Functional Protein Concentrates Extracted from the Green Marine Macroalga *Ulva* sp., by High Voltage Pulsed Electric Fields and Mechanical Press

Arthur Robin, Meital Kazir, Martin Sack, Alvaro Israel, Wolfgang Frey, Georg Mueller, Yoav D. Livney, and Alexander Golberg

**ABSTRACT:** With decreasing available land and fresh-water resources, the oceans become attractive alternatives for the production of valuable biomass, comparable to terrestrial crops. Seaweed cultivation for food, chemicals, and fuels is already under intensive development, yet efficient technologies for separation of major components are still missing. We report a food-grade process for the extraction of proteins from a green macroalga, *Ulva* sp., using high-voltage pulsed electric field (PEF) cell-membrane permeabilization, coupled with mechanical pressing to separate liquid and solid phases. We showed that a PEF treatment, at 247 kJ/kg fresh *Ulva*, delivered through 50 pulses of 50 kV, applied at a 70.3 mm electrode gap on the 140 g fresh weight of *Ulva* sp., resulted in an ~7-fold increase in the total protein extraction yield compared to extraction by osmotic shock. The PEF extract of 20% protein content showed 10–20 times higher antioxidant capacity than β-Lactoglobulin (β-Lg), bovine serum albumin, and potato protein isolates. The protein concentration per dry mass in the residual biomass after PEF treatment was increased compared to the control because of the removal of additional nonprotein compounds from the biomass during the extraction process. These results provide currently missing information and technological development for the use of macroalgae as a source of protein for promoting sustainable human nutrition and health.

**KEYWORDS:** Pulsed electric field, Protein concentrate, Macroalgae, *Ulva*, Antioxidant, Seaweed

**INTRODUCTION**

The demand for food is predicted to increase by 70% by the year 2050. However, not only the total amount of food needed will rise, but also the types of food required are expected to change because of lifestyle changes, such as rising income, urbanization, and aging population. The demand for proteins is expected to double, reaching 943 MMT by 2054. This rising demand for both animal and plant protein is expected to further increase the pressures on arable land use for agriculture and grazing, leading to further deforestation, land erosion, eutrophication, and biodiversity loss. One of the approaches to meet the challenge of protein demand is sustainable mariculture (seagriculture) of protein-rich marine macroalgae (seaweeds) in seas and oceans. Based on climate simulations and metabolic modeling, we have estimated that offshore grown biomass could provide 5–24% of the protein demand in 2050.

Marine macroalgae, some of which are consumed “as is” in the Far-East, have a significantly higher content of proteins in comparison with terrestrial plant proteins sources such as soy, nuts, and cereals. Up to 50% protein from the total macroalgae dry weight have been reported. In addition to their high potential availability, sustainability, and nutritional benefits, marine macroalgae derived peptides have shown additional value, because of their nutraceutical properties such as antioxidant, antihypertensive, immune-modulatory, anticoagulant, and hepato-protective attributes. Yet, the global market share of seaweed for nonhydrocolloid use is still below 1%. In many cases, the only use of the harvested or cultivated seaweed biomass is a single hydrocolloid product, and the remaining biomass (sometimes up 92%) is disposed back to the
environment as waste.13,14 This calls for developing biorefinery processes, which would enable multiple streams of products for the higher valorization of marine algae biomass.6,13,15,16 Toward the “zero waste” vision. However, today, macroalgae protein use is hindered because of lack of sufficient production and extraction technologies. Moreover, current regulations restrict macroalgae protein use as a food ingredient, by classifying algae as "novel foods”.17

