Effect of Pulsed Electric Field Treatments on Permeabilization and Extraction of Pigments from *Chlorella vulgaris*

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Abstract The effect of pulsed electric field (PEF) treatments of different intensities on the electroporation of the cytoplasmatic membrane of *Chlorella vulgaris,* and on the extraction of carotenoids and chlorophylls were investigated. Staining the cells with propidium iodide before and after the PEF treatment revealed the existence of reversible and irreversible electroporation. Application of PEF treatments in the range of 20–25 kV cm^{-1} caused most of the population of *C. vulgaris* to be irreversibly electroporated even at short treatment times (5 pulses of 3 μs). However, at lower electric field strengths (10 kV cm^{-1}), cells that were reversibly electroporated were observed even after 50 pulses of 3 μs. The electroporation of *C. vulgaris* cells by PEF higher than 15 kV cm^{-1} and duration is higher than 15 μs increased significantly the extraction yield of intracellular components of *C. vulgaris.* The application of a 20 kV cm^{-1} for 75 μs increased the extraction yield just after the PEF treatment of the carotenoids, and chlorophylls *a* and *b* 0.5, 0.7, and 0.8 times, respectively. However, further increments in electric field strength and treatment time did not cause significant increments in the extraction yield. The extraction of carotenoids from PEF-treated *C. vulgaris* cells after 1 h of the application of the treatment significantly increased the extraction yield in comparison to the yield obtained from the cells extracted just after the PEF treatment. After PEF treatment at 20 kV cm^{-1} for 75 μs, extraction yield for carotenoids, and chlorophylls *a* and *b* increased 1.2, 1.6, and 2.1 times, respectively. A high correlation was observed between irreversible electroporation and percentage of yield increase when the extraction was conducted after 1 h of the application of PEF treatment (R: 0.93), but not when the extraction was conducted just after PEF treatment (R: 0.67).

Keywords Microalgae · Carotenoids · Chlorophylls · Extraction · PEF · Permeabilization

Introduction

Microalgae are a diverse group of microorganisms with a great potential for the production of valuable biologically active products such as carotenoids, chlorophylls, phycobilins, fatty acids, vitamins, sterols, etc. (Pulz and Gross 2004). The current consumer demands for more natural products with fewer synthetic additives together with their wide range of biological activities of the products produced by these microorganisms have made microalgae bioproducts the focus of interest of the food, cosmetic, and pharmaceutical industries (Olaizola 2003).

In recent years, production of higher yields of microalgae-specific bioproducts has been improved by advances based on molecular biology and optimization of cultivation factors (temperature, pH, light, carbon source, salinity, nutrients, etc.) (Gassel et al. 2014; Gao et al. 2013; Jeon et al. 2013). These advances together with the possibility of operating large photobioreactors that are able to handle biomass and metabolites at sufficiently high levels are key factors in the economic viability of commercial exploitation of different products from microalgae (Del Campo et al. 2007). However, there are presently still several obstacles to fully taking advantage of bioproducts-producing microalgae such as the ability to successfully extract these compounds from the cell biomass. (Cooney et al. 2009)

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Bioproducts produced by microalgae are generally localized in the intracellular space or accumulated in organelles (e.g., pigments), vesicles, or in the cytoplasm. The presence of a cell wall surrounding the cells, and especially of an intact cytoplasmic membrane that acts as a semipermeable barrier, influences the extraction of these compounds from cells (Vanthoor-Koopmans et al. 2013). Traditionally, extraction of microalgae bioproducts is mainly conducted from dried biomass with organic or aqueous solvents, depending on the polarity of the compound to be extracted (Ceron et al. 2008). Conventional liquid extraction of compounds from microalgal matrices is time consuming, and a relatively large amount of solvents has to be used, which, in the case of organic solvents, is expensive and potentially harmful. Generally, in order to reduce time and solvent volumes, cells are mechanically disrupted prior to the extraction process. Mechanical disruption of microalgae can be accomplished in a variety of ways such as bead milling, homogenization, and ultrasound (Prabakaran and Ravindran 2011). However, these mechanical cell disruption methods are characterized by a lack of specificity that causes a range of cell debris or other impurities to be released with the compound of interest. This negatively affects the purification operation downstream (Balasundaram et al. 2009). Moreover, some of these treatments need to be performed in batch mode (bead milling), making it difficult to scale up the technology and they involve high power consumption (ultrasound). The use of supercritical CO_2 extraction has gained acceptance in recent years to extract high-value products from microalgae. The main advantage of this procedure is that the extracts are free of potentially harmful solvent residues (Macias-Sanchez et al. 2010). However, in some cases, extracts with relatively poor selectivity are obtained, and the cost of supercritical fluids and the associated equipment makes it difficult to compete with classical solvent extraction especially because this technology requires working with dry biomass (Cheng et al. 2011; Mendes et al. 2003). Drying microalgal biomass requires a significant amount of energy and may cause losses of valuable food compounds (Cooney et al. 2009).

