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Towards marine biorefineries: Selective proteins extractions from marine macroalgae *Ulva* with pulsed electric fields

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ABSTRACT

Macroalgae are potential feedstock for biorefineries. However, integration of macroalgae into biorefinery network requires new processing technologies that will lead to energy efficient and zero waste conversion of macroalgae biomass into food, chemicals and fuels. Here we report on the selective extraction of proteins from green macroalgae from *Ulva* genus by electroporation with energy efficient pulsed electric field (PEF) process. We show that application of 75 pulses with an average electric field strength of 2.964 ± 0.007 kV cm^{-1}, and pulse duration 5.70 ± 0.30 μs, delivered at approximately 0.5 Hz, combined with hydraulic pressing of the treated samples for 5 min with force of 45 daN cm^{-2} led to the total protein concentration of 59.13 ± 3.82 μg mL^{-1} in the extracted juice. The final temperature of the extracted juice was 35.50 ± 2.02 °C. The energy consumption of the process is 251 ± 3 kWh kg^{-1} of protein. We show that PEF process is selective and its extraction efficiency and damage are protein specific.

1. Introduction

Global population growth combined with the increase in quality of life in the era of changing climate will increase the demand for food, chemicals and fuels. The global demand for plant proteins is expected to grow from 4.73 in 2014 to 9.44 · 10^8 ton protein in 2054 (Stice, 2014). This growth in protein demand is expected to require additional 100 · 10^6 arable land hectares (Stice, 2014). Previous studies clearly show the positive impact of plant proteins consumption on sustainability and reduction of land, water, fertilizers and energy consumption (Pimentel & Pimentel, 2003). There is a consciously growing interest in exploring different plant sources for direct proteins use in the diet, either directly as entire plant or combined in the processed food products (Tuso, Ismail, Ha, & Bartolotto, 2013). In the last 5 decades microalgae biomass gained a lot of interest as a feedstock for proteins production (Becker, 2007). More recently food protein production is considered as a valuable co-product with biofuels in the algal biorefineries (Vanthoor-Koopmans, Wijffels, Barbosa, & Eppink, 2013). The production of microalgae biomass, however, is still cost prohibitive and further advances in the cultivation and harvesting technologies are required. Macroalgae, large multicellular organisms, have been mostly overlooked as a feedstock for protein production for many years (Golberg et al., 2014; Harnedy & FitzGerald, 2011; Lehahn, Ingle and Golberg, accepted for publication). However, many of the marine red and green macroalgae species have shown significantly higher content of proteins in comparison to the terrestrial plant proteins sources such as soy, nuts, and cereals (Fleurence, 2004; Harnedy & FitzGerald, 2011). In addition to their high yields and nutritional properties, marine macroalgae derived proteins and peptides have shown additional value because of their nutraceutical, pharmaceutical and cosmeceutical properties such as antioxidant, antihypertensive, immune-modulatory, anticoagulant and hepeto-protective substances (Fleurence, 2004; Harnedy & FitzGerald, 2011).

The value of the macroalgae as a protein source depends on the yields and functional properties. Previous work on the microalgae food proteins has shown that the economic viability of the algae proteins critically depends on the extracted protein yields (Vanthoor-Koopmans et al., 2013). In addition to yield, to achieve nutraceutical, pharmaceutical and cosmeceutical properties it is vitally important to preserve native proteins function. The complex, viscous and often charged macroalgae cell wall and extracellular matrix make the extraction process challenging (Joubert & Fleurence, 2007). Osmotic shock, mechanical grinding, high shear force, ultrasonic treatment, acid and alkaline pretreatment and polysaccharidase aided digestion and their combinations have been used to increase the extraction yields (Barbarino & Lourenço, 2005; Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995; Galland-Irmouli et al., 2000; Harnedy & FitzGerald, 2013; Rouxel, Daniel, Jérôme, Etienne, & Fleurence, 2001; Wong & Chikeung Cheung, 2001). Although the mentioned methods were shown to increase the extraction yields, they involve either thermal or

