 °C using a water bath. The biomass:solvent ratio was 1:20 (w/w) and the total volume was 0.2 L. The extractions took place during 60 min with continuous stirring at 350 rpm. The different solvents were assessed in triplicate. Once the extraction was completed, the mixtures were centrifuged at 8000 × *g* for 10 min and the resulting extracts were stored at 4 °C until further analysis. The concentration of phycocyanin in the extracts was determined spectrophotometrically using a standard curve and a BioTek Epoch 2 Microplate Spectrophotometer (BioTek Instruments Inc., VT, USA). The standard curve was generated with C-Phycocyanin PhycoPro™ (ProZyme Inc., USA) at concentrations of 0–9 $\mu g \cdot mL^{-1}$. The recovery yield was calculated as the amount of phycocyanin recovered in the extract per g of biomass on a dry weight basis. These standards were prepared in the same eutectic solvents used for extraction, and their absorbance was measured at 620 nm. Three determinations were done per natural replicate. The two NADES that led to the highest recovery yields were further investigated.

Table 1
Composition of the natural deep eutectic solvents used.

Abbreviations	Components	Molar ratio
A	Proline: lactic acid	1:1
B	Betaine: glycolycerol	1:2
C	Betaine: glycol: sucrose: water	2:3:1:5
D	Betaine: sucrose: polyurethane: water	5:2:2:21
E	Proline:Glycerol:Sorbitol:Water	1:1:1:13
F	Citric acid: arginine:water	1:1:7
G	Betaine: lactic acid	1:5
H	Thymol: Lauric acid	1:1
I	Menthol: lauric acid	4:1
J	Lactic acid: glucose: water	5:1:3
K	Betaine:Levulinic acid	1:2
L	Glycerol: sucrose: sorbitol: water	2:1:2:10
M	Citric acid: glycolerol	1:1
N	Lactic acid: glycol: water	3:1:3
O	Betaine: ethylene glycol	1:3
P	Carvacrol: decanoic acid	1:1

2.3. Cell wall disruption

The energetic requirements of the process were studied at concentrations of 1, 10 and 100 $g \cdot L^{-1}$. These concentrations are in line to those typically obtained from the initial culture, after ultrafiltration, and after continuous centrifugation, respectively. The lyophilised biomass was resuspended in water to reach the target concentrations. The cell wall disruption was done using a SONOPLUS GM 4200 sonicator (Bandelin electronic GmbH & Co., Berlin, Germany) equipped with a TS109 titanium sonotrode (Bandelin electronic GmbH & Co., Berlin, Germany) and operating at 130 W and 20 kHz. To maintain a low temperature during sonication, cold water (4 °C) was continuously recirculated. The disruption efficiency was estimated by measuring changes in the supernatant's optical density at 680 nm during sonication as described elsewhere (Villaró et al., 2023). This wavelength corresponds to the characteristic absorption peak of chlorophyll a. When the cell walls are disrupted, this pigment is released into the surrounding medium and as disruption progresses, more pigment diffuses into the supernatant. The optical density was measured using a Genesys 10S UV–Vis spectrophotometer (Thermo Fisher Scientific, Spain). The process was done in triplicate.

2.4. Extraction of phycocyanin

A 100 $g \cdot L^{-1}$ microalgae slurry was disrupted for 3 min as described above. The disrupted biomass was then frozen at −20 °C, freeze-dried, vacuum sealed, and stored at −20 °C until further use. Once the biomass was disrupted, the first step was to define the ranges for the main extraction parameters. A time curve (10 - 360 min) at 60 °C was performed to evaluate the behaviour of the recovery yield over time.

After determining the optimal time interval, a response surface methodology (a central composite face-centered design) was used to examine the impact of the two independent variables temperature (20–60 °C) and extraction time (30–360 min) on the phycocyanin recovery yield (expressed on a dry weight basis). The optimization was done with Design Expert v13 (Stat-Ease Inc., Minneapolis, MN, USA) software as described elsewhere (Valero-Vizcaino et al., 2024). The response variable was phycocyanin recovery yield, determined spectrophotometrically as described above.

2.5. Extraction of chlorophylls

The leftovers from the phycocyanin extraction were used as feedstock for the recovery of chlorophylls. A washing step using a biomass: distilled water ratio 1:10 (w:v) was evaluated, observing no influence on the chlorophyll content of the extract (data not shown). Briefly, the leftovers were mixed with 95 % (v/v) ethanol at a sample:solvent ratio of 1:10 (w/v), homogenised using a T-18 ULTRA-TURRAX™ disperser (IKA, Barcelona, Spain) operated at 10,000 rpm for 1 min, and further stirred at 350 rpm in the dark for 2 h. The extract was recovered by centrifugation at 5000 × *g* for 10 min using a Digicen 22 Universal centrifuge (Ortoalresa, Madrid, Spain). The extraction was done at room temperature (22 ± 1 °C) in a controlled environment. The extraction process was repeated until a colourless extract was obtained. Once the extraction was complete, all the extracts were combined and concentrated using an RV 8 rotary evaporator (IKA, Staufen, Germany). The leftovers from the chlorophyll extraction were frozen, freeze-dried and vacuum-sealed until further use. The chlorophyll and total carotenoid concentrations (TCC) were quantified as described in previous work (Chini Zittelli et al., 2022) using a Genesys™ 10 S UV spectrophotometer (Thermo Fisher Scientific, Madrid, Spain).

2.6. Pigmentation capacity of the extracts

Different products were developed using the phycocyanin- and chlorophyll-rich extracts obtained. Commercial blue and green products were purchased from local retailers. The selected blue product was The London N°1 Original Blue Gin (Grupo González Byass, Jerez de la Frontera, Spain) and the green products were Ampersand Sweet Melon Premium Gin (Grupo Osborne SA, El Puerto de Santa María, Spain) and alcohol-free Pi.ømka Green Apple Liquor (Hacendado, Valendia, Spain). The pH of these products was 7.71 ± 0.08, 7.78 ± 0.04, and 3.52 ± 0.10, respectively. A commercial uncoloured product (gin and liquor) was also purchased. The pigmentation capacity of the Spirulina-derived extracts was assessed by adding increasing amounts of the extracts to the selected uncoloured products and comparing their colour to the commercial products pigmented using artificial pigments. The colour of the samples, containing different pigment concentrations, was measured (L*, a*, and b* values) using a C400 chroma meter (Konika Minolta, Tokyo, Japan) and the D65 illuminant, which approximates to daylight. Eight measurements were taken per sample, and the results are expressed as mean values ± SD. The pigment concentration in the end products was calculated based on the amount of extract added and its concentration.

2.7. Biostimulant effect of the pigment extraction leftovers

The biostimulant capacity of the co-product left after the extraction of phycocyanins and chlorophylls was determined by means of assessing its effect on the germination of four different commercial seeds. The germination index (GI) was assessed as reported previously (Morillas-España et al., 2022b) using cherry tomato (*Solanum lycopersium*), cucumber (*Cucumis sativus*), sweet pepper (*Capsicum annuum*), and basil (*Ocimum basilicum*). Briefly, 25 seeds were placed on Whatman No. 5 filter paper inside a sterile Petri dish (90 mm in diameter). Then, 0.7 mL of either distilled water, a solution containing 1 mg·L^{-1} of gibberellin (control), or the biomass leftovers at a concentration of 0.5 g·L^{-1} in water were added to the Petri dish. Each treatment was assessed in triplicate. The Petri dishes were then incubated in the dark at 24 °C for three days.