The value of a protein sourced from macroalgae depends on the efficiency of the extraction process and on the functional and nutritional properties of the protein obtained. To achieve good physicochemical functionality, nutritional, and nutraceutical properties, it is important to preserve the native protein structure. However, the rigid and often charged macroalgal cell walls and the complex extracellular matrix make the extraction process challenging.18 Osmotic shock, mechanical grinding, high shear force, ultrasonic treatment, acid or alkaline pretreatment, enzymatic polysaccharides digestion aided extraction, and their combinations have been attempted to increase the extraction yields.19–25 Although the mentioned methods were shown to increase the extraction yields, they generally involve either thermal or chemical procedures that could adversely affect the functionality of the extracted proteins and peptides. Enzymatic digestion of the polysaccharides decreases their value, hence the overall added value of the extraction process. To bridge the gaps in knowledge and technology, we proposed to develop a chemical-free, nonthermal, extraction/separation process based on pulsed electric fields (PEFs) coupled with mechanical pressing. PEFs are an emerging, nonthermal, energy-efficient food processing technology already used for extraction of proteins from microalgae, yeast, bacteria, and plants.26 Recently, in another study from our group, PEFs have been proposed for protein extraction from the green macroalgae Ulva and were shown to have lower energy consumption than alternative extraction processes.28 In the current work, we show that a PEF, in combination with a mechanical press, enables extraction of proteins from the green macroalgae, Ulva. Following extraction, the protein was purified and concentrated. The concentrate extracted using PEFs showed superior antioxidant activity compared to reference protein isolates. This study paves a way toward industrial extraction and purification of macroalgal proteins for uses in the food and chemical industries.

### EXPERIMENTAL SECTION

**Macroalgal Biomass Production.** In this study the green marine macroalgae Ulva sp. was used as the primary source of biomass. Ulva is a seaweed of worldwide distribution, and in Israel it is found in the intertidal and shallow waters within the Mediterranean Sea shores. The initial inoculum was taken from an outdoor seaweed collection at the Israel Oceanographic & Limnological Research (IOLR) Institute, Haifa, Israel. The inoculum comprised a mixture of two closely related (both morphologically and molecularly) Ulva species: Ulva rigida and Ulva ohnoi.27 The biomass was cultivated in 40 L tanks supplied with running seawater, aeration, and weekly additions of 1 mM NH4Cl and 0.1 mM NaH2PO4. With each nutrient addition, the water exchange was stopped for 24 h to allow for their absorption. About 3.0 kg (fresh weight) of Ulva were packed in a sealed plastic bag and delivered to Karlsruhe Institute of Technology, reaching the destination within 48 h. Upon arrival, the seaweeds were quickly immersed for 2 days in a 400 L aquarium filled with seawater and exposed to natural sunlight.

**Protein Extraction from Seaweed Biomass with Pulsed Electric Fields and Mechanical Press.** Using a manual kitchen centrifuge, fresh Ulva biomass was centrifuged 3 times, 1 min each time, to remove the external surface-wetting water, so that <1 g of water was removed after the third run, meaning we had removed most of the surface water. About 140 g of fresh biomass (referred as FW, fresh weight) were loaded into the PEF treatment chamber with a volume of 232 cm3. The distance between the electrodes was 70.3 mm. Deionized water was added to fill the chamber completely (<100 mL). Submerging the seaweed biomass into deionized water may lead to partial disruption of the tissue by osmotic shock. We have considered this effect, when choosing the control conditions, as described below. Pressurizing the biomass in the chamber with water aimed to prevent the formation of bubbles that could lead to nonhomogeneous field distribution. The PEF parameters were charging voltage (0, 20, 35, 50 kV) and pulse numbers (0, 10, 20,

### Table 1. Protein Extracted from *Ulva* sp. Biomass (140 g FW) with PEF in a 232 cm3 Chamber with a 70.3 mm Gap between Electrodes