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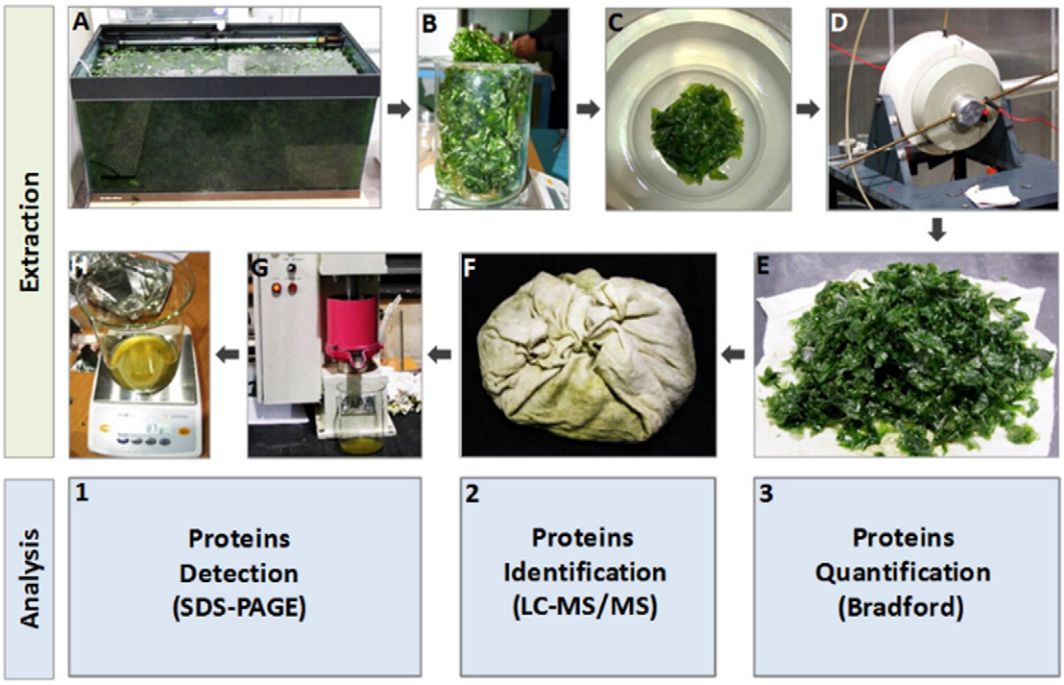

Fig. 1. Water soluble proteins extraction from macroalgae *Ulva* with pulsed electric field system. Extraction. A. Macroalgae storage system. B. External water removal. C. Biomass loading in the PEF treatment chamber. D. Application of pulsed electric fields for cell membrane electroporation. E. Treated biomass F. PEF treated biomass packing for mechanical extraction. G. Extraction of macroalgae intracellular liquid with mechanical press. H. Weighing of the extracted juice. Analysis. 1. Proteins detection with SDS-PAGE 2. Protein identification. 3. Total protein quantification.

chemical procedures that could affect the functionality of the extracted proteins and peptides. In this work, for the first time, we report on the chemical-free, non-thermal pulsed electric field (PEF) aided extraction of macroalgae water soluble proteins.

PEF is an emerging, non-thermal food processing technology already used to the energy-efficient extraction of proteins from microalgae (Goettel, Eing, Gusbeth, Straessner, & Frey, 2013; Parniakov et al., 2015), yeast (Ganeva & Galutzov, 1999; Ganeva, Galutzov, & Teissié, 2003), bacteria (Haberl Meglic, Marolt, & Miklavcic, 2015) and plants (Bluhm & Sack, 2008; Doevenspeck, 1961; Martin Sack & Bluhm, 2008; Vorobiev & Lebovka, 2010; Zagorulko, 1958). Although the exact mechanism of biological tissue permeabilisation by PEF is not fully understood, PEF technology is currently used in multiple applications in medicine and biotechnology (Kotnik et al., 2015; Rubinsky, 2007; Yarmush, Golberg, Serša, Kotnik, & Miklavčič, 2014). The current theory suggests that the membrane permeabilisation is achieved through the formation of aqueous pores on the cell membrane, a phenomenon known as electroporation (Weaver & Chizmadzhev, 1996). In the recent years significant advances in the industrial scale PEF system enabled the large scale use of the PEF process for biomass processing the sugar industry, saving up to 50% of the downstream energy investment in the process (Bluhm & Sack, 2008; Sack et al., 2009, 2010a,2010b;Sack & Bluhm, 2008; Sack et al., 2010a,2010b; Sack, Schultheiss, & Bluhm, 2005). Encouraged by this non-thermal, chemical-free, scalability and energy efficiency properties of PEF processes, we set out to test the working hypothesis that PEF will enable selective protein extraction from green macroalgae from *Ulva* genus, which has a potential to become a feedstock for marine biorefineries (Korzen, Abelson, & Israel, 2015b; Korzen, Peled, et al., 2015a).