2.8. Statistical analysis

The results are expressed as mean values ± standard deviation (SD). Data were analysed using analysis of variance and a Fisher's LSD post hoc test ($p < 0.05$) and the software Statgraphics Centurion v19 (Statgraphics Technologies Inc., VA, USA).

3. Results and discussion

3.1. Extraction of microalgae-derived pigments (initial screening) and cell wall disruption

Different NADES, listed in Table 1, were screened as candidates for recovering phycocyanin from dried *A. platensis*. The biomass was dried because most of the microalgal biomass being processed in Europe is imported from Asia as a dry powder and to preserve the stability of the pigments during storage. The results, shown in Fig. 1, revealed that the type of solvent used had a striking effect on the phycocyanin recovery yield ($p < 0.05$). Two solvents, solvent E and solvent L, showed the greatest potential for being used in the recovery of phycocyanin. The recovery yields were 1.19 ± 0.04 and 1.23 ± 0.05 g·100 g^{-1} on a dry weight basis, respectively. Previous studies achieved similar phycocyanin recoveries, reaching between 17.2 and 18.5 mg · g^{-1} (Liao et al., 2011). However, the value is low compared to previous work that achieved recovery yields of 103 mg· g^{-1} (Pan-utai and Iamtham, 2019) or 78 mg· g^{-1} (Jaeschke et al., 2019). The main reason for the low recovery achieved in this study was the lack of a cell wall disruption step. Phycocyanins are integral components of the phycobilisome, which is attached to the thylakoid membrane and associated with other pigments. Thus, cell disruption is indispensable for total phycocyanin extraction from *A. platensis*, being the key factor influencing extract yield and purity.

Different methods have been evaluated to disrupt cells of *A. platensis*, namely freeze and thaw cycles (at the laboratory scale),

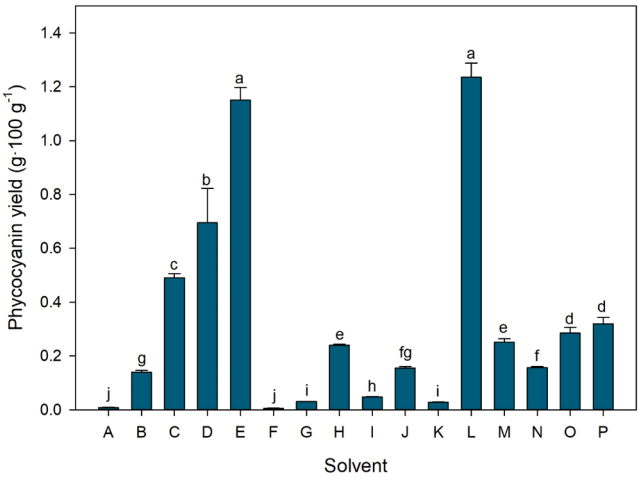

Fig. 1. Effect of the solvent used on the phycocyanin recovery yield. Data represent the mean value of three independent determinations ± SD. A: Proline: lactic acid, B: Betaine: glycolycerol, C: Betaine: glycol: sucrose: water, D: Betaine: sucrose: polyurethane: water, E: Proline:Glycerol:Sorbitol:Water, F: Citric acid: arginine:water, G: Betaine: lactic acid, H: Thymol: Lauric acid, I: Menthol: lauric acid, J: Lactic acid: glucose: water, K: Betaine:Levulinic acid, L: Glycerol: sucrose: sorbitol: water, M: Citric acid: glycolerol, N: Lactic acid: glycol: water, O: Betaine: ethylene glycol and P: Carvacrol: decanoic acid.

homogenisation, bead milling, high pressure homogenisation, microwaves, pulsed electric fields, and sonication (Jaeschke et al., 2021). Sonication led to high recovery yields in previous studies. For example, Sánchez-Zurano et al. (2020) found that adding a sonication step before protein extraction from *A. platensis* increased the solubilized protein from 60 to 96 %. Also, a previous work led to a phycocyanin recovery yield of 105.3 mg· g^{-1} after sonication during 30 min followed by up to 24 h of extraction (Pan-utai and Iamtham, 2019). One of the main limitations of this technology is its relatively high energetic requirement, mainly because of the attenuation of energy within the medium (Lee et al., 2017). To minimise this issue, it is necessary to process concentrated solutions. Higher algal biomass concentrations can enhance the energy efficiency of ultrasound treatment. This is attributed to negligible ultrasound attenuation in the aqueous phase, leading to more effective cell-cavitation interactions (Liu et al., 2022). Moreover, another study found that increased biomass concentration positively affected the yield of released components. Maximum yields for proteins (94 %), carbohydrates (40 %), and lipids (26 %) were achieved at the