<table>
<thead>
<tr>
<th>sample no.</th>
<th>charging voltage (kV)</th>
<th>number of pulses (N)</th>
<th>temperature after pulsatian (°C)</th>
<th>total energy input (kJ)</th>
<th>mass of protein extracted (mg)</th>
<th>liquid extract protein concentration (mg/mL)</th>
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<tr>
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<tr>
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<td>50</td>
<td>69.7</td>
<td>34.56</td>
<td>184.45</td>
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<td>27.9</td>
<td>0.00</td>
<td>18.98</td>
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</table>
treated sample was derived from the current and voltage measurements (RCR, Reliant EMC, CA), both connected to an oscilloscope (Pearson Electronics, CA) and a voltage divider (HILO-Test HVT 240 RCR, Reliant EMC, CA), both connected to an oscilloscope (Tektronix TDS 640A, Tektronix, Inc. OR). The resistance of the treated sample was derived from the current and voltage measurements based on Ohm's law. The total energy consumed for the PEF treatment was calculated based on the energy stored in the pulse capacitor using eq 1:

\[
E_t = 0.5 \times C \times (V)^2 \times N
\]

where \(E_t\) (J) is the total energy consumed for the treatment, \(C\) is the discharging capacitor capacitance (F), \(V\) (V) is the applied voltage, and \(N\) is the total number of pulses. Additional losses of the capacitor charger have not been considered. All combinations of charging voltage and number of pulses were applied on at least two replicates. Two samples were used as controls and correspond to the treatment condition of 0 pulse of 0 kV mentioned above. They were loaded into the PEF devices for the same duration as the longest PEF treatment; however, no pulses were applied.

The PEF-treated and control (0 pulses of 0 kV) biomass samples were wrapped in a fabric filter and placed in the mechanical press (HAPA type SPM 2.5S) to obtain the liquid extract. An additional step is often used after the pulsed electric field treatment, to increase the amount of extracts and take advantage of the increased mass transfer due to the electropermeabilisation of the tissues. Among the techniques used, diffusion in stirred solvent is the most popular in the literature, but we chose pressing for its convenience, short duration, scalablility (screw press, extrusion, etc.), and as a first solid/liquid separation step. A constant pressure of 4.5 MPa was applied for 5 min. The liquid extract from pressing was collected in a 2 L beaker and weighed. The "press-cake" was weighed after pressing. Liquid extract was collected and kept at -20 °C. Cakes were spread on a plate, dried at 40 °C for 24 h, and then kept at 5 °C. Throughout the article, the control conditions for the study that correspond to the biomass being placed in the chamber with deionized water, but without electrical treatment, were referred to as either "control conditions", "treatment of 0 pulses of 0 kV", or "osmotic shock" and detailed as sample nos. 8 and 20 in Table 1.

**Dry Matter and Ash Content Determination.** Liquid extract (1-2 mL) and press-cake (0.5-1 g) samples were weighed and then dried at 105 °C using a conventional oven for 24 h in preweighed clean crucibles. The crucibles were cooled down in a desiccator, weighed, and ignited at 550 °C for 3 h in a muffle furnace (Thermolyne muffle furnace, Thermo Scientific, MA) and then cooled down to 105 °C. Finally, the crucibles were removed from the furnace, kept in a desiccator to cool them down at room temperature, and weighed. Analysis was done in triplicate. Dry matter (DM) and ash content (AC) were calculated according to eqs 2 and 3.

\[
\text{DM} (\%) = 100\% \times (m3 - m2)/(m1 - m2)
\]

\[
\text{AC} (\%) = 100\% \times (m4 - m2)/(m3 - m2)
\]

where \(m1\) is the mass of the liquid extract or cake sample plus the crucible (mg), \(m2\) is the mass of the crucible, \(m3\) is the mass of the sample plus the crucible after drying at 105 °C, and \(m4\) is the mass of the sample plus the crucible after combustion at 550 °C. 