2. Experimental

2.1. Biomass material

Ulva biomass was obtained from Alga Plus Company, Portugal (cultivated in the certified integrated aquaculture facility). Macroalgae were then stored for 2 days in a 400 L aquarium with a salt concentration of 3.5%.

2.2. PEF treatment

The fresh biomass was centrifuged at about 840 rpm for 3 runs of 1 min each to remove the external water, so that <5 g of water has been removed during the third run. 140 g of *Ulva* biomass in a 2 L becher was weighted with scale of type KERN 440-49N. This amount of macroalgae was then poured and pushed into the PEF treatment chamber with a volume of 232 cm^3 for the application of a homogeneously distributed pulsed electric field. Water was added to the macroalgae to fill the chamber completely. The chamber was closed to begin the PEF treatment. After the PEF treatment was applied, the macroalgae were collected and returned to the becher. The electroporated macroalgae were weighted again. The treatment parameters were:

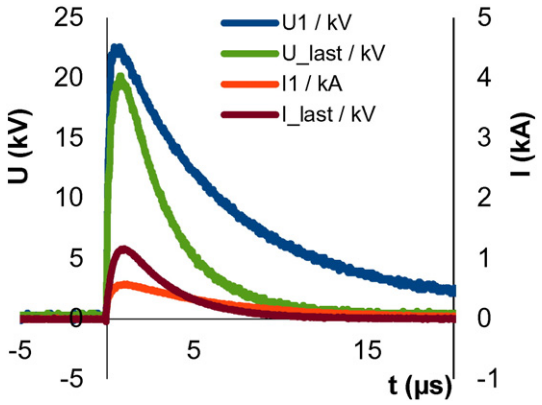

Fig. 2. Shape and magnitude of the single electric pulse delivered for macroalgae biomass electroporation. The shape for actually delivered voltage and measured current is shown for the first and last pulse in the series of 75 pulses applied on the biomass.

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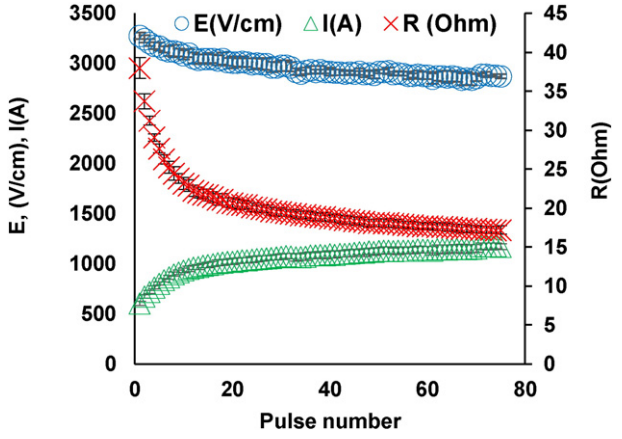

Fig. 3. Pulsed electric field and sample resistance behavior during macroalgae biomass treatment. Critical process parameters such as electric field (E), current (A) and biomass resistance (R) were monitored during each pulse. The experiment was done in triplicate. Error bars show ± SEM.

average field strength (average was taken between all pulses at all repeats, 225 measurements) 2.964 ± 0.007 kV cm^{-1}, and pulse duration 5.70 ± 0.30 μs, delivered at 0.5 Hz. These parameters were chosen based on the previous studies with PEF dehydration of various types of biomass with this system (Sack et al., 2009,2010a,2010b; Sack et al., 2008). Temperature was measured with a digital thermometer (TFA Type 30.1018). Current and voltage across the electrodes of the treatment chamber during each pulse were measured with a current probe (PEARSON 110 A) and a voltage divider (HILO-Test HVT 240 RCR), both connected to an oscilloscope (Tektronix TDS 640A). The impedance of the treated sample was derived from the current and voltage measurements.

