



C-phycocyanin extraction assisted by pulsed electric field from *Artrosphira platensis*

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ABSTRACT

This paper assesses the application of pulsed electric fields (PEF) to the fresh biomass of *Artrhospira platensis* in order to enhance the extraction of C-phycocyanin into aqueous media.

Electroporation of *A. platensis* depended on both electric field strength and treatment duration. The minimum electric field intensity for detecting C-phycocyanin in the extraction medium was 15 kV/cm after the application of a treatment time 150 μ s (50 pulses of 3 μ s). However higher electric field strength were required when shorter treatment times were applied. Response surface methodology was used in order to investigate the influence of electric field strength (15–25 kV/cm), treatment time (60–150 μ s), and temperature of application of PEF (10–40 °C) on C-phycocyanin extraction yield (PEY). The increment of the temperature PEF treatment reduced the electric field strength and the treatment time required to obtain a given PEY and, consequently decreased the total specific energy delivered by the treatment. For example, the increment of temperature from 10 °C to 40 °C permitted to reduce the electric field strength required to extract 100 mg/g d_w of C-phycocyanin from 25 to 18 kV/cm, and the specific energy input from 106.7 to 67.5 kJ/Kg.

Results obtained in this investigation demonstrated PEF's potential for selectively extraction C-phycocyanin from fresh *A. platensis* biomass. The purity of the C-phycocyanin extract obtained from the electroporated cells was higher than that obtained using other techniques based on the cell complete destruction.

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1. Introduction

C-phycocyanin is a blue-colored water-soluble protein of great commercial and industrial significance. It is widely used as colorant in both food and cosmetic industries, but also as fluorescent marker in biomedical research. Furthermore, its potential as a therapeutic agent in oxidative stress-induced diseases has been demonstrated (Zhao, Yi-liang, Jia-mei, & Wei-min, 2014).

The blue-green microalga *Artrosphira platensis* is an excellent source of C-phycocyanin. This compound that serves as a principal photoreceptor for this cyanobacterium's photosynthesis is arranged, along with other phycobiliproteins, into supramolecular complexes called phycobilisomes, located in the thylacoid membranes (Sekar & Chandramohan, 2008). It has been estimated that this cyanobacterium's protein fraction may contain up to 20% of C-phycocyanin. The commercial exploitation of this colored substance requires its extraction from the phycobilisomes, and subsequent purification. Pigment purity is of utmost importance, particularly for fluorescent applications in clinical and immunological analysis (Sekar & Chandramohan, 2008).

It has been reported that the extraction of phycobiliproteins from cyanobacteria is notoriously difficult because of the extremely resistant cell wall (Wyman, 1992). Although many different methods have been assayed for the extraction of C-phycocyanin from *A. platensis*, none of them are considered as standard procedure.

C-phycocyanin can be extracted from dry or wet *A. platensis* biomass into aqueous media. In order to improve the extraction yield and to reduce extraction time, different procedures that cause the breakage of cell envelopes such as freeze/thaw cycles, homogeneization in mortar and pestle, sonication, bead milling and lysozyme disintegration of the cell wall have been assayed (Duangsee, Phoopat, & Ningsanond, 2009). However, all of these cell disruption methods are characterized by a lack of specificity that causes the release of cell debris or other impurities that could negatively affect the quality and purity of the extracts, as well as downstream recovery and purification operations. On the other hand, it has been reported that the drying of *A. platensis* biomass resulted in approximately 50% loss of C-phycocyanin (Sarada, Pillai, & Ravishankar, 1999).

Pulsed electric fields (PEF) cause the increment of cell membrane permeability (electroporation), via the application of high-intensity electric field pulses of short duration – generally in the order of microseconds to milliseconds (Kotnik, Kramar, Pucihar, Miklavcic, & Tarek, 2012). Several studies have demonstrated that the electroporation of

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bacteria, yeast and microalgae improves the extraction of intracellular compounds of interest, such as lipids, proteins, carbohydrates, or pigments with low energy consumption (Barba, Grimi, & Vorobiev, 2015; Coustets et al., 2015; Ganeva, Galutsov, & Teissie, 2003; Goettel, Eing, Gusbeth, Straessner, & Frey, 2013; Luengo, Martínez, Bordetas, Álvarez, & Raso, 2015; Parniakov et al. 2015a and b; Postma et al., 2016; Zbinden et al., 2013). Treatment of fresh *A. platensis* biomass by PEF could improve the current extraction process of C-phycoerythrin from fresh biomass by reducing the release of cells debris, facilitating subsequent purification operations, and minimizing the energetic costs and losses associated with the drying of the biomass.

Temperature of application of PEF has been demonstrated to be a key parameter affecting cell membrane electroporation. Several studies have demonstrated that increasing the temperature decreases the critical electric field required to cause electroporation in both eukaryote and prokaryote cells and enhance effects derived from PEF treatment such as microbial inactivation or improving extraction of intracellular compounds (Lebovka, Praporscic, Ghnini, & Vorobiev, 2005; Saldaña, Álvarez, Condón & Raso, 2014).

This study aims to evaluate the potential of the application of PEF to fresh *A. platensis* biomass to improve the extraction of C-phycoerythrin into aqueous media, assessed both in terms of extraction yield and purity of the extract. Here we have evaluated the effects of PEF electric field strength, treatment time and application temperature, in order to establish which are the optimal conditions for C-phycoerythrin extraction.