**Total Protein Quantification by the Lowry Method.** Total protein was quantified using a modified Lowry assay that has been adapted to a microplate following an application note from the Biotek Instruments company. The liquid extract was centrifuged 2 min at 2350 × g. The supernatant was then directly analyzed or dialyzed prior to analysis with ultrapure water. Standard curves were produced using bovine serum albumin (BSA) at different concentrations (0-500 μg/mL). Diluted extracts and standards were analyzed by adding 100 μL in a well of a 96-well plate. Biuret reagent was prepared by mixing 0.5 mL of 1% cupric sulfate with 0.5 mL of 2% sodium potassium tartrate, followed by the addition of 50 mL of 2% sodium carbonate in 0.1 N NaOH. After standards and samples were diluted and transferred to the microplate, 200 μL of biuret reagent were added to each well and mixed thoroughly by repeated pipetting. The mixture was then equilibrated at room temperature for 10–15 min prior to the addition of 20 μL per well of 1.0 N Folin-Ciocalteu reagent. Samples were mixed immediately by repeated pipetting following each addition. The color was allowed to develop for 30 min at room temperature, and then the absorbance was measured at 650 nm on a spectrophotometer (Infinite 200 Pro, TECAN, Switzerland). As a blank, a water-only control was used. Analyses were done in triplicate, and results were expressed as BSA Equivalent.

**Amino Acid Analysis.** The analysis of amino acid composition was carried out by High-Performance Ion Chromatography (HPIC), according to Application Note 163 “Determination of Protein Concentrations Using AAA-Direct” from Dionex Inc. (Thermo Fischer Scientific, MA, USA) with some modification. Briefly, 1 mL of 6 M HCl was added to 1 mg of protein extract and thermochemical hydrolysis was conducted in a dry bath (Bio-Base, China) (16 h, 112 °C). At the end of the thermochemical hydrolysis, the acid was evaporated by nitrogen. The dry samples were reconstituted with ultrapure water, vortexed multiple times, and equilibrated for at least 1 h in sealed vials. Dilutions of each sample (1/10 and 1/50) were then filtered (0.22 μm) into HPIC vials. The total amino acid content was analyzed by HPAEC-PAD (High-Performance Anion-Exchange Chromatography coupled with Pulsed Amperometric Detection) using a Dionex ICS-5000 platform (Dionex, Thermo Fischer Scientific, MA, USA) with an analytical column (Aminopack 10) and its corresponding guard column. An electrochemical detector with a gold AAA electrode and an AgCl reference electrode was used for detection. The eluent gradient program and the waveform for the electrochemical detector used were as described in the above-mentioned Application Note 163. Other conditions were as follows: flow rate (0.25 mL/min), injection volume (10 μL), column temperature (30 °C), and autosampler temperature (5 °C). The program was validated by using a commercial amino acid mix (AAS18, Sigma-Aldrich, MO, USA), and four dilutions of the mix (1/50, 1/100, 1/250, and 1/1000) were used to build a calibration curve for 17 amino acids (alanine, arginine, aspartate, cystine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine) with a correlation factor \(R^2 > 99\%\) for each calibration curve. As methionine, cysteine, and its dimer cystine are sensitive to acid hydrolysis, oxidative degradation could occur, and thus, their amino acid content for methionine and cystine are underestimated. All samples were hydroyzed in triplicate, and each hydrolysate was injected twice for HPIC analysis.

**PEF Extracted Protein Purification and Concentration.** Total protein extract was obtained by PEF treatment, followed by dialysis against deionized water (MWCO 100–500 Da, Spectrum Laboratories Inc., USA) and freeze-drying. The protein content in the dry powder was measured using the Lowry method as described above.

**Determination of the Antioxidant Properties of PEF Extracted Protein.** The antioxidant activity of the extracted and concentrated proteins was measured using two different methods: the ferric reducing antioxidant power (FRAP) assay and the oxygen radical absorption capacity (ORAC) assay. Briefly, for the FRAP assay, FRAP reagent was freshly prepared by dissolving 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, Sigma-Aldrich Ltd., Israel) (10 mM in 40 mM HCl) and FeCl3·6H2O (20 mM in DW) solutions in acetate buffer (300 mM, pH 3.6), at 1:1:10 v/v ratio. A 210 μL aliquot of FRAP reagent was transferred into each sample well of a 96-well microplate, containing 7 μL of each tested sample. To create a calibration curve for FeSO4·7H2O (2000 μM) was diluted in distilled water for a concentration range of 0–2000 μM and 210 μL of FRAP reagent were added to 7 μL of each diluted sample. The absorption at 593 nm was measured, for all samples, 4
min after adding the FRAP reagent, using a plate reader (Eon, BioTek Instruments, Inc., USA).