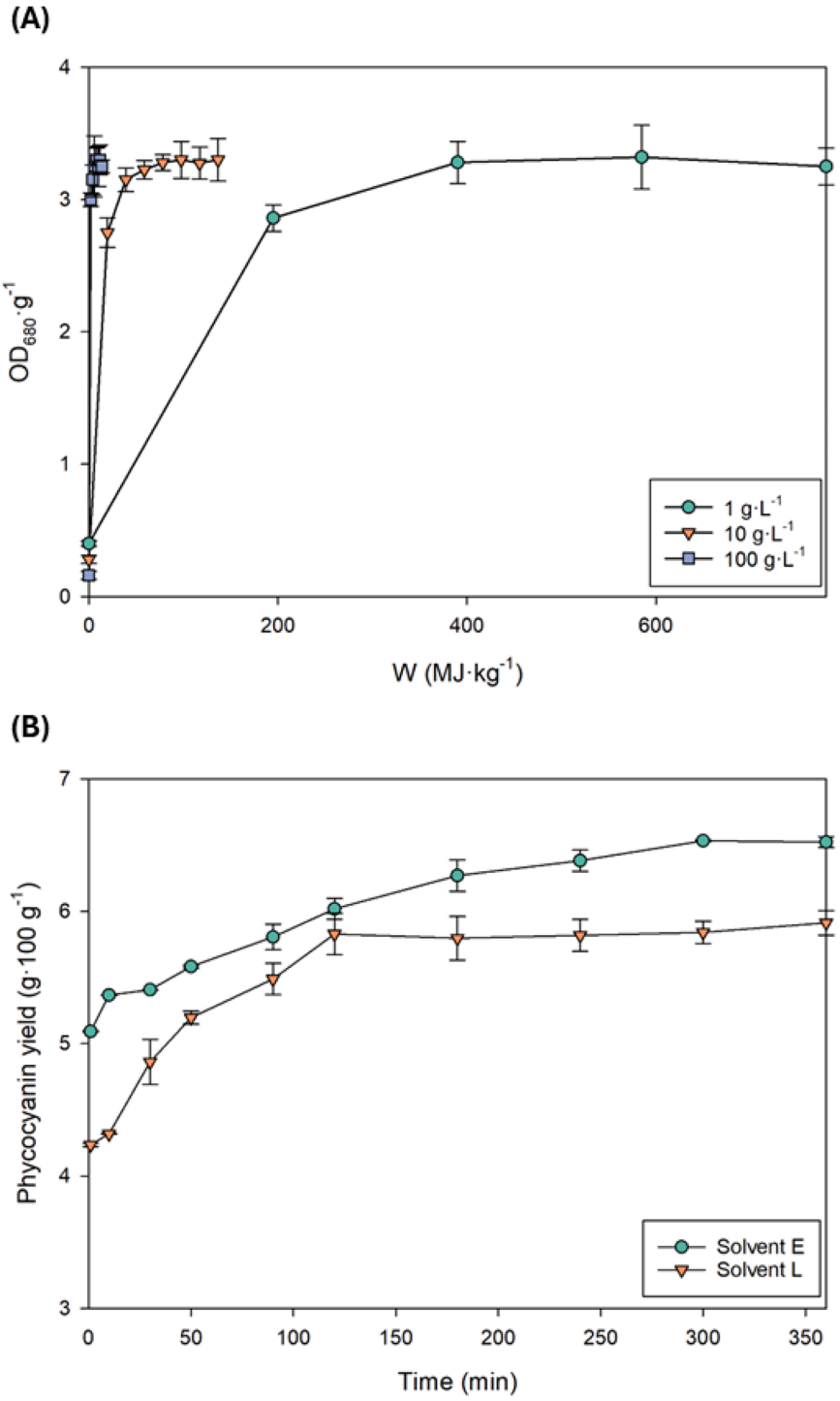

Fig. 2. (A) Effect of biomass concentration on the energetic requirements of cell wall disruption, and (B) effect of cell wall disruption and extraction time on the phycocyanin recovery yield. Data represent the mean value of three independent determinations ± SD. Solvent E: Proline:Glycerol:Sorbitol:Water and Solvent L: Glycerol: sucrose: sorbitol: water.

highest biomass concentration of 75 g·L^{-1} (González-Balderas et al., 2020).

Fig. 2A shows the effect of biomass concentration on the efficiency of the disruption and on the energetic requirements. While disruption efficiency (OD_{680}·g^{-1}) generally increases with specific energy input before plateauing, the energy required to reach this plateau, and the maximum efficiency attained, varied significantly with biomass concentration. More precisely, all concentrations achieved a similar maximum level of cell breakdown, but the 10 and 100 g·L^{-1} concentrations reached this peak with significantly less energy. This indicates that the use of concentrated mixtures led to significantly lower energetic requirements ($p < 0.05$) mainly because the volume that needs to be processed to achieve a given product is lower. Furthermore, in all cases, beyond a certain energy input, cell disruption reached a maximum, and further

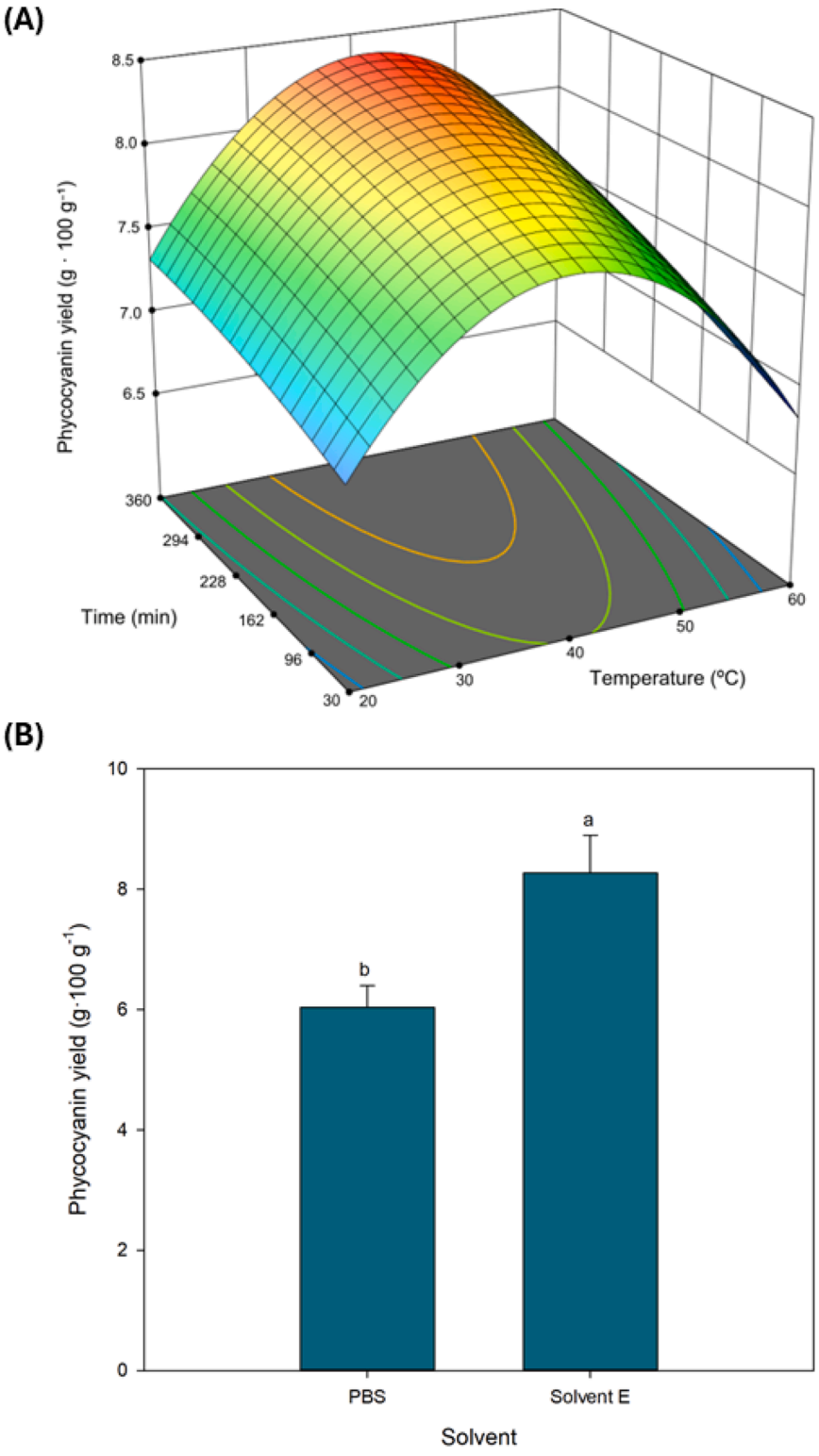

Fig. 3. (A) Effect of extraction time and temperature on the phycocyanin recovery yield and (B) effect of the optimal conditions on the phycocyanin recovery yield. Data represents the mean value of three independent determinations ± SD. Solvent E: Proline:Glycerol:Sorbitol:Water.

energy application did not result in additional breakdown. These results are in line with previous studies where the most concentrated solution led to lower energy consumption (Chegukrishnamurthi et al., 2025).

