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PEF-assisted Supercritical CO_2 Extraction of Pigments from Microalgae *Nannochloropsis oceanica* in a Continuous Flow System

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This study aimed at evaluating the effect of pulsed electric fields (PEF) pre-treatment on the extractability of pigments (total carotenes, chlorophyll *a*) from microalgae *Nannochloropsis oceanica* by supercritical CO_2 ($scCO_2$) extraction. Aqueous microalgal suspensions (1% w/w) added with 10% ethanol were treated in a continuous flow unit with either PEF at different field strengths (10–30 kV/cm) and total specific energy inputs (20–100 kJ/kg), or $scCO_2$ at different pressures (8-20 MPa), with fixed temperature (35°C), CO_2/biomass ratio (53.3 kg_{CO2}/kg_{DW}) and contact time (7 min). Extracts from conventional solvent extraction process and PEF-assisted $scCO_2$ extraction were analyzed and compared with those achieved upon the application of single PEF and $scCO_2$ extraction processes. Results showed that in PEF-assisted extraction the recovery yield of the target compounds significantly increased with increasing the treatment severity, even though the effect of the field strength appeared less important than the energy input. After PEF treatment at 10 kV/cm and 100 kJ/kg, the extraction yield for total carotenes and chlorophyll *a* increased by 1.6 and 1.4 times, respectively. During single $scCO_2$ extraction process at 14 MPa the extraction yield for total carotenes and chlorophyll *a* increased up to 10.3 and 13.6 times, respectively, as compared to the untreated samples. The use of PEF prior to $scCO_2$ extraction remarkably increased the extractability of the two pigments showing a clear synergistic effect. The results obtained in this work suggested that the application of PEF prior to $scCO_2$ extraction could represent a suitable approach for the efficient recovery of pigments from microalgal biomass.

1. Introduction

Microalgae are evaluated as a rich source of compounds of commercial relevance such as proteins, carbohydrates, lipids and pigments (e.g., chlorophylls and carotenoids), with potential applications as natural additives or active ingredients for food, feed, cosmetic, and pharmaceutical products (Zhang et al., 2018). The main limit to the exploitation of active ingredients from microalgae biomass is represented by the difficulties associated with their selective recovery, being tingly locked in the cytoplasm or in internal organelles inside the algae cells, protected by the rigid cell wall and membranes, which act as a barrier that greatly limit the penetration of the solvent into the cells and the diffusion of the solubilized intracellular compounds during extraction process (Carullo et al., 2018). Conventional extraction of bioactive compounds from biological matrices, including microalgae, is traditionally based on solvent extraction process. The latter offers a simple approach, but typically suffers from several drawbacks such as high organic solvent consumption, long extraction times, relatively high temperature as well as low yield and selectivity (purity), which have led to advancements in the search for novel and ecofriendly extracting technologies (Poojary et al., 2016). Among these, pulsed electric fields (PEF)-assisted extraction and supercritical CO_2 ($scCO_2$) extraction are gaining increasing interest in the frame of microalgae biorefinery, due to the possibility to ensure high recovery yields and selectivity with reduced operative costs (Poojary et al., 2016). In PEF-assisted extraction process, wet microalgae biomass is exposed to repetitive short (of the order of μs) duration pulses of high intensity electric fields (E=10-50 kV/cm), which induce the permeabilization of cell membranes by electroporation, thus facilitating the penetration of the solvent into the cells and the release of intracellular matter (Poojary et al.,