For the ORAC assay, Fluorescein and 2,2′-Azobis (2-methylpropionamide) dihydrochloride (AAPH, Sigma-Aldrich Ltd., Israel) were dissolved in potassium phosphate buffer (75 mM, pH 7.4) and diluted to final concentrations of 30 ng/mL and 21.7 mg/mL, respectively. At the first step, 25 μL of each tested sample were added to a well of a 96-well microplate, containing 150 μL of fluorescein (30 ng/mL). The 96-well microplate was placed in a fluorescence plate reader (VarioskanTM Flash, Thermo Scientific) and incubated for 15 min at 37 °C. Then, 25 μL of AAPH (21.7 mg/mL) were added to each well and the microplate was shaken for 20 s at 180 rpm. Fluorescence readings were taken every 25 s for 90 min with an excitation wavelength of 485 nm and an emission wavelength of 528 nm.

Statistical Analysis. Statistical analysis was performed using the Excel (ver. 13, Microsoft, WA) Data analysis package and R software (ver.2015, RStudio Inc., Boston, MA). The standard error (±SE) is shown by error bars. At least two replicates were done for each experimental condition.

RESULTS AND DISCUSSION

The initial biomass composition was 18.5% DM (after removal of surface-wetting seawater), including 29 ± 0.1% ash and 20.2 ± 1.3% protein. The procedure for macroalgae biomass production, protein extraction, and concentration using a pulsed electric field is shown in Figure 1a. The procedure included the following: (1) biomass cultivation which can be done in land-based tanks (as in this study), or offshore; (2) removal of surface-wetting seawater; (3) PEF treatment; (4) mechanical pressing for liquid extraction from the biomass; (5) separation of small molecules (mainly salts) by dialysis (100–500 Da MWCO); and (6) extract concentration by freeze-drying.

The temporal sensitivity of protein and amino acids contents after each step, as reported by Beal et al. (2013) for lipid extraction, was not determined here and is planned to be observed in a subsequent study.

The shape and values of the applied electric pulses are shown in Figure 1b, c. An example of the voltage applied in one of the treatments is shown in Figure 1b. The maximum voltage (of the first pulse) was 40.68 kV, and the maximum voltage of the last pulse in the series (N = 50) was 38.44 kV. This resulted in electric field intensities of 581 to 549 V mm⁻¹ with maximum currents of 4528 A in the first pulse and 6384 A in the last pulse. Under these conditions, the resistance of electro-permeabilized algae decreased from 8.98 ohm in the first pulse to 6.02 ohm in the last pulse.

The effect of pulsed electric fields on lipid membranes is a partially known phenomenon that results in the breakdown of the lipid membrane. This induces an increase in the conductivity through the membrane as well as a loss of its selective permeability. Although the phenomenon is not fully understood, the current consensus is that aqueous pores of various sizes and lifetimes are formed, allowing materials to flow freely between the extracellular media and the intracellular space. Under our conditions of dozens of pulses of 4 to 6 μs in the range of 2 to 6 kV/cm, we expected to induce irreversible electro-permeabilization of the membrane, to a effect its resistivity and capacitance properties, which are coupled, as the pores increase in size during the treatment leading to permanent damage of the membranes, and, thus, to cell lysis. Following cell lysis, extraction of intracellular components, such as protein, would be facilitated. Indeed, evidently, these changes of resistance, capacitance, and media composition have probably led to the change of current vs time (Figure 1c), showing a higher current, and a more steep reduction in current over time at the last pulse than in the first pulse.

Application of a PEF and mechanical press led to the extraction of 34–46% of total intracellular ash, in comparison with only 18% extracted by osmotic shock and mechanical
press (control condition) (Figure 2a, Table 1). The same treatment led to extraction of 11–21% of the total dry matter from the Ulva biomass in comparison to extraction of only 6–7% by osmotic shock followed by the same mechanical pressing (control conditions) (Figure 2b). No significant differences in the yields of ash or dry matter were observed when varying PEF parameters (Figure 2a, b), suggesting complete electroporation of the biomass.