2.3. Energy consumption

The total energy consumed for the PEF treatment was calculated based on the energy stored in the pulse capacitor with the following Eq.1:

$$E_t = 0.5 \cdot C \cdot 10^{-9} \cdot \left(V \cdot 10^3\right)^2 \cdot N \quad (1)$$

where E_t (J) is the total energy consumed for the treatment of one treatment chamber, C is the discharging capacitor capacitance (nF), V (kV) is the applied voltage and N is the total number of pulses. Additional losses of the capacitor charger have not been considered.

The energy consumed with PEF for protein extraction was then calculated with Eq.2:

$$E_p = \frac{E_t}{m} / (C_p \cdot m_{PEF}) \quad (2)$$

where E_p (kWh kg^{-1}) is the PEF energy required to extract 1 g of protein, E_t (kWh kg^{-1}) is the total energy consumed to treat the PEF chamber, m (kg) is the raw mass of treated macroalgae, C_p (kg mL^{-1}) is the concentration of the proteins in the extracted juice and m_{PEF} (mL) is the volume of the extracted juice.

2.4. Mechanical juice extraction

The electroporated algae were placed in a cloth material that was folded so that the algae could not escape during pressing. The algae wrapped up in the fabric were placed in the mechanical press (HAPA type SPM 2.5S). A force of 45 daN cm^{-2} was applied for a determined time of 5 min using the automatic mode of the press that keeps the pressure applied to the piston constant. Extracted juice from pressing was collected in a 2 L becher and weighted at the end of the pressing. The pressed material was taken out of the press, weighted, reorganized

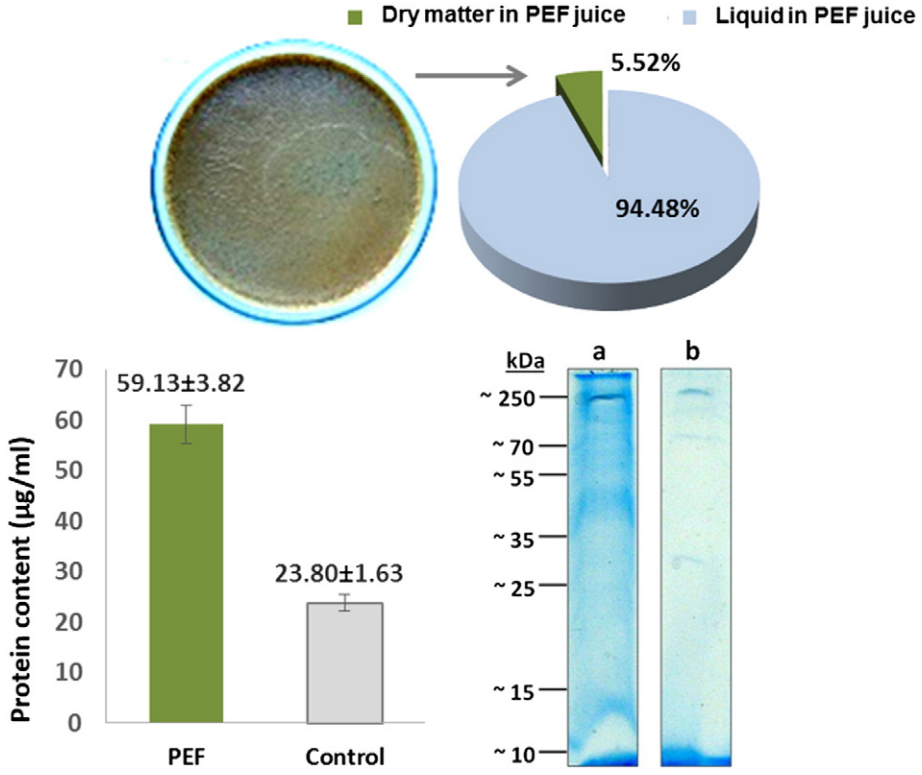

Fig. 4. Proteins extraction from *Ulva*. SDS gel electrophoreses (right) and total protein quantification with Bradford (left) Process parameters and total yields for 24 kV, 75 pulses applied. Error bars show ± SEM. Insert in the top row shows the total water soluble solids extracted from *Ulva* with PEF. The complete composition of the extract is still to be determined.