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2016). On the other hand, $scCO_2$ extraction has been considered as a “green” alternative to conventional extraction methods, where organic solvents are replaced by $scCO_2$, which has several advantages including low toxicity, flammability and cost, and high purity when compared to other fluids. During $scCO_2$ extraction process the biomaterial, typically in dried form, is placed in contact with CO_2 for a certain time in a batch, semi–batch or continuous system, where the solvating power (polarity) of supercritical fluid can be adjusted by either manipulating the temperature and pressure of the fluid or by adding a co-solvent, such as ethanol, allowing the selective extraction of a wide range of compounds (Kitada et al., 2009). For both PEF and $scCO_2$ treatments the ability to extract valuable compounds from microalgae, including lipids, pigments, carbohydrates and proteins has been widely demonstrated (Carullo et al., 2018; Kitada et al., 2009; Luengo et al., 2014; Macías-Sánchez et al., 2005; Zhang et al., 2018). However, in the case of “hard structured” microalgal cells, where the cell wall/membrane system greatly limits the mass transfer phenomena, it is necessary to apply intense processing conditions (high field strengths and energy inputs for PEF; high pressure, contact time and temperature for $scCO_2$) to recover substantial amounts of valuable intracellular compounds. Therefore, in order to reduce the operative costs and to maximize the selective extraction of high value components, the use of either PEF or $scCO_2$ in a hurdle or cascade biorefinery approach has been suggested (Macías-Sánchez et al., 2019; Postma et al., 2016). In particular, the use of PEF and $scCO_2$ in a hurdle approach has been successfully used in previous works (Pataro et al., 2014), but only for microbial inactivation purposes. Interestingly, it was found that the combination of PEF and pressurized CO_2 resulted in a marked increase in the microbial inactivation showing a clear synergistic effect. Therefore, this work represents the first attempt to apply PEF and $scCO_2$ in a cascade approach for the intensification of the extractability of target intracellular compounds from microalgae.

The aim of this research was to assess the effectiveness of the application of PEF treatment prior to $scCO_2$ extraction on the recovery yield of pigments (total carotenes and chlorophyll *a*) from wet *Nannochloropsis oceanica* microalgae using a continuous flow system.

In the experimental campaign, the effect of either PEF electric parameters (field strength and energy input) or $scCO_2$ processing variables (pressure) on the extractability of the compounds of interest was investigated, in order to select optimal processing conditions to be used in the PEF-$scCO_2$ cascade approach.

2. Materials and methods

2.1 Microalgae suspension

Nannochloropsis oceanica is a marine green algae with an almost spherical shape and mean diameter of about 2 μm belonging to the Eustigmataceae family. For the experiments an algae paste of *Nannochloropsis oceanica* (24% solid content), purchased from Archimede S.r.l (Genova, Italy), was used. Before each extraction process, the biomass was centrifuged at 6500 rpm for 10 min and subsequently resuspended in a citrate-phosphate McIlvaine buffer (pH = 7) to which ethanol (10% v/v) was added as a co-solvent in order to adjust the solvating power of $scCO_2$ (Kitada et al., 2009). The final concentration of the algae suspension was 1% dry weight (DW) and its electrical conductivity was 1.5 mS/cm at 25°C (conductivity meter HI 9033, Hanna Instrument, Milan, Italy).

2.2 Experimental apparatus

All the experiments were carried out in a bench-scale continuous flow unit, described in detail in a previous work (Pataro et al., 2014). The unit allowed to process algae suspensions by applying a single PEF treatment, a single $scCO_2$ treatment or a PEF pre-treatment followed by a $scCO_2$ treatment.

Briefly, it consisted of a peristaltic pump used to transfer the microalgal suspension through a stainless steel coiled tube submerged into a water heating bath before passing through two co-field PEF treatment chambers (3.9 mm inner diameter, 5 mm electrode gap) hydraulically connected in series. PEF treatments were carried out by means of a high voltage pulsed (20 kV, 25 kW) power generator (Diversified Technology Inc., Bedford, WA, USA) able to generate either mono- or bipolar square wave pulses (1-10 μs, 1-1000 Hz). Two T-thermocouples were used to measure the product temperature at the inlet and at the exit of the PEF chamber. The actual voltage and current signals at the treatment chambers were measured by special probes connected to an oscilloscope. After passing through the PEF chamber, a three way valve allowed the mixing of biomass with liquid CO_2 (99.995% purity) pumped into the system by means of a volume displacement pump operating in constant flow mode. Then the biomass-CO_2 mixture enters the holding tube (3.9 mm in diameter) submerged in a water bath used to maintain constant the processing temperature. The length of the tube can be adjusted in order to achieve the required residence time. Two thermocouples measured the temperature of the mixture at the inlet and at the exit of the holding tube. Three metering valves placed immediately before and after the PEF chamber as well as at the end of the holding tube were used to both pressurize the system and collect the treated samples.