3.2. Extraction of phycocyanin

3.2.1. Selection of time range

The following assay was performed using only the selected solvents E and L, as they had previously shown the highest phycocyanin recovery. The results reported in this study are similar to those of Hilali et al. (2022), who also found that, among all 60 NADES, glycerol-based ones were the best solvents for phycocyanin extraction. Notably, the glycerol-based NaDES with glucose and either betaine or proline (4:1:1) showed the highest phycocyanin recovery and strong radical scavenging activity. The extraction process was repeated after adding an initial sonication step. The mixtures were then mixed at 60 °C during 6 h (Fig. 2B). In both cases, the initial cell wall disruption step led to an increase in the recovery yield of approximately 400–450 % compared to the values shown in Fig. 1 (without sonication). The main reason is that the cell wall of Spirulina consists of four layers made of glucan and peptidoglycan polymers and acidic polysaccharides providing high resistance (Chen et al., 2020). The recovery yield was slightly higher when using solvent E than solvent L ($p < 0.05$); for this reason, solvent E was selected for further optimisation. These recoveries are in line with other studies. For example, another study applying different US frequencies (20, 30, and 40 kHz) for 20 min obtained 64, 93, and 129.5 $mg{\cdot}g^{-1}$ of phycocyanins from Spirulina, respectively (Berrouane et al., 2022). Phycocyanin extraction began with a high recovery yield, as the cells were already broken, allowing for easy liberation of the compounds. A significant increase in recovery was seen in the first 60 min. From 60 to 200 min, the recovery increased at a slower rate, and after 200 min, the yield became stable with only a very slight further increase. A time range of 30 to 360 min was selected for the following optimisation. This range includes the initial rapid extraction phase and the saturation point, both crucial to obtain the maximum yield.

3.2.2. Temperature and extraction time optimization

To further improve the phycocyanin recovery yield, both temperature and extraction time were optimised using a response surface methodology. Both, extraction time but mainly temperature had a striking effect on the recovery yield ($p < 0.05$). While extraction time had a linear effect on the phycocyanin recovery yield ($p = 0.0002$), the relationship between temperature and extraction yield was mainly quadratic ($p < 0.0001$), which can be seen in Fig. 3A. Unlike the quadratic effect of temperature (which peaks and then decreases), the extraction time has a positive and constant influence on phycocyanin yield, which increases uniformly as the process is prolonged. Overall, the Model F-value (150.7) implied that the model was significant and explained a large proportion of the variability of the phycocyanin recovery yield. The quadratic temperature has the most significant factor, suggesting that there is an optimal temperature to maximise the extraction yield. The ANOVA results showed that the Lack of Fit F-value was 0.59, which means that the Lack of Fit was not significant relative to the pure error. Both the predicted R^2 (0.9585) and the adjusted R^2 (0.9836) were in reasonable agreement. Overall, the application of the RSM led to the following quadratic equation, which was suitable to navigate the design space:

$$Phycocyanin\,recovery\left(mg{\cdot}100\,g^{-1}\right) = 4121.38 + 191.17{\cdot}T + 0.57{\cdot}t + 0.02{\cdot}T{\cdot}t - 2.44{\cdot}T^2$$

where T and t refer to the extraction temperature (°C) and extraction time (min).

As was mentioned above, the phycocyanin yield initially increased as temperature rised, but then started to drop once a specific temperature was exceeded. The model indicates that increasing the temperature from 20 °C to 40 °C substantially improves extraction efficiency. This initial improvement is due to enhanced mass transfer and increased protein solubilisation at higher temperatures. However, further temperature increases led to a decrease in phycocyanin recovery, primarily because of denaturation. This overall behaviour is since cyanobacterial pigment protein stability is highly temperature dependent. This thermal instability is likely caused by the disruption of the protein-pigment complex, which compromises structural integrity and function. These findings align with other studies. For example, Sadewo et al. (2025) observed that increasing the temperature to 50 °C improved extraction efficiency of phycocyanins; however, yields declined beyond 55 °C due to protein degradation. Chentir et al. (2018) also found that temperatures above 50 °C dramatically reduced the concentration of C-Phycocyanin.

The model was validated using a set of 10 conditions randomly selected (data not shown; R^2=0.9357) and optimised, achieving a theoretical maximum recovery yield of 8.06 $g{\cdot}100\ g^{-1}$ at the optimal temperature and extraction time, that is 39.7 °C and 127.9 min, respectively. These optimal conditions were validated (Fig. 3B) and compared against an extraction using PBS, which is the standard solvent used in the literature (Jaeschke et al., 2021). Solvent E led to a higher recovery yield compared to PBS ($p < 0.05$). This is consistent with the results reported by Li et al. (2024), who found that all five glycerol-based DESs tested showed a 13.5-fold higher phycoerythrin yield than water. The phycocyanin recovery was 8.26 ± 0.63 $g{\cdot}100\ g^{-1}$, which is a similar value to that predicted using the above-described model (8.06 $g{\cdot}100\ g^{-1}$). This extract also contained a small amount of chlorophyll a (44.1 ± 4.3 $mg{\cdot}100\ g^{-1}$). The phycocyanin-rich extract was stored and used as a food colourant, as described in the following section. The phycocyanin-rich extract was stored and used as a food colourant as describes in the following section.

3.3. Extraction of chlorophylls

The leftovers from the phycocyanin extraction were used as feedstock to recover chlorophylls and carotenoids using a conventional organic solvent. This second extraction step led to an extract with a chlorophyll concentration of 94.9 $mg{\cdot}L^{-1}$ and an overall recovery yield of 300.81 $mg{\cdot}100\ g^{-1}$. The chlorophyll concentration was achieved by concentrating the initial extract using a rotary evaporator, this step was not optimised. The concentration before the concentration was 21.8 $mg{\cdot}L^{-1}$. The overall chlorophyll-a recovery was lower than that obtained in previous work; for example, previous studies achieved yields of 570 $mg{\cdot}100\ g^{-1}$ (Marzorati et al., 2020) or 684 $mg{\cdot}100\ g^{-1}$ (Tong et al., 2011) using supercritical CO_2 as the solvent. When compared to similar solvents, the chlorophyll-a concentration achieved in previous studies was 5–18 $mg{\cdot}L^{-1}$ (Mohammed et al., 2023), compared to 21.8 $mg{\cdot}L^{-1}$ in this study. This could be explained by the fact that a significant percentage of these non-polar metabolites were either extracted during the first step or degraded due to high temperatures. Moreover, it is important to highlight that the chlorophyll content depends largely on the strain being used and the cultivation conditions. In contrast, a similar chlorophyll recovery was achieved in another study that used a sequential extraction process from Spirulina. The authors first used a polar NaDES followed by a non-polar NaDES, yielding two distinct extracts: a phycocyanin-rich extract (90.85 $mg{\cdot}g^{-1}$) and a chlorophyll-rich extract (2.3 $mg{\cdot}g^{-1}$) (Hilali et al., 2024). The green concentrated extract obtained in this study was used as a food colourant as described below. The leftovers from the chlorophyll recovery were further studied as plant biostimulants.

3.4. Colouring capacity of the extracts

The phycocyanin-rich extract was used to colour gyn with the goal of mimicking the colour of a commercial blue gyn that was pigmented using gardenia flower extract and E-133, a synthetic dye known as brilliant blue that is one of the oldest FDA-approved colour additives.