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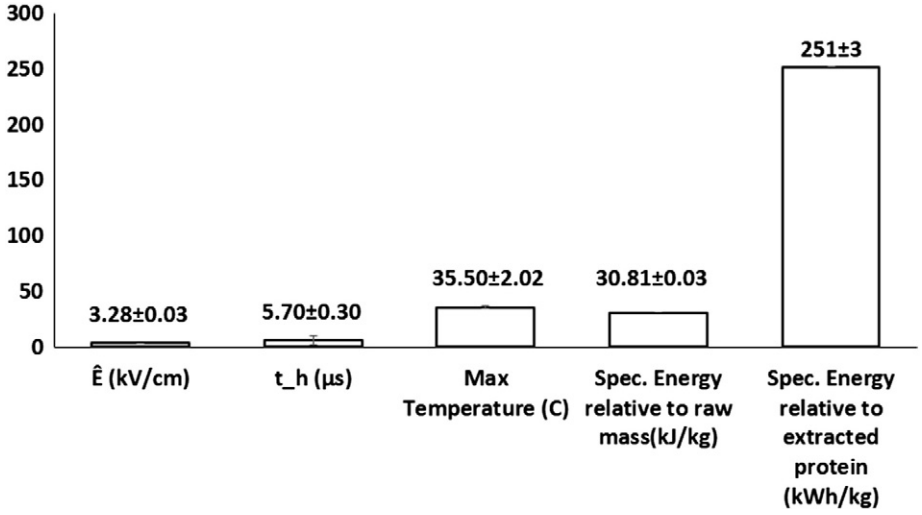

Fig. 5. Process parameters and energy requirement for proteins extraction from *Ulva* thalli.

and put back into the press for a second pressing step. The extracted juice and the pressed algae were again weighted.

2.5. Crude protein quantification

Bovine serum albumin (BSA) in DDW calibration curve was done in following concentrations:

1 mg mL^{-1}, 500 μg mL^{-1}, 250 μg mL^{-1}, 200 μg mL^{-1}, 150 μg mL^{-1}, 150 μg mL^{-1}, 100 mL^{-1}, 50 μg mL^{-1}, 25 μg mL^{-1}, 0 μg mL^{-1}. Every 10 μl BSA concentration mixed with 115 μl Bradford buffer. Extracted juice was filtered with 0.2 μm filter, 10 μL of samples mixed with 115 μL Bradford buffer. The BSA concentrations and the extracted juice samples with Bradford buffer were measured at optical density (OD) 450 nm and 590 nm. The numbers of OD 450 parts 590, were the basis for linear calibration curve. OD detection was done with an EL808, BioTek spectrophotometer (Winooski, VT, USA).

2.6. Gel electrophoresis

Extracted juice from pressing with/without PEF treatment was filtered with a 0.2 μm filter. Protein precipitation was made: 1 volume of Trichloroacetic acid (TCA) 100% (*w*/*v*) added to 4 volumes extracted juice samples. Then moved to 1.5 mL tubes. The samples incubated in 10 min at 4 °C. The tube spin in microcentrifuge at 14 K rpm, 5 min. Supernatant was removed, protein left in the pellet intact. Pellet was washed with 200 μL cold acetone. Spin in microfuge at 14 K rpm, 5 min. We made total of 2 acetone washes. Pellet was dried by placing tube in 95 °C heat block for 5–10 min. SDS-PAGE, 4X sample buffer (with βME) and sample boiled for 10 min in 95 °C heat block. The samples were run on SDS gel 12% agarose 200 V, 30 min.

2.7. Extracted proteins identification quantification with LS–MS/MS

2.7.1. Proteolysis

200 μL of the samples was brought to 8M Urea. The protein in 8M Urea, was reduced with 2.8 mM DTT (60 °C for 30 min), modified with 8.8 mM iodoacetamide in 100 mM ammonium bicarbonate (in the dark, room temperature for 30 min) and digested in 2 M Urea, 25 mM ammonium bicarbonate with modified trypsin (Promega) at a 1:50 enzyme-to-substrate ratio, overnight at 37 °C. One microgram from each sample was injected into a LC–MS/MS device.