Treatment of fresh microalgal biomass by pulsed electric field (PEF) could replace the conventional techniques that aim at improving the bioproduct extraction from microalgae. PEF is a technology that causes electroporation of the cell membranes by application of intermittent electric field strength of high intensity for periods of time in the order of microseconds. Electroporation causes the increment of the cell membrane permeability to ions and macromolecules due to the formation of local defects or pores in the cell membranes. Depending on the intensity of the treatment and cell characteristics, reversible or irreversible pores can be formed (Weaver and Chizmadzhev 1996).

This technology has been proved to be an effective method for irreversible permeabilization of cell membranes of both eukaryotes and prokaryotes (Boussetta et al. 2013; Donsi et al. 2010; Monfort et al. 2012). It has been demonstrated that PEF increases the extraction rates and yield of different intracellular compounds of interest from plant cells such as sugar, polyphenols, anthocyanins, chlorophylls, carotenoids, and betalains (Puertolas et al. 2012).

The application of PEF for improving microalgal lipid extraction has been previously observed (Goettel et al. 2013; Grimi et al. 2014; Sheng et al. 2011; Zbinden et al. 2013). However, a better understanding of the process conditions required for microalgae electroporation and the mechanisms involved in this effect is required to define the processing conditions necessary for obtaining the maximum extraction yield of metabolites of microalgae with lower energetic consumption.

Chlorella vulgaris is a unicellular *Chlorophyta* alga that is able to accumulate high levels of the carotenoid lutein and other pigments such as chlorophylls *a* and *b* (Gouveia et al. 1996). The objective of this study was to investigate the relationship between reversible or irreversible electroporation of *C. vulgaris* cells, loss of viability, and enhanced extraction of carotenoids, and chlorophylls *a* and *b*.

Materials and Methods

Cell Culture

Chlorella vulgaris (BNA 10-007, National Bank of Algae, Canary Islands, Spain) were grown in BG-11 medium containing the following components: 15 g L^{-1} NaNO$_3$, 4.0 g L^{-1} K$_2$HPO$_4$, 7.5 g L^{-1} MgSO$_4$·7H$_2$O, 3.6 g L^{-1} CaCl$_2$·2H$_2$O, 0.6 g L^{-1} citric acid, 6 g L^{-1} ammonium ferric citrate green, 0.1 g L^{-1} EDTA. Na$_2$, 2.0 g L^{-1} Na$_2$CO$_3$, and trace metal solution (H$_3$BO$_3$ 2.86 g L^{-1}, MnCl$_2$·4H$_2$O 1.81 g L^{-1}, ZnSO$_4$·7H$_2$O 0.22 g L^{-1}, Na$_2$MoO$_4$·2H$_2$O 0.39 g L^{-1}, CuSO$_4$·5H$_2$O 0.08 g L^{-1}, and Co(NO$_3$)2·6H$_2$O 0.05 g L^{-1}). For solid medium, 1.5 g of technical agar was added to 100 mL of medium. Medium BG 11 (liquid and solid) was autoclaved at 121 °C for 20 min.

Cells were cultured photoautotrophically in 1-l Roux flask bubbled with air (6 mL s^{-1}) at 30 °C, in light:dark cycles (12:12 h) with white fluorescent lamps (15 mmol m^{-2} s^{-1}). The cultivation medium was initially inoculated at a concentration of 1×10^6 cells mL^{-1} using a pre-culture obtained from a single colony. Cell density was determined by microscope (microscope L-Kc, Nikon, Tokyo, Japan) in a Thoma cell chamber (ServiQuimia, Constantí, Spain). Experiments were performed with cells at the stationary phase of growth after an incubation time between 10 and

20 days. Dry weight of microalgae was determined by vacuum drying (GeneVac Ltd, UK) at 60 °C using 1 mL of the microalgal suspension.