The results, shown in Fig. 4 showed that the colour difference was minimal at a phycocyanin concentration of 0.037 mg mL^{-1}. At this concentration the colour difference was 4.53, meaning that the colour difference between the two samples was noticeable to the human eye even for an untrained observer. Cserhalmi et al. (2006) established an analytical classification for perceptible colour differences, dividing them into five categories: not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), very visible (3.0–6.0), an extremely remarkable difference (6.0 – 12.0) and above 12.0 a colour or shade difference. Other studies suggest that ΔE values below 3 indicate that the colour of the sample is almost indistinguishable from the reference when viewed with the naked eye (Mezquita et al., 2015). The results were comparable to those reported in a previous study where phycocyanin extracted using phosphate buffer led to a colour difference between commercial isotonic drinks and phycocyanin-coloured isotonic drinks of between 5 and 10 (García et al., 2021). Similarly, the colour difference between blue commercial beverages (wine, isotonic drink) and the same beverage coloured using phycocyanin derived from *Oscillatoria* sp. (and extracted using phosphate buffer) was in the range 5–15 (Morillas-España et al., 2022c). Although the colour differences were perceptible these differences were minimal. In fact, the substitution of the artificial colourant with a natural one is a key advantage that aligns with the growing market demand for natural ingredients. Several studies have shown that colours derived from microalgal extracts are well-accepted by consumers and are commercially viable, even when they differ from traditional products (Villaró et al., 2023). In addition to their colouring potential, Spirulina pigments, particularly phycocyanin, exhibit notable antioxidant activity, which enhances their value as multifunctional ingredients for food applications (Jaeschke et al., 2021). The addition of the phycocyanin-rich extract to the alcoholic beverage did not affect the pH of the product, which was 7.70 ± 0.11. This is relevant as pH has a striking effect on the stability of phycocyanin, which is more stable at pH varying from 5.0 to 7.5 (Pez Jaeschke et al., 2021). The stability of the colour will be further investigated in future studies.

The colour difference was smaller for the beverages coloured using the chlorophyll-rich extract, with colour difference values of 3.93 and 1.65 for the apple liquor (Fig. 5A) and the green gin (Fig. 5B), respectively. The addition of the chlorophyll-rich extract did not affect the pH of the products, which was 7.79 ± 0.12 and 3.50 ± 0.11 for the green gin and apple liquor, respectively. The chlorophyll concentration needed to achieve these values was 0.29 mg mL^{-1} in the apple liquor and 0.07 mg mL^{-1} in the green gin, respectively. The colour achieved in both cases was not the same and this was partially attributed to the lower stability of chlorophylls at acidic pH (Villaró et al., 2023). Under acidic conditions, the central magnesium ion in the chlorophyll molecule is replaced by hydrogen ions to form olive-coloured pheophytin, which partially explains the different colour in both products. The photographs provided in Figs. 4 and 5 offer a visual representation of the beverages' colours. It is important to note that, although they were taken in the same place, with the same illumination, and at the same distance (objective-beverage surface), the perceived colour in these figures may vary due to factors such as monitor calibration or camera angle relative to the beverage. The colour difference between the commercial and the Spirulina-coloured apple liquor was below 2.0, suggesting that the difference was perceptible only through close observation. The commercial alcohol-free apple liquor was coloured using E-102, E-131, and E-104. In the case of the green gin, the colour difference was higher, meaning that it was perceptible at a glance although the difference can be still considered as small. There are few scientific articles that use chlorophylls extracted directly as a green colorant. For example, a 0.05 % concentration of a hydroalcoholic *Tetraselmis chuii* extract was applied to fondant, resulting in a green colour with minimal impact on product quality (Pereira et al., 2024). The food industry uses microalgae as green colourants in the formulation of numerous green coloured foods (Lafarga, 2019). While the colouring capacity of the whole biomass and the extraction of chlorophylls have been widely studied, the colouring capacity of microalgae-derived chlorophyll-rich extracts has not been widely studied.

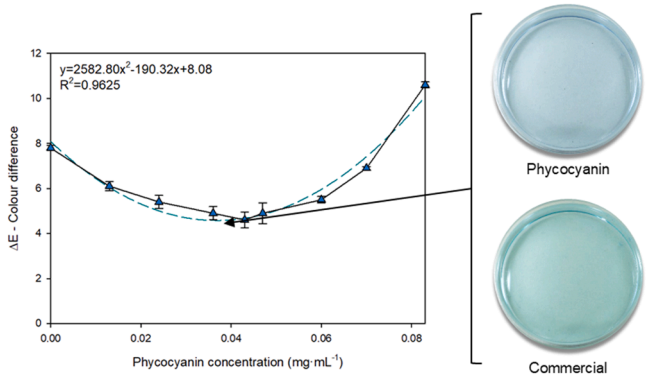

Fig. 4. Evaluation of the colouring capacity of phycocyanin-rich extract. The colour difference indicates the variation between the commercial product and the phycocyanin-rich extract with gin. Values are means of measurements in triplicate measurements ± SD.

3.5. Biostimulant effect of the pigment extraction leftovers

The leftovers from the phycocyanin and chlorophyll extraction were assessed as plant biostimulants by means of assessing the GI of different seeds. Seed treatments such as priming, coating, or soaking are common techniques to promote seed germination and represent a solution to achieve seedling establishment under abiotic stress conditions (Gupta et al., 2022). For example, Kelpak® (Kelp Products Ltd., South Africa), an extract derived from Kelp promoted the germination of *Ceratotheca triloba* seeds under low temperature and osmotic potential (Masondo et al., 2018). In this study, the compounds studied as plant biostimulants were the leftovers from the extraction of phycocyanin and chlorophylls. A previous study evaluated the biostimulant effects of the leftovers obtained after the extraction of proteins from *A. platensis* (Villaró et al., 2023). In that study, proteins were recovered by isoelectric solubilisation/precipitation and the leftovers from that process showed a higher effect on root formation and chlorophyll content compared to the whole biomass. The results obtained in this study are shown in Fig. 6. The leftovers obtained after the pigment extraction showed a significant increase in the GI of all the seeds studied. It is important to highlight that the graph represents the percentage of increase compared to water alone. The GI of all the seeds was around 80–150 % higher and was especially high for pepper and basil seeds. In addition, when compared with the commercial hormone, the residual biomass decreased the GI by 32 % in tomato, but increased it by 24 %, 10 %, and 42 % in cucumber, pepper, and basil, respectively. The results are in line with other studies that demonstrated the GI promotion capacity of microalgae-derived extracts. For example, extracts of different strains belonging to the *Chlorella* genus increased the GI of cucumber seeds by 75–138 % (Ferreira et al., 2021). Similarly, an extract obtained from *Chlorella vulgaris* increase the GI of wheat seeds to 147 % (Viegas et al., 2021). In a different study, the treatment of seeds with an algal extract showed 100 % germination in 3 days, higher than the germination percentage of the untreated seeds (Supraja et al., 2020). Overall, the findings described in this study suggest that extracts derived from microalgae, even those considered leftovers from pigment (phycocyanin and chlorophyll) extraction, hold significant potential as effective biostimulants for enhancing seed germination across various plant species. This highlights a promising route for valorising co-products generated during the downstream processing of *A. platensis* into valuable and sustainable agricultural inputs.