2.7.2. Mass spectrometry analysis

The tryptic peptides were desalted using C18 tips (Homemade stage tips) dried and re-suspended in 0.1% formic acid. The peptides were resolved by reverse-phase chromatography on 0.075× 180-mm fused silica capillaries (J&W) packed with Reprosil reversed phase material (Dr Maisch GmbH, Germany). The peptides were eluted with linear 60 minute gradient of 5 to 28% 15 minute gradient of 28 to 95% and 15 min at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.15 μL/min. Mass spectrometry was performed by a Q Exactive plus mass spectrometer (Thermo) in a positive mode using repetitively full MS scan followed by collision induces dissociation (HCD) of the 10 most dominant ions selected from the first MS scan.

2.7.3. Computational analysis

The mass spectrometry data was analyzed using either the MaxQuant software 1.5.1.2 (Mathias Mann's group) or Peaks 7 software (Bioinformatic Solutions). The analyses were done vs. the green algae section of the NCBI-nr database with 1% FDR. The data was quantified by label free analysis using the same software. Intensity parameter mean: Summed up eXtracted Ion Current (XIC) of all isotopic

Table 1
Proteins detected only after PEF treatment.

Protein identification	NCBI accession number (GI)	Protein found in the species	MW (kDa)	Average normalized intensity (N = 3)
Calreticulin	255089467	*Micromonas sp. RCC299*	48	2.57E + 07
Ferredoxin-NADP+ reductase	545356935	*Coccomyxa subellipsoidea C-169*	38	6.26E + 07
Fructose-1,6-bisphosphatase	145345160	*Ostreococcus lucimarinus*	35	3.53E + 07
Fructose-bisphosphate aldolase 1	302831241	*Volvox carteri f. nagariensis*	41	3.42E + 07
Phosphoglycerate kinase	654120603	*Tetraselmis sp. GSL018*	45	2.30E + 07
Ribosomal protein L12 (chloroplast)	11467764	*Nephroselmis olivacea*	18	4.70E + 07
Predicted protein	145346523	*Ostreococcus*	32	3.41E + 07
Predicted protein	612389598	*Bathycoccus prasinos*	78	4.32E + 06

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Table 2
Proteins extracted with water without PEF treatment. Not detected in the PEF treated samples.

Protein identification	NCBI accession number (GI)	Protein found in the species	MW (kDa)	Average normalized intensity (N = 2)
Amidohydrolase 2	693500897	*Ostreococcus tauri*	40	2.14E + 08
Calmodulin	654126732	*Tetraselmis sp. GSL018*	16	2.66E + 07
Cytosolic 80S ribosome and 40S small subunit	302839477	*Volvox carteri f. nagariensis*	16	3.45E + 07
Histone H4	761973387	*Monoraphidium neglectum*	11	1.33E + 07
Photosystem I iron–sulfur center, partial (plastid	269925003	*Volvox carteri f. nagariensis*	3	2.56E + 07
Hypothetical protein	612393855	*Bathycoccus prasinos*	36	3.28E + 07
Predicted protein	158274897	*Chlamydomonas reinhardtii*	58	1.33E + 07
Predicted protein	303280339	*Micromonas pusilla CCMP1545*	40	1.97E + 07

clusters associated with the identified AA sequence. For specific protein quantification, the intensity measured for each protein was normalized to the total intensity of all proteins from the same sample.

2.8. Statistical analysis

Statistical analysis was performed with Excel (ver. 13, Microsoft, WA) Data analysis package. All experiments and controls were done in triplicates unless stayed differently. Standard error of the mean (SEM) is shown in error bars. One side Student's t-test was performed for compare the total protein extraction yield to controls. MS statistical analysis for proteins identification was done as described in 2.7.3. The criterion for inclusion was that the same protein was identified in at least two repeats from three.