PEF Treatments

The PEF equipment and treatment chamber used in this investigation were previously described by Saldana et al. 2010. Microalgae were treated in a tempered batch parallel-electrode treatment chamber (25.0 ± 0.1 °C) with a distance between electrodes of 0.25 cm and an area of 1.76 cm^2. The temperature of the treatment medium was measured with a thermocouple before and after PEF treatment, and the temperature variation was always lower than 2 °C. The energy per pulse (W) was calculated using the following equation:

$$W = \int_0^i k \cdot E(t)^2 \mathrm{d}t, \tag{1}$$

where k (S m^{-1}) is the electrical conductivity of the treatment medium, E (V m^{-1}) 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 by the number of pulses. The total specific energy (kJ kg^{-1}) applied was determined by dividing the total energy by the mass of the treated medium.

Before the treatments, microalgae were centrifuged at $3,000 \times g$ for 10 min at 25 °C and re-suspended in a citrate-phosphate McIlvaine buffer (1 mS cm^{-1}; pH 7). With this conductivity (1 mS cm^{-1}), the resistance of the treatment chamber (140 Ω) was in the range of resistances that permits to obtain square wave pulses with the PEF equipment used in this investigation. The microalgal suspension (0.5 mL) at a concentration of 10^9 CFU mL^{-1} was placed into the treatment chamber by means of a 1-mL sterile syringe (TERUMO, Leuven, and Belgium). C. vulgaris cells were subjected to up to 50 square waveform pulses of 3 μs at 10, 15, 20, and 25 kV cm^{-1} corresponding with the specific energies per pulse of 0.30, 0.66, 1.2, and 1.86 kJ L^{-1} of culture (0.009, 0.021, 0.038, and 0.059 kJ kg^{-1} dry weight). Frequency of pulse delivery was 0.5 Hz.

Enumeration of Viable Cells

PEF-treated and control cell suspensions were serially diluted in McIlvaine buffer (1 mS cm^{-1}; pH 7) sterile solution. From the selected dilutions, 20 μL was streak plated into solid media. Plates were incubated at 30 °C for 7 days with the same light regime used for the liquid culture. Longer incubation times did not increase the microalgal counts. Then, incubation colonies were counted to determine the number of survivors.

Staining Cells with Propidium Iodide

Detection of electroporation of C. vulgaris cells was performed with the uptake of the fluorescent dye propidium iodine (PI) (Sigma-Aldrich, Barcelona, Spain). PI is a small (660 Da) hydrophilic molecule that is unable to cross through an intact cytoplasmatic membrane. The cells stained by PI were observed using an epi-fluorescence microscope (Nikon, Mod. L-Kc, Nippon Kogaku KK, Japan), and the fluorescence of the whole population was measured with a spectrofluorophotometer (mod. Genios, Tecan, Austria) using a 535-nm excitation filter (523–547 nm) and a 625-nm emission filter (608–642 nm). Two alternative staining protocols were followed under the same experimental conditions to detect reversible and irreversible electroporation.

Staining Cells Before PEF Treatments

Before PEF treatments, microalgae were centrifuged at $3,000 \times g$ for 10 min at 25 °C and re-suspended in a citrate-phosphate McIlvaine buffer (1 mS cm^{-1}; pH 7) to a final concentration of approximately 10^9 cells mL^{-1}. After that, PI was added to cell suspensions to a final concentration of 0.8 mM and the suspension was treated by PEF. After PEF treatment, microalgae in contact with PI were incubated for 10 min. Previous experiments showed that longer incubation times did not influence the fluorescence measurements. After incubation, the cell suspensions were centrifuged and washed two times until no extracellular PI remained in the buffer. PI trapped inside the cells was quantified by spectrofluorophotometry. Results were expressed as the percentage of permeabilized cells based on the fluorescence value obtained for cells permeabilized by the most intense PEF treatment (150 μs at 25 kV cm^{-1}) used in this investigation. Under these conditions, the permeabilization of individual cells was also checked using an epi-fluorescence microscope.

The degree of permeabilization evaluated following this protocol corresponds to the sum of the irreversible and reversible permeabilized cells.

Staining Cells After PEF Treatment

PI was added to a final concentration of 0.8 mM after application of the PEF treatment to the microalgae suspension. After the addition of the PI, suspension was incubated for 10 min, and centrifuged and washed two times until no extracellular PI remained in the buffer; then, the fluorescence was measured. The degree of permeabilization when cells were stained after the PEF treatment corresponded to irreversible permeabilized cells. Reversible permeabilization was determined by comparing the fluorescent measured following the two staining protocols.

Fluorescence measures were based on mean values obtained from at least two independent experiments.