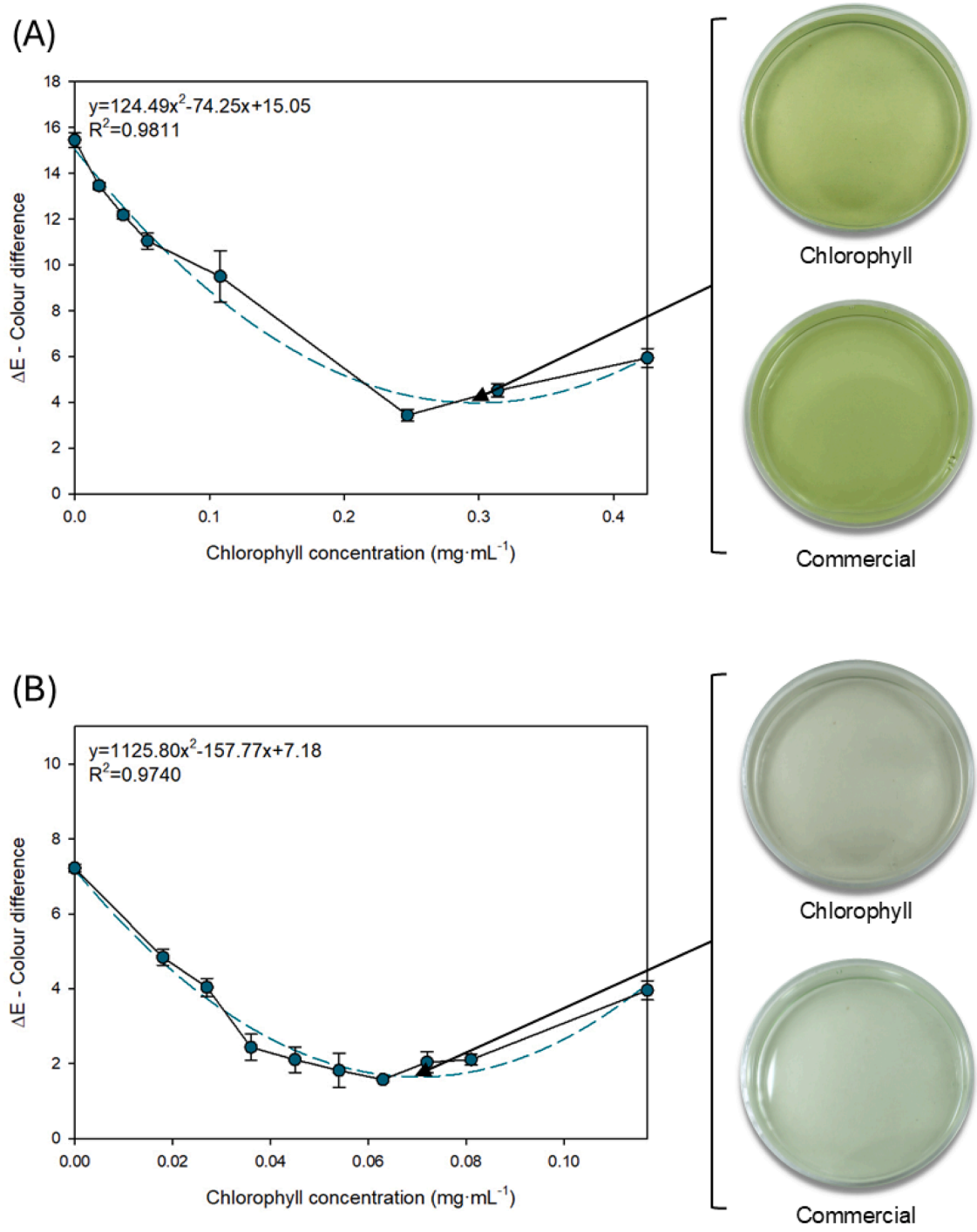

Fig. 5. Colouring capacity of the chlorophyll-rich extract in (A) apple liquor and (B) gin. Data represents the mean value of three independent determinations ± SD.

4. Conclusions

This study developed and validated a comprehensive zero-waste biorefinery strategy for *A. platensis*, maximising the value derived from its biomass. The optimised NADES-based extraction, coupled with ultrasound-assisted cell disruption, proved highly effective for recovering phycocyanin, achieving yields comparable to or exceeding conventional methods. The subsequent extraction of chlorophylls from the residual biomass further enhanced the overall valorisation. Both phycocyanin- and chlorophyll-rich extracts demonstrated promising colouring capacities, effectively mimicking commercial beverage colours with acceptable differences and natural colours. The final co-product, the pigment-free biomass, exhibited plant biostimulant effects, promoting seed germination across various crops. This integrated approach not only provides sustainable alternatives to synthetic colorants but also offers a valuable agricultural input, contributing to a more circular bioeconomy and reducing waste in microalgae processing.

Ethical statement - studies in humans and animals

Not applicable.

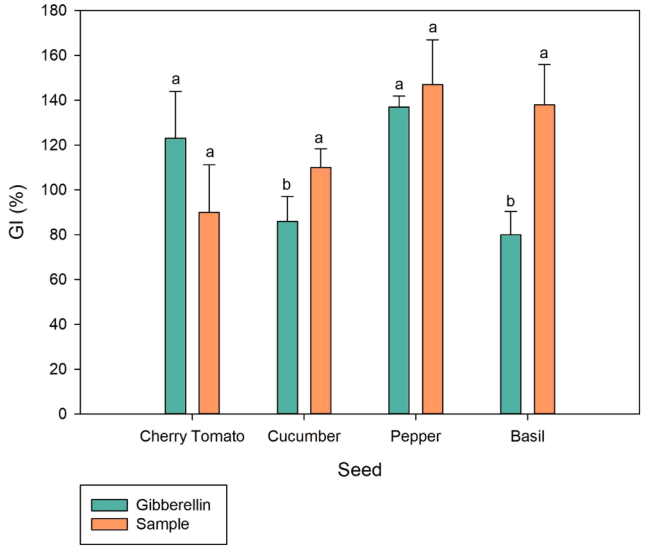

Fig. 6. Germination index. All values represent the percentage of variation with respect to distilled water. Data represent the mean value of three independent determinations ± SD. Different letters indicate statistical differences between the commercial hormone and the leftovers from the pigment's extraction ($p < 0.05$). No comparison was made between different seed type.

CRediT authorship contribution statement

Silvia Villaró-Cos: Writing – original draft, Visualization, Investigation, Formal analysis. **Luisa Gouveia:** Supervision, Resources. **Jelena Vladić:** Resources, Investigation. **Ana Sánchez-Zurano:** Investigation, Formal analysis. **Irene Martínez-García:** Investigation. **Tomás Lafarga:** Writing – original draft, Visualization, Supervision, Funding acquisition, Formal analysis.

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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Data availability

Data will be made available on request.

References

Berrouane, N.E.H., Attal, F.S., Benchabane, A., Saghour, I., Bitam, A., Gachovska, T., Amiali, M., 2022. Freeze–thaw-, enzyme-, ultrasound-and pulsed electric field-assisted extractions of C-phycocyanin from Spirulina platensis dry biomass. J. Food Meas. Charact. 16, 1625–1635. https://doi.org/10.1007/s11694-021-01264-3.

Bortolini, D.G., Maciel, G.M., Fernandes, I., de, A.A., Pedro, A.C., Rubio, F.T.V., Branco, I. G., Haminiuk, C.W.I., 2022. Functional properties of bioactive compounds from Spirulina spp.: current status and future trends. Food Chem. Mol. Sci. 5, 100134. https://doi.org/10.1016/j.fochms.2022.100134.

Bürck, M., Fratelli, C., Assis, M., Braga, A.R.C., 2024. Naturally colored ice creams enriched with C-phycocyanin and spirulina residual biomass: development of a fermented, antioxidant, tasty and stable food product. Fermentation 10, 304. https://doi.org/10.3390/fermentation10060304.

Castro, V., Teixeira, A., Simões, L., Chamorro, F., Lourenço-Lopes, C., Parreira, C., Badenes, S.M., Costa, L., Prieto, M.A., Oliveira, R., Dias, A.C.P., 2025. Chemical characterization and antioxidant potential of arthrospira sp., thalassiosira sp., and raphidonema sp. Food Chem. 469, 142554. https://doi.org/10.1016/j.foodchem.2024.142554.