3. Results and discussion

3.1. Process of proteins extraction from macroalgae Ulva with PEF

The process of protein extraction from macroalgae with PEF system for cell membrane disruption is shown in Fig. 1. First we analyzed the shape of the individually delivered electric pulse. Because of the cell membrane electroporation, the resistance of the treated macroalgae biomass reduces. Therefore we expected mild changes in the shape and pick values of each individual pulse. Fig. 2 shows the shape of delivered voltage and current at the first (U1, I1) and last (U_last, I_last) pulse in the delivered series of 75 pulses. The pulse source has been designed such, that a series of pulses of equal energy are applied. A pulse circuit based on a capacitor discharge has been employed. For a series of pulses the charging voltage of the capacitor has been kept constant. Pulses with an aperiodically damped shape are applied to the load. The peak current of the pulse is influenced by the resistance of the electrode system inside the treatment chamber and the stray inductance of the pulse circuit. The resistance of the treatment chamber decreases with the number of applied pulses, as discussed later. As a consequence, the pulse shape changes with the decreasing resistance, and the voltage across the electrode system decreases slightly with increasing number of pulses.

3.2. PEF parameters and changes in the macroalgae biomass during extraction

Next, we analyzed the changes in the peak electric field and current during the whole treatment (Fig. 3). We observed the decrease of the actual delivered electric field strength per pulse and increase of the current per pulse (Fig. 3). These changes are expected because of treated media conductivity increase (Fig. 3 shows the decrease of the resistance), which follows cell membrane electroporation and release of intracellular cell content. In the application of the 75 pulses on the biomass, the actual peak electric field decreased from 3.215 ± 0.033 kV cm^{-1} at the beginning of the treatment (first five pulses) to 2.864 ± 0.040 kV cm^{-1} at the end of the treatment (last five pulses in the series). The current increased from 713.6 ± 16.8 A at the beginning of the pulse series (first five pulses) to 1173.86 ± 30.8 A. These changes in the actual electric field and current are explained by the 46% drop of the sample resistance during the application of pulsed electric fields. Interestingly, we observed that the major decrease in the resistance (35%) and increase in the current from 713.6 ± 16.8 A to 1024 ± 26.7 A took place during the first twenty pulses. This is probably the number of pulse required to electroporation the majority of cells in the treated *Ulva* thalli. Previously, we have observed similar pattern of rapid resistance decrease and current increase in the skin tissue, where electric fields also electroporated cells inside the complex extracellular matrix (Golberg et al., 2013) and also in sugar beet tissue (Bluhm & Sack, 2008).

3.3. Extracted proteins yield

With the treatment parameters used in this study the average electric field strength applied on the macroalgae thalli was 2.964 ± 0.007 kV cm^{-1}, the pulse duration was 5.70 ± 0.30 μs (Fig. 5). The total dry matter (Fig. 4) consisted 5.52 ± 0.20% of the total extracted juice weight (Fig. 4). PEF increased the total protein extraction yields (p-val < 10^{-6}) (Fig. 4). The total protein concentration in the extracted

Table 3
Proteins detected in samples with PEF and without PEF treatment.

Protein identification	NCBI accession number (GI)	Protein found in the species	MW (kDa)	Average normalized intensity	
				(−PEF) (N = 2)	(+PEF) (N = 3)
Actin	116222105	*Pterosperma cristatum*	38	8.96E + 07	3.40E + 07
Heat shock protein 70	304555563	*Ulva pertusa*	73	2.80E + 07	4.81E + 06
Iron-superoxide dismutase 1	149275667	*Ulva fasciata*	25	3.04E + 07	9.46E + 06
Plastocyanin	3024399	*Ulva pertusa*	11	1.59E + 09	1.27E + 09
Plastocyanin precursor	48526878	*Ulva pertusa*	15	8.24E + 08	2.14E + 09
Sedoheptulose-1,7-bisphosphatase	545353814	*Coccomyxa subellipsoidea C-169*	37	1.19E + 07	1.02E + 07
Ubiquitin	552821086	*Chlorella variabilis*	8	3.82E + 07	2.57E + 07
Uroporphyrin-III C-methyltransferase	303285200	*Micromonas pusilla CCMP1545*	32	1.66E + 08	1.01E + 07
Hypothetical protein	761971964	*Monoraphidium neglectum*	113	1.82E + 08	1.03E + 07
Predicted protein	145348138	*Ostreococcus lucimarinus CCE9901*	39	2.10E + 09	3.78E + 08

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juice was 59.13 ± 3.82 μg mL^{-1} (Fig. 4). This is in contrast to 23.80 ± 1.33 μg mL^{-1} observed in the control samples. Importantly, the developed process has almost no thermal effects on the produced proteins as the maximum observed temperature was 35.50 ± 2.02 ^{0}C.