Pigment Extraction

For pigment extraction, 100 µL of non-treated or PEF-treated suspension just after the PEF treatment or after 1 h of incubation in the treatment medium at 20 °C was added to 1 mL of 96 % ethanol and vortexed. The mixture was incubated in the dark at room temperature for 20 min and centrifuged at 6,000×g for 90 s. The absorbance of the supernatant was measured at 470, 649, and 664 nm against a 96 % ethanol blank. The concentrations of total carotenoids, and chlorophylls a and b were calculated according to the following equations (Lichtenthaler 1987):

$$\text{Chlorophyll } a\,(Ca) : (13.36 \times A664) - (5.19 \times A649), \tag{2}$$

$$\text{Chlorophyll } b\,(Cb) : (27.43 \times A649) - (8.12 \times A664), \tag{3}$$

Total carotenoids:
$$(1,000 \times A470 - 2.13 \times Ca - 97.64 \times Cb)/209. \tag{4}$$

Statistical Analysis

The results correspond to the average of two independent experiments conducted with two different microalgae suspensions. The presented results are mean ± standard deviation. One-way analysis of variance (ANOVA) using the Tukey's test was performed to evaluate the significance of differences between the mean values. Differences were considered significant at $p < 0.05$. GraphPad PRISM (GraphPad Software, San Diego, CA USA) was used to perform the statistical analysis.

Results and Discussion

Figure 1 shows the influence of treatment time at different electric field strengths on the electroporation of the cytoplasmatic membrane of C. vulgaris, when PI was added before (1A) and after (1B) the PEF treatment. Independent of the staining protocol, the uptake of PI increased with the treatment time and intensity of the electric field strength. However, at 10 kV cm^{-1} and after treatment times equal to or lower than 75 µs at 15, 20, and 25 kV cm^{-1}, PI uptake was higher when the dye was added before the PEF treatment. For example, after 10 kV cm^{-1} for 75 µs, the PI uptake was near 80 % when PI was added before the PEF treatment but it was only 12 % when it was added after the treatment. The difference between the PI uptakes under the same PEF treatment conditions reveals the existence of
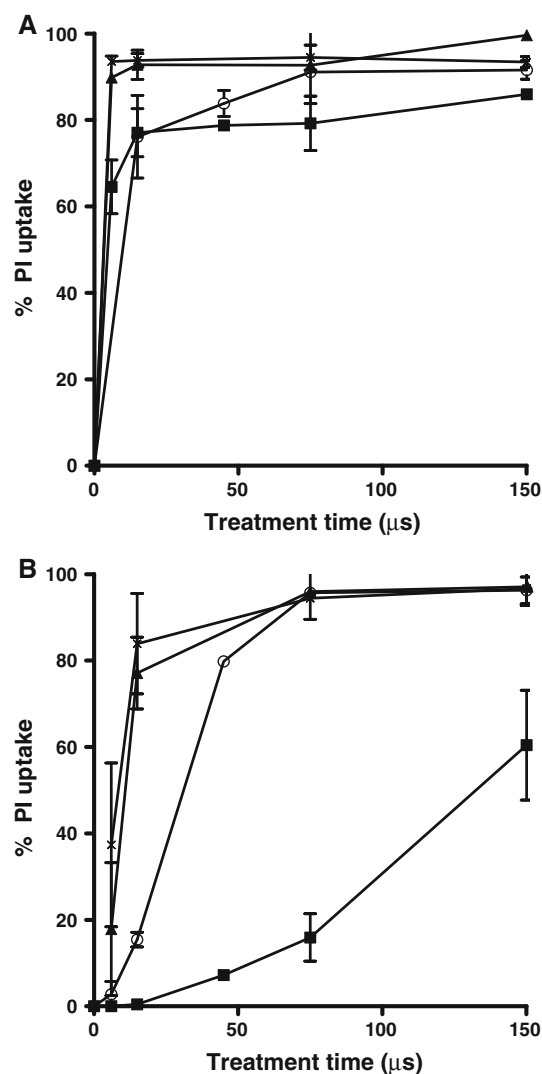