2. Material and methods

2.1. Culture conditions of *Arthrospira platensis*

Arthrospira platensis (BNA 0007B, National Bank of Algae, Canary Islands, Spain) were grown in a modified *Spirulina* medium (Aiba & Ogawa, 1977).

Cells were cultured photoautotrophically in 2-L tubes of 8 cm diameter and 53 cm height, bubbled with air (6 mL/s) at 30 °C, in light:dark cycles (12:12 h) using white fluorescent lamps (15 mmol/m² s). The culture medium was initially inoculated at an optical density of 0.1 at 560 nm using a pre-culture. To determine biomass concentration, samples were taken every 24 h. Experiments were performed using cells at the stationary phase of growth after an incubation time between 7 and 9 days. Biomass concentration at the stationary phase was around 1 g_{dw}/L. Dry weight (dw) of microalgae was determined by vacuum drying (GeneVac Ltd., UK) at 60 °C using 1 mL of the cell suspension until reaching constant weight (around 1 h).

2.2. Cell disruption

In order to determine the total amount of C-phycoerythrin, an aliquot of 150 µL of wet cell biomass was blended with 1350 µL of distilled water, and the mixture was disrupted by bead-beating using a bead beater (bead diameter 0.1 mm, BioSpec Products INC, USA) at a speed of 4800 rpm (10 cycles of 10 s). Following each cycle, the sample was cooled down in water at 0 °C to avoid overheating of the sample.

2.3. PEF treatments

The PEF equipment used in this investigation was previously described by Saldaña et al., 2010. Fresh biomass of *A. platensis* was treated at different temperatures in a tempered batch parallel-electrode treatment chamber with a distance between electrodes of 0.25 cm and an area of 1.76 cm². The inner part of the electrodes was empty and dielectric oil (conductivity: 1.4 µS/cm) tempered was recirculated through both electrodes to temper the treatment medium at (10.0, 25.0, 40.0 °C) and to maintain a constant temperature in the medium during the PEF treatments. The temperature of the treatment medium was

measured with a thermocouple before and after the PEF treatment; temperature variations were always lower than 2 °C.

Before treatment, fresh biomass of *A. platensis* was centrifuged at 3000 × g for 10 min at 25 °C and suspended in distilled water. Three cycles of centrifugation and resuspension in distilled water were conducted to obtain a final conductivity of 1.0 mS/cm at 25 °C. This conductivity did not change at 10 °C mS/cm and increased to 1.1 at 40 °C mS/cm. The *A. platensis* suspension (0.44 mL) was placed in the treatment chamber by means of a 1 mL sterile syringe (TERUMO, Leuven, Belgium). The suspension was subjected from 15 to 50 monopolar square 3 µs waveform pulses of 3.75, 5 and 6.25 kV. These voltages resulted in electric field strengths of 15, 20, 25 kV/cm respectively that corresponded with total specific energies that ranged from 13.5 to 110.1 kJ/kg of suspension. The energy per pulse (*W*) was calculated using the following equation:

$$W = \int_0^t \sigma \cdot E(t)^2 dt \quad (1)$$

in which σ (S/m) is the electrical conductivity of the treatment medium; E (V/m) is the electric field strength; and t (s) is the duration of the pulse. The total energy (kJ) applied was calculated by multiplying the energy per pulse (*W*) by the number of pulses. The total specific energy (kJ/kg) applied was determined by dividing the total energy by the mass of treated medium. Frequency of application of the treatments was 0.5 Hz.

2.4. C-phycoerythrin extraction

For C-phycoerythrin extraction, 1 mL of the untreated or PEF-treated *A. platensis* suspension was added to 19 mL of distilled water. The extraction was conducted in a rotary shaker at 20 °C in the dark. In order to obtain extraction curves, samples were gradually collected until 420 min. After centrifugation (6000 × g for 90 s), the supernatant's optical density was measured at 615 and 652 nm. C-phycoerythrin concentration was calculated according to the following equation (Bennett & Bogorad, 1973):

$$PC = OD_{615} - 0.474 \times (OD_{652})/5.34 \quad (2)$$

in which PC is the C-phycoerythrin concentration (mg/mL), OD_{615} is the optical density of the sample at 615 nm, and OD_{652} is the optical density of the sample at 652 nm.

The purity of C-phycoerythrin extract was monitored spectrophotometrically by the following equation (Abelde, Betancourt, Torres, Cid, & Barwell, 1998):

$$EP = OD_{615}/OD_{280} \quad (3)$$

in which EP is the protein extract purity and OD_{615} indicates the phycoerythrin concentration; OD_{280} is the optical density of the sample at 280, indicating the total concentration of proteins in the solution.

The C-phycoerythrin extraction yield (PEY) was calculated as:

$$PEY = PC \times V/d_w \quad (4)$$

in which PC is the C-phycoerythrin concentration (mg/mL), *V* is the volume of solvent (mL), and *d_w* is the dried biomass (g).

2.5. Experimental design

Response surface methodology (RSM) was used to evaluate the effect of electric field strength (15–25 kV/cm), treatment time (60–150 µs), and temperature (10–40 °C) on the C-phycoerythrin extraction yield (PEY) from *A. platensis* after 360 min of extraction.

The data obtained after having treated the cells under the conditions described in Section 2.2 were modeled with the following second-order

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