3.4. PEF process energy consumption

An important parameter in the production of proteins for food and feed application is the energy consumptions. Non-thermal pulsed electric field has been shown previously to reduce the total energy consumption of the sugar extraction by 30–50% at the industrial scale (Bluhm & Sack, 2008; Sack et al., 2010a,2010b;). Here we analyzed the energetic consumption of the pulsed electric field process for water soluble solids, including proteins, extraction from macroalgae *Ulva*. The specific energy consumed relative to raw mass of macroalgae was 8.56 ± 0.01 Wh kg^{-1} (30.81 ± 0.03 kJ kg^{-1}) and the specific energy relative to the extracted proteins was 251 ± 3 kWh kg^{-1}(Fig. 5).

3.5. PEF extracted proteins identification

Previous works have shown the use of PEF to extract proteins from microalgae (Goettel et al., 2013; Parniakov et al., 2015), yeast (Ganeva & Galutzov, 1999; Ganeva et al., 2003), bacteria (Haberl Meglic et al., 2015) and plants (Bluhm & Sack, 2008; Doevenspeck, 1961; Sack & Bluhm, 2008; Vorobiev & Lebovka, 2010; Zagorulko, 1958). However, to the best of our knowledge these were reported as crude protein extraction. In this work we identified and quantified specific proteins extracted from *Ulva* genus with PEF using LC/MS/MS (Supplementary information Table 1S shows all identified proteins in at least one sample). Proteins that have been uniquely identified in the PEF treated sample extracts that appear in Table 1 (criteria for inclusion werethe same protein that has been identified in at least two repeats from three). Proteins detected only in samples from *Ulva* biomass treated only with water, that appear in Table 2 and criteria for inclusion were the same protein that has been identified in at least two repeats from two). These proteins were not observed in the PEF treated samples and probably have been damaged by electric fields. In Table 3 we report on the proteins that have been detected in both PEF treated and non-treated samples. Interestingly, we observed that besides Plastocyanin precursor, PEF reduced the quantities of proteins extracted from *Ulva* biomass with tap water (based on intensity parameter). Our previous work with DNA showed that specific PEF parameters lead to DNA nicking (Golberg & Rubinsky, 2010). Additional studies showed that specific PEF parameters could lead to either activation or inactivation of enzymes, depending on enzyme type (Ohshima, Tamura, & Sato, 2007). However, most of the studies until today showed that PEF increases the extraction yields. Indeed, in our study PEF increased ~3 times the extracted proteins yields if measured in a bulk. However, the detailed identification and quantification of individual proteins, reported here for the first time, reveals a more complex scenario. Some proteins are extracted with PEF (Table 1 and Table 3), but some, which can be extracted with tap water are partially (Table 3) or completely (Table 2) degraded by the treatment. To the best of our knowledge this is the first report that identified proteins extracted from biomass with PEF; previous studies used bulk proteins characterization (Coustets et al., 2015; Ganeva et al., 2003). These findings are new and important, as they open a possibility to optimize PEF parameters for the extraction of specific proteins from macroalgae and other biomass. An important future application could be the inactivation of the ingenious proteases during protein extraction processes. Additional future studies will address the effect of PEF extraction on the functional properties of the extracted proteins. PEF provides a potentially unique non-thermal, chemicals-free proteins extraction method that could preserve the functional properties of the proteins, important for food and pharmaceutical applications.

4. Conclusions

Macroalgae are promising, but challenging sustainable feedstock for biorefineries. Complete zero waste conversion of macroalgae into food, chemicals and fuels will reduce the burden of the agriculture from arable land. Here we report on a new technology to extract green macroalgae *Ulva* proteins with electroporation by PEF. PEF is an emerging, energy efficient technology for biomass processing. We showed that PEF increases ~ by 3 times the total protein extraction, and is selective, as it increases the extraction yields of some specific proteins but damages others. This study demonstrates the scalable, energy efficient technology for extraction essential for food supply chemicals-proteins.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ifset.2016.03.013.

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