Fig. 1 Influence of treatment time at different electric field strengths on the PI uptake when PI was added before (a) and after (b) the PEF treatment. 10 kV cm^{-1} (black square), 15 kV cm^{-1} (white circle), 20 kV cm^{-1} (black triangle), and 25 kV cm^{-1} (times)

reversible electroporation. It means that in a proportion of microalgal cells, which correspond to the reversibly electroporated population, the permeabilization caused by PEF disappeared after the treatment. Consequently, in these cells, PI could enter into the cytoplasm during the PEF treatment but it was not able to cross the cytoplasmic membrane if PI was added after the treatment. It is generally accepted that a specific transmembrane voltage threshold exists for the manifestation of the electroporation phenomenon. This threshold depends not only on the intensity of the external electric field applied but also on the size and dimension of the cell. When the external voltage applied generates a cell transmembrane voltage around the critical value, reversible electroporation occurs; if the transmembrane voltage generated is higher than the

critical value, the electroporation is irreversible (Ivorra 2010). In this study, it has been observed that PEF treatments of an electric field strength ≥ 20 kV cm^{-1} even with short treatment times (2 pulses of 3 µs) caused the irreversible electroporation of most of the population of *C. vulgaris* (small differences were observed in the percentage of PI uptake when the PI was added before or after the treatment). However, at lower electric field strengths, the PEF treatment caused both reversible and irreversible electroporation in the population of cells of *C. vulgaris*. Similar results have been obtained by other authors investigating the electroporation of different bacteria by PEF (Garcia et al. 2007; Wouters et al. 2001). The existence of both types of electroporated microalgal cells could be explained because the induced transmembrane voltage at lower electric field strength was not high enough for causing irreversible electroporation in the smaller size cells of the microalgal population.

The relationships between the percentage of PI uptake when the PI was added before (Fig. 2a) or after (Fig. 2b) the PEF treatment and the percentage of dead cells estimated by plate counting after the treatment are shown in Fig. 2. A theoretical straight line with slope 1 and intercept 0, which represents a perfect agreement between percentage of PI uptake and cell death has been included in Fig. 2. According to the results shown in Fig. 2a, cell death was not correlated with the percentage of PI uptake. While the percentage of PI uptake ranged from 60 to 100 %, the percentage of dead cells ranged from 0 to 100 %. For example, a treatment that permeabilized 70 % of the cells when PI was added before the treatment did not cause significant death in the population of *C. vulgaris* (Fig. 2a). According to Fig. 2a, when the % of dead cells was lower than 80 %, the number of permeabilized microalgal cells was higher than the number of dead cells. Therefore, a percentage of electroporated cells closed the pores after the treatment and as consequence they survived. Gram-positive bacteria capable of resealing their pores after the PEF treatment and surviving have been also observed by other authors (Garcia et al. 2007; Wouters et al. 2001). On the other hand, when the cells were stained after the PEF treatment (Fig. 2b), no correlation was observed between the percentage of PI uptake and dead cells when the percentage of dead microalgal cells was lower than 80 %. However, in this case, the percentage of irreversible permeabilized cells was lower than the percentage of death cells. Hence, according to these results, a percentage of the cells that were dead during the treatment were able to recover the integrity of the membrane becoming the cytoplasmatic membrane not permeable to PI when the dye was added after the treatment. Other authors have also observed the presence of dead cells with impermeabilized cytoplasmatic membranes within a population of Gram-
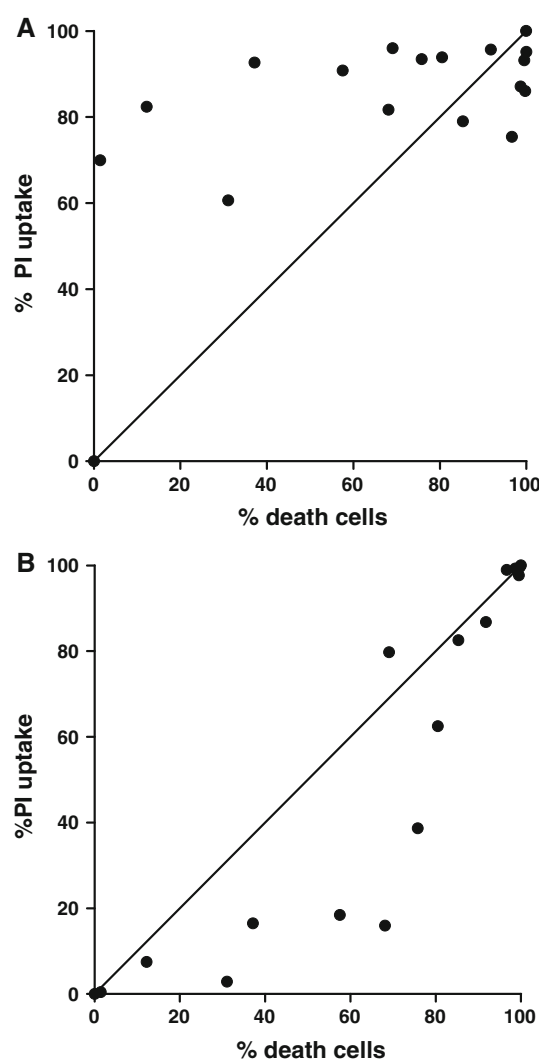