Chegukrishnamurthi, M., Nagarajan, S., Ravi, S., Mudliar, S.N., Ranade, V.V., 2025. Hydrodynamic cavitation mediated Spirulina valorisation with insights into phycocyanin extraction and biogas production. Commun. Biol. 8, 326. https://doi.org/10.1038/s42003-025-07702-y.

Chen, W., Xu, J., Yu, Q., Yuan, Z., Kong, X., Sun, Y., Wang, Z., Zhuang, X., Zhang, Y., Guo, Y., 2020. Structural insights reveal the effective Spirulina platensis cell wall dissociation methods for multi-output recovery. Bioresour. Technol. 300, 122628. https://doi.org/10.1016/j.biortech.2019.122628.

Chentir, I., Hamdi, M., Li, S., Doumandji, A., Markou, G., Nasri, M., 2018. Stability, bio-functionality and bio-activity of crude phycocyanin from a two-phase cultured Saharian Arthrospira sp. strain. Algal. Res. 35, 395–406. https://doi.org/10.1016/j.algal.2018.09.013.

Cserhalmi, Z., Sass-Kiss, A., Tóth-Markus, M., Lechner, N., 2006. Study of pulsed electric field treated citrus juices. Innov. Food Sci. Emerg. Technol. 7, 49–54. https://doi.org/10.1016/j.ifset.2005.07.001.

Chini Zittelli, G., Mugnai, G., Milia, M., Cicchi, B., Silva Benavides, A.M., Angioni, A., Addis, P., Torzillo, G., 2022. Effects of blue, orange and white lights on growth, chlorophyll fluorescence, and phycocyanin production of Arthrospira platensis cultures. Algal. Res. 61, 102583. https://doi.org/10.1016/j.algal.2021.102583.

European Parliament and Council, 2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Off. J. Eur. Union 354, 16–33. L. https://eur-lex.europa.eu/eli/reg/2008/1333/oj.

Ferreira, A., Melkonyan, L., Carapinha, S., Ribeiro, B., Figueiredo, D., Avetisova, G., Gouveia, L., 2021. Biostimulant and biopesticide potential of microalgae growing in piggery wastewater. Env. Adv.. 4, 100062. https://doi.org/10.1016/j.envadv.2021.100062.

García, A.B., Longo, E., Bermejo, R., 2021. The application of a phycocyanin extract obtained from Arthrospira platensis as a blue natural colorant in beverages. J. Appl. Phycol. 33, 3059–3070. https://doi.org/10.1007/s10811-021-02522-z.

González-Balderas, R.M., Velásquez-Orta, S.B., Valdez-Vazquez, I., Orta-Ledesma, M.T., 2020. Intensified recovery of lipids, proteins, and carbohydrates from wastewater-grown microalgae desmodesmus sp. by using ultrasound or ozone. Ultrason. Sonochem. 62, 104852. https://doi.org/10.1016/j.ultsonch.2019.104852.

Gupta, S., Doležal, K., Kulkarni, M.G., Balázs, E., Van Staden, J., 2022. Role of non-microbial biostimulants in regulation of seed germination and seedling establishment. Plant Growth Regul. 97, 271–313. https://doi.org/10.1007/s10725-021-00794-6.

Hilali, S., Wils, L., Chevalley, A., Clément-Larosière, B., Boudesocque-Delaye, L., 2022. Glycerol-based NaDES as green solvents for ultrasound-assisted extraction of phycocyanin from Arthrospira platensis—RSM optimization and ANN modelling. Biomass. Convers. Biorefin. 12, 157–170. https://doi.org/10.1007/S13399-021-02263-6.

Hilali, S., Van Gheluwe, L., Yagmur, M., Wils, L., Phelippe, M., Clément-Larosière, B., Montigny, B., Jacquemin, J., Thiery, E., Boudesocque-Delaye, L., 2024. NaDES-based biorefinery of Spirulina (Arthrospira platensis): a new path for sustainable high value-added metabolites. Sep. Purif. Technol. 329, 125123. https://doi.org/10.1016/J.SEPPUR.2023.125123.

Hynstova, V., Sterbova, D., Klejdus, B., Hedbavny, J., Huska, D., Adam, V., 2018. Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing chlorella vulgaris and spirulina platensis using high performance thin layer chromatography. J. Pharm. Biomed. Anal. 148, 108–118. https://doi.org/10.1016/j.jpba.2017.09.018.

Jaeschke, D.P., Rocha Teixeira, I., Damasceno Ferreira Marczak, L., Domeneghini Mercali, G., 2021. Phycocyanin from Spirulina: a review of extraction methods and stability. Food Res. Int. 143, 110314. https://doi.org/10.1016/j.foodres.2021.110314.

Jaeschke, D.P., Mercali, G.D., Marczak, L.D.F., Müller, G., Frey, W., Gusbeth, C., 2019. Extraction of valuable compounds from Arthrospira platensis using pulsed electric field treatment. Bioresour. Technol. 283, 207–212. https://doi.org/10.1016/j.biortech.2019.03.035.

Lafarga, T., 2019. Effect of microalgal biomass incorporation into foods: nutritional and sensorial attributes of the end products. Algal. Res. 41, 101566. https://doi.org/10.1016/j.algal.2019.101566.

Lee, S.Y., Cho, J.M., Chang, Y.K., Oh, Y.K., 2017. Cell disruption and lipid extraction for microalgal biorefineries: a review. Bioresour. Technol. 244, 1317–1328. https://doi.org/10.1016/j.biortech.2017.06.038.

Liao, X., Zhang, B., Wang, X., Yan, H., Zhang, X., 2011. Purification of C-phycocyanin from spirulina platensis by single-step ion-exchange chromatography. Chromatographia 73, 291–296. https://doi.org/10.1007/s10337-010-1874-5.

Li, K., Jiang, C., Han, S.Il, Kang, S., Chen, J., Won, D., Kang, Y., Bae, B., Choi, Y.E., Kim, H.S., Lee, J., 2024. Green and efficient method to acquire high-value phycobiliprotein from microalgal biomass involving deep eutectic solvent-based ultrasound-assisted extraction. Food Chem. 449. https://doi.org/10.1016/J.FOODCHEM.2024.139196.

Lin, J., Pang, Y., Huo, Y., Jiang, J., Zhou, B., Shang, C., 2025. Extraction, purification and characterization of Spirulina phycocyanin. Algal. Res. 85, 103861. https://doi.org/10.1016/j.algal.2024.103861.

Liu, Y., Liu, X., Cui, Y., Yuan, W., 2022. Ultrasound for microalgal cell disruption and product extraction: a review. Ultrason. Sonochem. 87, 106054. https://doi.org/10.1016/J.ULTSONCH.2022.106054.

Martins, M., Albuquerque, C.M., Pereira, C.F., Coutinho, J.A.P., Neves, M.G.P.M.S., Pinto, D.C.G.A., Faustino, M.A.F., Ventura, S.P.M., 2021. Recovery of chlorophyll a derivative from Spirulina maxima: its purification and photosensitizing potential. ACS Sustain. Chem. Eng. 9, 1772–1780. https://doi.org/10.1021/acssuschemeng.0c07880.

Masondo, N.A., Kulkarni, M.G., Finnie, J.F., Van Staden, J., 2018. Influence of biostimulants-seed-priming on Ceratotheca triloba germination and seedling growth under low temperatures, low osmotic potential and salinity stress. Ecotoxicol. Env. Saf. 147, 43–48. https://doi.org/10.1016/j.ecoenv.2017.08.017.