Increasing the charging voltage from 20 to 50 kV led to an increase from 145 ± 15 mg to 203 ± 23 mg of protein released from the Ulva biomass after pressing was applied, respectively. In comparison, 33 ± 20 mg were extracted with osmotic shock (Figure 3a, Table 1). Increasing the number of pulses from 10 to 50 led to an increase from 161 ± 20 mg (N = 10) to 170 ± 17 mg (N = 50) of protein released, in comparison to 33 ± 20 mg in the control (N = 0) (Figure 3b, Table 1). Interestingly, in the tested protocols, increasing the energy input using PEFs (26–246 kJ/kg FW) increased the total amount of proteins released in comparison with the osmotic shock control, but the ratio of protein released with respect to the control was similar between various PEF protocols tested in this study without noticeable effect of increasing the energy input per kg of biomass (Figure 3c, Table 1). This suggests electroporation alone is not sufficient to increase the extracted protein yield, and optimization of extraction conditions (such as solvent pH and polarity) will be needed in future work. Moreover, the PEF-induced temperature increase (due to the Joule effect) above 30 °C did not increase the yield of protein extraction in the tested range (Figure 3d, Table 1).

Measuring the total amino acid content of both liquid extract and biomass solid residue is a reference method to quantify protein in seaweed.19 Moreover it can provide some information on the extraction of peptides or free amino acids, and on the nutritional value, such as essential amino acids content. Interestingly, amino acid analysis showed a larger yield of total protein in the liquid extract (173 ± 6 mg/140 g FW for 50 kV, 50 pulses, vs 43 ± 22 mg/140 g FW for the osmotic shock control (Figure 4a)). The total amino acids extraction yield was 0.9 ± 0.3% and 2.9 ± 0.03% of the initial protein (total amino acids) for the control and PEF treatment (50 kV, 50 pulses), respectively. The 2.9% is a very low

Figure 2. (a) Ash and (b) dry matter in PEF extract as percent of the ash and dry matter in the Ulva biomass, respectively. Conditions of the extractions are detailed in the x-axis: charging voltage (in kV) and number of pulses (N). The last bar (0 pulses of 0 kV) corresponds to the control conditions, where the biomass was only submerged in pure water and no pulses were applied. n = 6–9 (2–3 experimental repeats per condition, with each result determined in triplicate). Error bars represent ± SE.

Figure 3. Impact of PEF process parameters on yield of protein extraction from Ulva biomass. The impact of (a) applied voltage; (b) number of pulses; (c) total energy applied; and (d) final process temperature (temperature increase is due to heat release by the Joule effect during treatment, and is correlated with total energy applied). The total number of samples was 20. Analysis of proteins in each sample was performed in triplicate. Quantification was done with Lowry method. Error bars show ± SE.
tuning the time and number of extraction steps,31 and/or using different pulse parameters,28 and/or by using different solvents (such as alkali, or water–ethanol mixtures).42}

Protein in the PEF extract was concentrated from ∼3.5% protein (DW basis) solution to ∼20% protein (DW basis) in a powder form (Figure 5a). This is the first time, to the best of our knowledge, that PEF extracted protein-rich product was purified from macroalgae biomass. This protocol is the first step for integration of PEF derived algal proteins into the standard protein supply chain, where powders of various protein concentrations are used in food and other applications. Future work will address the increase of protein content in the powder, but this work shows the promise of using dialysis as a simple step for seaweed protein purification and concentration. Dialysis, or its industrial counterpart, diafiltration—using steps of dilution and concentration by ultrafiltration membranes—is a common food processing unit operation, which is used at industrial scale. Ultrafiltration is commonly used for concentration and/or removal of salt and other small molecules in the food, pharmaceutical, and bioprocessing industries.43,44