Fig. 2 Relationship between the percentages of cell permeabilization assessed by PI staining before (**a**) and after PEF (**b**) against the percentage of death cells. To show the degree to which each treatment causes membrane permeabilization, a theoretical *straight line* with slope = 1 and intercept = 0 is included

negative bacteria treated by PEF when they were treated in a medium of pH 7 (Aronsson et al. 2005; Garcia et al. 2007). According to these authors, death of these cells could be caused by secondary damages to other structures or functions.

In summary, the application of mild PEF treatment to a population of *C. vulgaris* may result in non-electroporated cells and electroporated cells. Between the electroporated cells we can find live reversibly electroporated cells, dead cells with their cytoplasmatic membranes not permeabilized, and dead cells with their cytoplasmatic membranes permeabilized. No relationship between the occurrence of membrane permeabilization by PEF and cell death would indicate that the quantification of the number of inactivated cells is not a good index for the estimation of the efficacy

Fig. 3 Influence of treatment time at different electric field strengths on the extraction yield of carotenoids (**a**), chlorophyll *a* (**c**), and chlorophyll b (**e**) from *C. vulgaris* just after the PEF treatment, and extraction yield of carotenoids (**b**), chlorophyll *a* (**d**), and chlorophyll *b* (**f**) from *C. vulgaris* after 1 h of incubation after the PEF treatment. Control (■); 10 kV cm^{-1} (▤); 15 kV cm^{-1} (▨); 20 kV cm^{-1} (□); 25 kV cm^{-1} (▧)
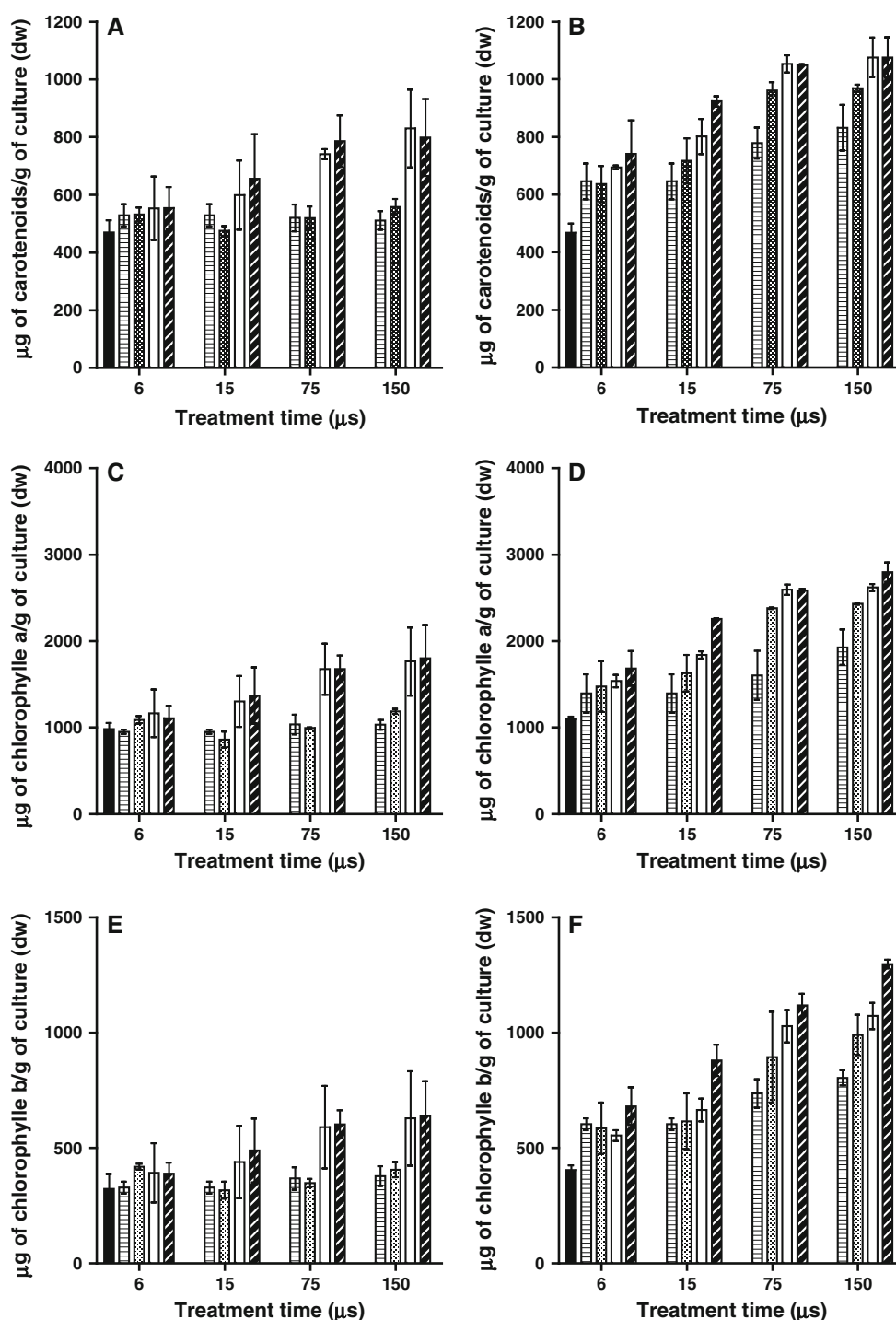