Marzorati, S., Schievano, A., Idà, A., Verotta, L., 2020. Carotenoids, chlorophylls and phycocyanin from Spirulina: supercritical CO 2 and water extraction methods for added value products cascade. Green. Chem. 22, 187–196. https://doi.org/10.1039/C9GC03292D.

Mezquita, P.C., Huerta, B.E.B., Ramírez, J.C.P., Hinojosa, C.P.O., 2015. Milks pigmentation with astaxanthin and determination of colour stability during short period cold storage. J. Food Sci. Technol. 52, 1634–1641. https://doi.org/10.1007/s13197-013-1179-4.

Milovanovic, S., Grzegorczyk, A., Świątek, Ł., Tyśkiewicz, K., Konkol, M., Stojanovic, D., 2025. Enhanced separation of valuable compounds from Spirulina using supercritical carbon dioxide: influence of pretreatments and co-solvent addition on composition and bioactivity of extracts. J. Supercrit. Fluids 220, 106545. https://doi.org/10.1016/j.supflu.2025.106545.

Mohammed, I.A., Ruengjitchatchawalya, M., Paithoonrangsarid, K., 2023. Cultivation manipulating zeaxanthin-carotenoid production in Arthrospira (Spirulina) platensis under light and temperature stress. Algal. Res. 76, 103315. https://doi.org/10.1016/j.algal.2023.103315.

Morillas-España, A., Lafarga, T., Sánchez-Zurano, A., Acién-Fernández, F.G., González-López, C., 2022a. Microalgae based wastewater treatment coupled to the production of high value agricultural products: current needs and challenges. Chemosphere 291, 132968. https://doi.org/10.1016/j.chemosphere.2021.132968.

Morillas-España, A., Ruiz-Nieto, Á., Lafarga, T., Acién, G., Arbib, Z., González-López, C. V., 2022b. Biostimulant capacity of chlorella and chlamydopodium species produced using wastewater and centrate. Biology 11, 1086. https://doi.org/10.3390/biology11071086.

Morillas-España, A., Bermejo, R., Abdala-Díaz, R., Ruiz, Á., Lafarga, T., Acién, G., Fernández-Sevilla, J.M., 2022c. Biorefinery approach applied to the production of food colourants and biostimulants from Oscillatoria sp. Biology 2022 (11), 1278. https://doi.org/10.3390/biology11091278.

Na, J., Jang, S., Song, M., Nam, S.E., Choi, W.Y., Shin, H., Kwon, S., Baek, Y., 2025. Unraveling the unique bioactivities of highly purified C-phycocyanin and allophycocyanin. J. Biol. Eng. 19, 34. https://doi.org/10.1186/s13036-025-00496-x.

Novais, C., Silva, S., Pinto, T., 2022. Natural food colorants and preservatives: a review. J. Agric. Food Chem. 70 (1), 1–15. https://doi.org/10.1021/acs.jafc.1c07533.

Pan-utai, W., Iamtham, S., 2019. Extraction, purification and antioxidant activity of phycobiliprotein from Arthrospira platensis. Process. Biochem. 82, 189–198. https://doi.org/10.1016/j.procbio.2019.04.014.

Pereira, T., Barroso, S., Pinto, F.R., Silva, F., Teixeira, P., Mendes, S., Gil, M.M., 2024. Application of microalgae as natural colorant for pastry and confectionary products. Food Sci. Nutr. 12, 9479–9492. https://doi.org/10.1002/fsn3.4394.

Pez Jaeschke, D., Rocha Teixeira, I., Ferreira Marczak, L.D., Mercali, G.D., 2021. Phycocyanin from Spirulina: a review of extraction methods and stability. Food Res. Int. 143, 110314. https://doi.org/10.1016/j.foodres.2021.110314.

Sadewo, B.R., Dewayanto, N., Suyono, E.A., Nisya, A.F., Parafianto, A.N., Zulhan, B.M., Budiman, A., 2025. Phycocyanin ultrasound assisted extraction from Spirulina (Arthrospira platensis) using sodium phosphate buffer solvent: mass transfer modelling and stability test. S. Afr. J. Chem. Eng 53, 103–116. https://doi.org/10.1016/j.sajce.2025.04.004.

Sánchez-Zurano, A., Morillas-España, A., González-López, C.V., Lafarga, T., 2020. Optimisation of protein recovery from Arthrospira platensis by ultrasound-assisted isoelectric solubilisation/precipitation. Processes 8, 1586. https://doi.org/10.3390/pr8121586.

Supraja, K.V., Behera, B., P., B, 2020. Efficacy of microalgal extracts as biostimulants through seed treatment and foliar spray for tomato cultivation. Ind. Crops. Prod. 151, 112453. https://doi.org/10.1016/j.indcrop.2020.112453.

Taragjini, E., Ciardi, M., Musari, E., Villaró, S., Morillas-España, A., Francisco, Alarcón, J., Lafarga, T., 2022. Pilot-scale production of A. platensis: protein isolation following an ultrasound-assisted strategy and assessment of techno-functional properties. Food Bioprocess Technol. 15, 1299–1310. https://doi.org/10.1007/s11947-022-02789-1.

Tong, Y., Gao, L., Xiao, G., Pan, X., 2011. Supercritical CO2 extraction of chlorophyll a from Spirulina platensis with a static modifier. Chem. Eng. Technol. 34, 241–248. https://doi.org/10.1002/ceat.201000379.

Valero-Vizcaino, A., Villaró-Cos, S., Morillas-España, A., Cerdá-Moreno, C., Lafarga, T., 2024. Production of techno-functional proteins and plant biostimulants from Nannochloropsis gaditana. Food Biosci. 59, 104000. https://doi.org/10.1016/j.fbio.2024.104000.

Viegas, C., Gouveia, L., Gonçalves, M., 2021. Evaluation of microalgae as bioremediation agent for poultry effluent and biostimulant for germination. Env. Technol. Innov. 24, 102048. https://doi.org/10.1016/j.eti.2021.102048.

Villaró, S., Acien, G., González-López, C.V., Clagnan, E., Lafarga, T., 2023a. Production of arthrospira platensis BEA 005B: biomass characterisation and use as a colouring additive in macarons. LWT 182, 114843. https://doi.org/10.1016/j.lwt.2023.114843.

Villaró, S., Morillas-España, A., Acién, G., Lafarga, T., 2022. Optimisation of operational conditions during the production of arthrospira platensis using pilot-scale raceway reactors, protein extraction, and assessment of their techno-functional properties. Foods 11, 2341. https://doi.org/10.3390/foods11152341.

Villaró, S., Acién, G., Alarcón, J., Ruiz, Á., Rodríguez-Chikri, L., Viviano, E., Lafarga, T., 2023b. A zero-waste approach for the production and use of Arthrospira platensis as a protein source in foods and as a plant biostimulant in agriculture. J. Appl. Phycol. 35, 2619–2630. https://doi.org/10.1007/s10811-023-02993-2.

Vladić, J., Kovačević, S., Aladić, K., Jokić, S., Radman, S., Podunavac-Kuzmanović, S., Duarte, A.R.C., Jerković, I., 2023. Innovative strategy for aroma stabilization using green solvents: supercritical CO2 extracts of Satureja montana dispersed in deep eutectic solvents. Biomolecules 13, 1126. https://doi.org/10.3390/biom13071126.