2.3 Extraction procedure

Three different extraction experiments were carried out: PEF-assisted extraction, $scCO_2$ extraction and a PEF pre-treatment followed by a $scCO_2$ extraction. During the PEF-assisted extraction the algae suspension (1% DW) was pumped through the system at constant flow rate (20 mL/min) and temperature (25°C) and subjected to PEF treatment with monopolar square wave (5 μs) pulses at different field strengths (E=10-30 kV/cm) and total specific energy inputs (W_T=20-100 kJ/kg). The temperature increase of the microalgal suspension due to Joule effect never exceeded 10 °C. The $scCO_2$ extraction experiments were carried out by pumping the algae suspension (13 mL/min) through the system with the pulse generator switched off, and by feeding $scCO_2$ immediately after the PEF chamber. Treatments were carried out at different pressure levels (P= 8, 14, and 20 MPa) and at a fixed temperature (35°C), CO_2/biomass ratio (53.3 kg_{CO2}/kg_{DW}) and holding time (7 min). Optimal treatment conditions for both PEF, in terms of E and W_T, and $scCO_2$ extraction process, in terms of P, were identified and used to assess the effect of using both technologies in a cascade approach on the extractability of valuable compounds from the microalgal cells.
The suspension was first treated by PEF and then subjected to $scCO_2$ extraction by feeding supercritical CO_2 immediately after the PEF chamber. After each single (PEF, $scCO_2$) or cascade (PEF-$scCO_2$) extraction process, samples were collected in 15 mL falcon tubes at the exit of the processing plant and allowed to stand for 1 h at 25°C under shaking (160 rpm) to ensure the diffusion of intracellular components out of the cells. Finally, the cell suspensions were centrifuged (10 min, 6500 rpm) and the supernatants were transferred to fresh tubes and stored at 4°C for further analysis.

2.4 Analysis of the extracts

Total carotenes and chlorophyll *a* content of supernatants were evaluated using the method described by Lichtenthaler & Wellburn (1983). The maximum absorbance of chlorophyll *a* (A_{ch}^{a}), chlorophyll *b* (A_{ch}^{b}) and total carotenes (A_{cr}) was detected at the wavelengths of 675 nm, 653 nm and 470 nm, respectively, by using a UV-Vis V-650 spectrophotometer (Jasco Inc., Easton, USA). The concentrations of pigments in the extract were calculated using the following equations:

$$C_{ch.}^{a} = 15.55\, A_{ch}^{a} - 7.34\, A_{ch}^{b} \quad (1)$$

$$C_{ch.}^{b} = 27.05\, A_{ch}^{b} - 11.21\, A_{ch}^{a} \quad (2)$$

$$C_{cr.} = (1000\, A_{cr} - 2.86\, C_{ch}^{a} - 85.9\, C_{ch}^{b})/245 \quad (3)$$

where C_{cr}, C_{ch}^{a}, and C_{ch}^{b} are the concentrations (in μg of pigment/g_{DW}) of total carotenes, chlorophyll *a*, and chlorophyll *b,* respectively.

2.5 Statistical analysis

All the experiments and analyses were performed in triplicate. Results in terms of mean values and standard deviations were obtained using SPSS 20 Software (SPSS Inc., Chicago, USA) with a further comparison among the mean values by Tukey's test. Any statistical difference was considered significant for $p \leq 0.05$.