of electroporation for improving extraction of intracellular compounds from *C. vulgaris*.

Effect of PEF on the Extraction of Carotenoids, and Chlorophylls *a* and *b* From *Chlorella vulgaris* Cells

The effects of the electric field strength and treatment time on the extraction of carotenoids, and chlorophylls *a* and *b* from *C. vulgaris* cells treated by PEF are shown in Fig. 3. As the extracted compounds are lipophilic, ethanol was used as a solvent. The extraction was conducted just after the PEF treatment (Fig. 3a–c) and after pre-incubating the cells for 1 h after applying the PEF treatment (Fig. 3c–e). Solid black bars correspond to the extraction from untreated *C. vulgaris* cells (control). Extraction yield increased by increasing electric field strength and treatment

time independent of the extraction protocol followed but the extraction protocol did not affect the extraction yield of the three compounds investigated for the control sample. A pre-incubation for 1 h before extraction did not increase extraction yield for control cells ($p > 0.05$). However, for the samples treated by PEF, the extraction yield of the investigated compounds was higher. For example, after a PEF treatment at 20 kV cm^{-1} for 75 µs, the extraction yields for carotenoids, and chlorophylls a and b were 42, 54, and 195 % higher, respectively, when extraction was conducted after 1 h of pre-incubation. Statistically significant increments of the extraction yields were not observed for increasing treatment time from 75 to 150 µs ($p > 0.05$) at any electric field strength applied. However, the influence of the electric field depended on the extraction protocol followed. When the extraction was conducted just after PEF treatment, the application of a PEF treatment of 15 kV cm^{-1} or lower did not increase significantly the extraction yield of the three compounds in comparison to the control ($p > 0.05$). However, the application of a PEF treatment of 15 kV cm^{-1} for 75 µs improved the extraction yield of the carotenoids, and chlorophylls a and b by 104, 142, and 176 %, respectively, when the samples were pre-incubated for 1 h. The application of a PEF treatment at 20 kV cm^{-1} for 75 µs increased significantly the extraction yield of the carotenoids, and chlorophylls a and b for 124, 164, and 218 %, respectively, but further increments of the electric field did not cause a significant increment in the extraction yields of the three compounds. The electric field strength applied to obtain the higher pigment extraction yield was intermediate between the 45 kV cm^{-1} used for enhancing lipid extraction from the microalgae *Ankistrodesmus falcatus* (Zbinden et al. 2013) and the 3–4.5 kV cm^{-1} used for extraction of proteins from *C. vulgaris* (Coustets et al. 2013). This difference in the electric field strengths required for microalgae electroporation could be related to the pulse duration used in the different studies. While in this research pulses with durations of microseconds were applied, pulses of nanoseconds and milliseconds in duration were used for the extraction of lipids and proteins, respectively. The relationship between the pulse duration and electric field strength required to cause cell electroporation needs further investigation for a better understanding of the influence of this parameter. On the other hand, the smaller size of *C. vulgaris* cells compared with eukaryote cells of plant tissues could explain the reason why higher electric fields were required for improving extraction from microalgae. Generally, electric field strengths lower than 7 kV are used to improve the extraction of different compounds from eukaryote cells of plant tissues when pulses in the microsecond range are used (Puertolas et al. 2012).
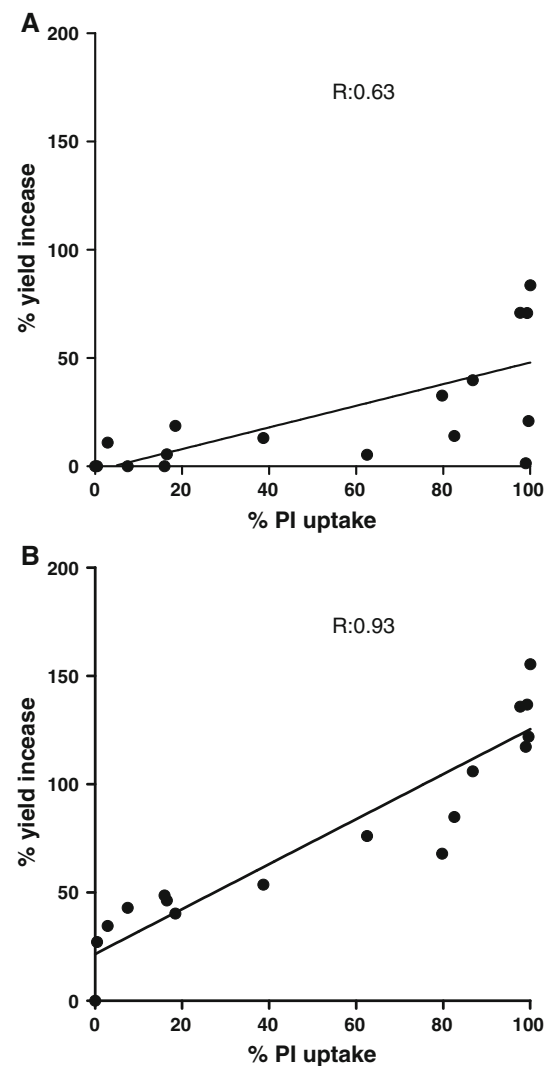
Fig. 4 Relationship between the percentages of cell permeabilization assessed by PI and after PEF treatment against the percentage of carotenoids extraction yield increase in comparison to the control when extractions were performed just after the PEF treatment (**a**) and 1 h after the PEF treatment (**b**)