3. Results and discussion

3.1 Effect of PEF pre-treatment parameters on the extractability of pigments

Figure 1 shows the concentration (on DW basis) of total carotenes and chlorophyll *a* detected in the supernatant of untreated and PEF treated *Nannochloropsis oceanica* suspensions at different field strength and energy input after 1 h of extraction at 25°C. Results show that the content of total carotenes and chlorophyll *a* detected in the extracts of untreated samples was 25.1 $\mu g/g_{DW}$ and 41.7 $\mu g/g_{DW}$, respectively. This leakage of intracellular pigments may be ascribed to either a spontaneous cell lysis (Carullo et al., 2018) or to the capability of ethanol to affect the membrane barrier properties, thus facilitating the release of a certain amount of intracellular compounds (Zhang et al., 2018). The permeabilization effect of the cell membranes induced by the application of PEF treatment likely reduced the mass transfer resistance of intracellular compounds, leading to a significantly higher release of both pigments. However, it is worth noting that among the PEF treated samples the effect of the applied field strength appeared less important than that of the energy input within the investigated range. The maximum recovery of total carotenes and chlorophyll *a* was 41.8 mg/g_{DW} and 60.2 mg/g_{DW}, respectively, which was detected in the extracts of microalgae suspension treated at 10 kV/cm and 100 kJ/kg. Recently, few authors have investigated the ability of PEF to enhance the extractability of pigments from microalgae obtaining controversial results. For example, when Luengo et al. (2014) evaluated the extraction yield of pigments from *C. vulgaris* cells using 96% ethanol as extraction solvent, they found a substantial higher amount of carotenoids (42%) and chlorophyll *a* (54%) in the

supernatant of PEF (20 kV/cm, 75 μs) treated samples as compared to control extracts. In contrast, Grimi et al. (2014) did not find any detectable amount of the same pigments in the water extracts of PEF (20 kV/cm, 13.3–53.1 kJ/kg) pre-treated cell suspensions of microalgae *Nannochloropsis sp*.

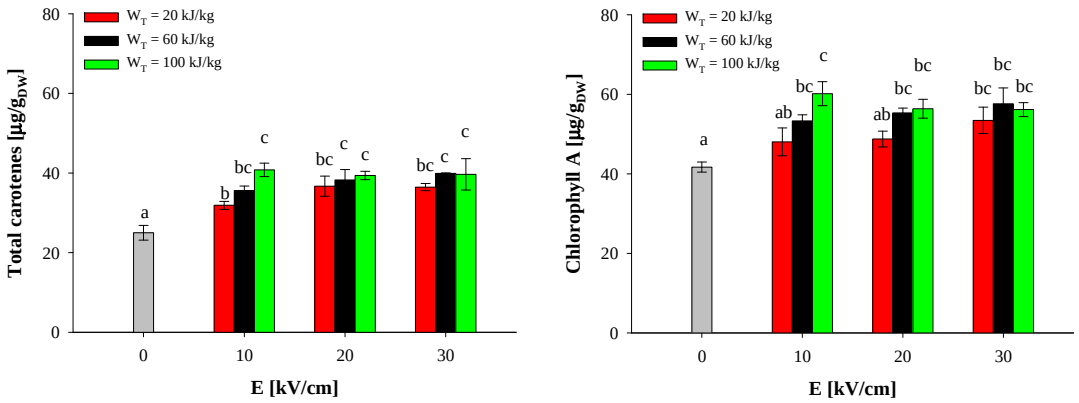

Figure 1: Total carotenes and chlorophyll a content of supernatants from untreated (0 kV/cm) and PEF treated Nannochloropsis oceanica suspension as a function of the field strength and for different energy inputs. Different letters above the bars indicate significant differences among the mean values (p≤0.05).

The explanation for these results might be attributed to the fact that these pigments are hydrophobic substances, soluble only in organic solvents or mixture of polar and nonpolar organic solvents, and practically insoluble in water. However, behind the solvent polarity, the observed scarce efficiency of PEF treatments also detected in the present research may be also ascribed to the fact that PEF merely electroporated the cytoplasmic membrane of algae cells while inducing no or only slight damages to the membranes of smaller intracellular organelles such as chloroplast to which pigments are bounded. It is known, in fact, that the critical electric field required to trigger electroporation is inversely related to cell size (Poojary et al., 2016). Therefore, the increase in the yield of pigments upon the application of PEF pre-treatment may be attributed to the subsequent plasmolysis of the chloroplast during the maceration time due to osmolytic disequilibrium in the cytoplasmatic space, as a consequence of the electroporation of the cytoplasmic membrane of the algae cells (Luengo et al., 2014).

3.2 Effect of $scCO_2$ processing parameters on the extraction of pigments

Pressure, along with temperature, is the most relevant parameter that affects the solvent power and the penetration capacity of the solvent into the matrix, thus affecting the extraction yield (Macías-Sánchez et al., 2005). The influence of the operative pressure (from 8 to 20 MPa) during $scCO_2$ extraction of total carotenes and chlorophyll *a* from *Nannochloropsis oceanica* cell suspension at a fixed temperature (35 °C), CO_2/biomass ratio (53.3 kg_{CO2}/kg_{DW}) and holding time (7min), is shown in Figure 2.

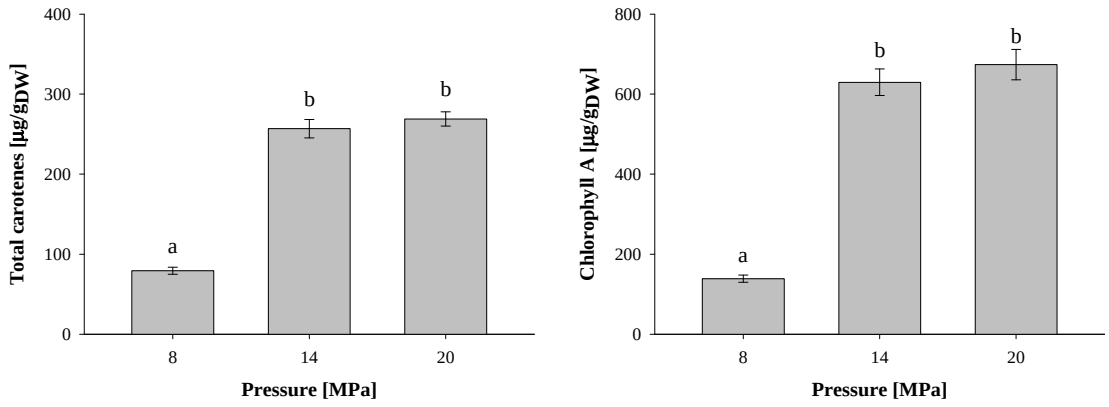

Figure 2: Total carotenes and chlorophyll a content in the supernatants after $scCO_2$ extraction at different pressure. Different letters above the bars indicate significant differences among the mean values (p≤0.05).

Results show that the amount of extracted pigments significantly (p≤0.05) increased when pressure was increased from 8 to 14 MPa. However, further increments of the pressure did not cause any significant increase in the extraction yields of the two compounds. Although any comparison with data found in the current literature is very difficult due to the different types of equipment, microalgae strains and experimental conditions used, our results appear consistent with those reported in previous works. In fact, it was shown that the maximum extraction yield of carotenoids and chlorophyll *a* from *Nannochloropsis gaditana* and *Scenedesmus almeriensis* was obtained at an intermediate pressure within the investigated range (10-60 MPa) (Macías-Sánchez et al, 2005, 2010). This behavior can be mainly attributed to the two effects that the increase in pressure has on CO_2 when temperature is maintained constant. In fact, on increasing the pressure increases the density of CO_2 and, consequently, the solvation power of the fluids, which in turn increases the solubility of the compounds and extraction yield. However, when pressure increases the diffusion coefficient decreases, thus reducing the penetration capacity of the solvent into the matrix and, hence, the extraction yield (Macías-Sánchez et al, 2005). The predominance of one or other effect explains the trend observed in the experimental data, from which it can be concluded that, in our case, a pressure of 14 MPa may be sufficient to determine the highest extractability of pigments from the *Nannochloropsis oceanica* cells.

3.3 PEF-assisted scCO2 extraction of pigments

Figure 3 reports the content of total carotenes and chlorophyll *a* detected in the supernatant of untreated (control) and treated algae suspension in a cascade process, with a PEF treatment (E=10 kV/cm; W_T=100 kJ/kg) stage applied prior to the $scCO_2$ extraction stage (P=14 MPa, T=35°C, R=53.3 $kgCO_2/kg_{DW}$, t=7min). For the sake of comparison, also the extraction yield of pigments detected after single PEF-assisted extraction and single $scCO_2$ extraction processes is reported.