The higher extraction yield of the three pigments after 1 h of incubation in the samples treated by PEF was not caused by an increment of the degree of permeabilization in the cells treated by PEF. No statistically significant ($p > 0.05$) differences between PI uptake just after application of PEF treatment and after 1 h of incubation were observed (data not shown). The increment observed could be caused by the plasmolysis of the chloroplast during the incubation time. As pigments such as carotenoids, and chlorophylls a and b are located in the chloroplast, their extraction requires that these compounds first cross the chloroplast membrane and then the cytoplasmatic membrane. The chloroplast plasmolysis could be due to osmolytic disequilibrium in the periplasmic space as a consequence of the loss of selective permeability of the

cytoplasmatic membrane in the electroporated cells. When the extraction was conducted just after the PEF treatment the cytoplasmatic membrane was permeabilized but not the chloroplast membrane. Nevertheless, after 1 h of incubation, both membranes could become permeabilized and, consequently, the extraction of the three pigments should be facilitated.

Figure 4 shows a high positive correlation $R = 0.93$ between the percentage of PI uptake when PI was added after the treatment (irreversible electroporation) and the percentage of yield increase when the extraction was conducted after 1 h of the application of the PEF treatment (Fig. 4b). However, no good correlation ($R = 0.67$) was observed when the extraction was conducted just after 1 h (Fig. 4 a). Similar results were obtained with chlorophylls a and b (data not shown). This behavior could also be related with the fact that pigments need to cross chloroplast and cytoplasmatic membranes for extraction. As the chloroplast membrane is intact after treatment, no correlation was observed between extraction and irreversible electroporation. However, as the integrity of the chloroplast membrane was reduced after 1 h, a high correlation was observed between the percentage of yield increase and irreversible electroporation.

As conclusion, results obtained in this investigation demonstrated the potential of PEF for improving extraction of compounds of interest from the microalgae C. *vulgaris*. The efficacy of PEF on extraction enhancement depended not only on the processing parameters (electric field strength and treatment time) but also on the elapsed time from the application of the treatment and the extraction process. Due to the differences in compounds of interest that may be extracted from microalgae and in cell size, cell shape and cell envelopes between different microalgae species definition of processing conditions for optimization extraction will require specific studies for each application.

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