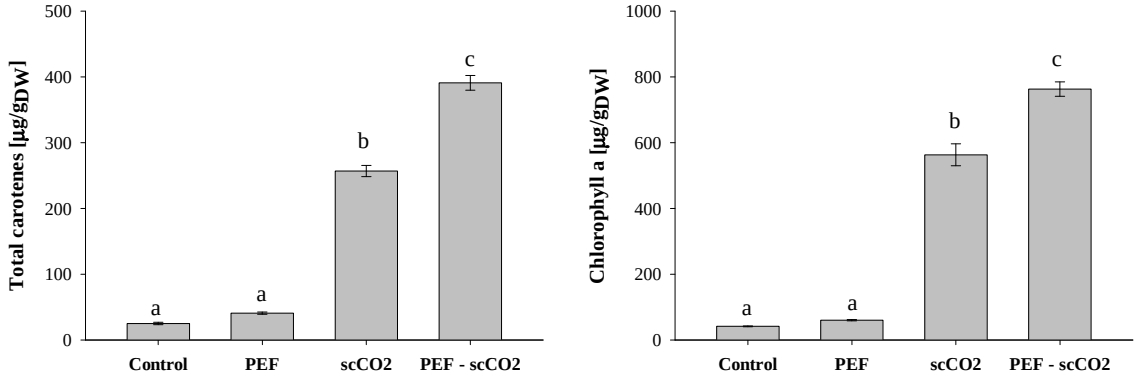

Figure 3: Total carotenes and chlorophyll a content of supernatants from untreated (Control), single PEF, single $scCO_2$ and cascade PEF-$scCO_2$ processed aqueous microalgal suspensions with 10% ethanol as co-solvent. Different letters above the bars indicate significant differences among the mean values (p≤0.05).

Results show that, as compared to the control extraction, the application of single $scCO_2$ treatment significantly (p≤0.05) increased the extraction yield of pigments, while only a slight but not significant (p>0.05) increment was detected after PEF-assisted extraction process. Moreover, the extraction yield of total carotenes and chlorophyll *a* of $scCO_2$ treated samples was found to be, respectively, 6.3 and 9.4 times higher than that detected in PEF-treated samples. This may be likely attributed to the higher solubility of pigments, especially carotenes and chlorophyll *a* in ethanol-$scCO_2$ than in ethanol-water solvent. In addition, it is known that ethanol in supercritical CO_2 may enhance its solvent power, thus facilitating the selective extraction of pigments as target components (Kitada et al., 2009). The exposure of algae biomass to an external electric field prior to the scCO2 extraction led to further increase of the extraction yield for both pigments. Interestingly, a clear synergistic effect between the applied technologies could be detected for both total carotenes and chlorophyll a, which led to a significant increase ($p < 0.05$) in the extraction yield of total carotenes (36%) and chlorophyll *a* (52%) with respect to the single scCO2 extraction process. These findings can be explained taking into account the mechanism occurring when PEF and $scCO_2$ processes are applied. It has been shown that the $scCO_2$ extraction process involves the diffusion of CO_2 into the cells and that the rate of diffusion is limited by the solubility of CO_2 in the suspending medium as well as by the mass transport resistance through the cell membrane (Pataro et al., 2014). However, since the data shown in Figure 3 were obtained by keeping constant the pressure of CO_2 in the processing line, it can be concluded that the mass transport resistance through the cell membrane is the main factor limiting the diffusion of CO_2 into the algae cells. Therefore, it is likely that when PEF and $scCO_2$ treatments were carried out in series, the permeabilization of the algae cell membranes induced by the application of the electrical pre-treatment facilitated the diffusion of pressurized

CO_2 through microalgal cells, thus improving the extraction efficiency of the $scCO_2$ process. To date, no previous works investigated the effect of PEF pre-treatment on the extraction efficiency of $scCO_2$ of intracellular compounds from microalgae. However, these findings are somewhat consistent with those reported by other authors, who found that the pretreatment of algae with physical or mechanical cell disintegration techniques such as crushing, sonication and ball milling play a crucial role in enhancing the extraction efficiency of pigment components by $scCO_2$ (Macías-Sánchez et al., 2010; Poojary et al., 2016).

4. Conclusions

In this work the effect of the PEF parameters (E, W_T) and $scCO_2$ processing conditions (P), as well as the use of both technologies in a cascade approach on the extractability of valuable compounds (total carotenes and chlorophyll *a*) from aqueous suspensions of *Nannochloropsis oceanica* microalgae was investigated.

Results confirmed that single PEF-assisted extraction process applied under relatively mild conditions (10 kV/cm, 100 kJ/kg) and using a polar solvent was unable to unlock and sufficiently solubilize high amount of pigments bounded to the chloroplast of the algae cells. Significantly higher extraction yields of total carotenes and chlorophyll *a* were, instead, detected when $scCO_2$ added with 10% ethanol as co-solvent, was used. However, within the range of $scCO_2$ processing conditions investigated, the main factor limiting the extractability of pigments from *Nannochloropsis oceanica* cells appeared to be the very low diffusion of $scCO_2$ through the intact cell membrane. However, when the two technologies were used in a cascade approach, the results clearly showed the existence of a synergistic action of PEF and $scCO_2$ combined process to intensify the extractability of pigments from microalgae cells.

These promising results confirm the potential of PEF technology to improve the penetration capacity of $scCO_2$ inside the algae cells, thus intensifying the extractability of valuable compounds from wet microalgae biomass with lower processing conditions and avoiding the need for energy-intensive drying.

References

Carullo D., Abera B.D., Casazza A.A., Donsì F., Perego P., Ferrari G., Pataro G., 2018, Effect of Pulsed Electric Fields and High Pressure Homogenization on the acqueous extraction of intracellular compounds from the microalgae Chlorella vulgaris, Algal Research, 31, 60–69.

Grimi N., Dubois A., Marchal L., Jubeau S., Lebovka N.I., Vorobiev E., 2014, Selective extraction from microalgae Nannochloropsis sp. using different methods of cell disruption, Bioresource Technology, 153, 254–259.

Kitada K., Machmudah S., Sasaki M., Goto, M., Nakashima Y., Kumamoto S., Hasegawa T., 2009, Supercritical CO2 extraction of pigment components with pharmaceutical importance from Chlorella vulgaris, J. Chem. Technol. Biotechnol., 84, 657-661.

Lichtentaler H., Wellburn A., 1983, Determination of Total Carotenoids and Chlorophyll a and b of Leaf Extracts in Different Solvents, Biochemical Society Transactions, 11, 591–592.

Luengo, E., Condon-Abanto, S., Alvarez, I., Raso, J., 2014, Effect of Pulsed Electric Field Treatments on Permeabilization and Extraction of Pigments from Chlorella vulgaris, J Membrane Biol, DOI 10.1007/s00232-014-9688-2.

Macías-Sánchez, M.D., Mantell, C., Rodriguez, M., Martinez de la Ossa, E., Lubian, L.M., Montero, O., 2005, Supercritical fluid extraction of carotenoids and chlorophyll a from Nannochloropsis gaditana, Journal of Food Engineering, 66, 242-251.

Macías-Sánchez, M.D.,Fernandez-Sevilla, J.M., Acien Fernandez, F.G., Ceron Garcia, M.C., Molina Grima, E., 2010, Supercritical fluid extraction of carotenoids from Scenedesmus almeriensis, Food Chemistry, 123, 928–935.

Pataro G., De Lisi M., Donsì G., Ferrari G., 2014, Microbial inactivation of E. coli cells by a combined PEF–HPCD treatment in a continuous flow system, Innovative Food Science and Emerging Technologies, 22, 102–109.

Poojary, M.M., Barba, F.J., Aliakbarian, B., Donsì, F., Pataro, G., Dias, D.A., Juliano, P., 2016, Innovative Alternative Technologies to Extract Carotenoids from Microalgae and Seaweeds, Marine Drugs, 14, 214.

Postma P.R., Pataro G., Capitoli M., Barbosa M.J., Wijffels R.H., Eppink M.H.M., et al., 2016, Selective extraction of intracellular components from the microalga Chlorella Vulgaris by combined pulsed electric field-temperature treatments, Bioresource Technology, 203, 80–88.

Zhang, R., Parniakov, O., Grimi, N., Lebovka, N., Marchal, L., Vorobiev, E., 2018, Emerging techniques for cell disruption and extraction of valuable bio-molecules of microalgae Nannochloropsis sp., Bioprocess and Biosystems Engineering, https://doi.org/10.1007/s00449-018-2038-5.