PEF also increased the protein concentration in the residual cakes because of the extraction of other nonprotein compounds (213 ± 0.33 mg/g DM for 50 kV, 50 pulses vs 164 ± 13 mg/g DM for the osmotic shock control (Figure 4b)). These results show for the first time that PEF, followed by the steps described above, produces two protein-enriched (concentrated) products. The first product is the extracted, dialyzed, and dried protein (Figure 5a), and the second product is the residual press cake, which has lower ash content than the original dry macroalgae biomass, hence a higher proportion of protein. Protein concentration in the residual biomass takes place as the PEF-assisted extraction also removes compounds other than protein, such as ash (>70% of the extracted dry matter) and other organic material while extracting about 3 times more dry matter than with osmotic shock (Figure 2). This protein-rich solid biomass residue can be of particular interest as animal feed;45 previous studies have already shown the application of raw Ulva sp. biomass as a feed for aquaculture,46 sheep,47 and broiler chicken.48

Concerning individual amino acids in the extracts (Figure 4c), we showed that PEF treatment (50 kV, 50 pulses) was

Figure 4. Concentrations of protein (as total amino acid) extracted by PEF in the liquid extract and in the residual Ulva biomass. (a) Protein content (as total amino acid) in the liquid extract, extracted from 140 g of fresh weigh of Ulva using 50 kV, 50 pulses protocol, with input of 247 kJ/kg FW. (b) Protein (as total amino acid) concentration in the residual biomass cake. n = 12 per point. (c) The yield of individual amino acids in the liquid extract extracted with PEF or osmotic shock (control conditions). Quantification was done with the total amino acid determination method. Results are expressed as mg of amino acids/g of initial dry seaweed material. Error bars show ± SE.
more efficient than osmotic shock in extracting valine, tyrosine, threonine, serine, proline, phenylalanine, lysine, leucine, isoleucine, histidine, glycine, glutamate, aspartate, arginine, and alanine.

Importantly, PEF-assisted pressing provides a quick, chemical-free, and mild-thermal extraction method, which makes it an easy and environmentally friendly process. Moreover, as the tissue integrity of the whole biomass is preserved, the separation and purification of the extracted fraction are easier than if the whole tissue was crushed, which is a distinct feature of PEF-assisted extraction. Furthermore, we expect minimal changes to protein structure and function under mild PEF treatment parameters. PEFs can induce proteins.50

Consequently, the nonprotein fraction is expected to contribute a significant part of the detected antioxidant activity.

Additionally, algae, and Ulva sp. in particular, are rich with sulfated polysaccharides, such as ulvan. It has been previously found62,53 that these sulfated polysaccharides have an antioxidant potential, which increases in correlation with the sulfate content.64 Although both sulfated polysaccharides and phenolic compounds can hinder the functionality of the extracts to some extent (lower bioavailability, etc.),65 they can also provide unique health and functional benefits.62,66 Thus, depending on the application, subsequent purification steps to improve the protein content might not be desired.41 Therefore, the nonprotein fraction is expected to contribute a significant part of the tested antioxidant activity.

One noteworthy potential safety issue is that applying a strong electric field in water can cause erosion of the electrode, releasing metal ions into the media. However, safety concerns activity of the extracted macroalgae protein concentrates. The antioxidative activity of the protein concentrates was measured and evaluated for two different mechanisms. The first tested mechanism was an oxygen radical absorption capacity (ORAC) assay that is based on a hydrogen atom transfer (HAT) mechanism (Figure 5b, Trolox was used as a standard). The second tested mechanism was a ferric reducing antioxidant power (FRAP) assay that is based on a single-electron transfer (SET) mechanism (Figure 5c, FeSO4 was used as a standard).

The antioxidant activity resulting from the protein itself can be explained by the presence of amino acids containing either nucleophilic sulfur-containing side groups, such as Cys and Met, or aromatic side groups, such as Tyr, Phe, and Trp.53 We found a higher content of phenylalanine in the PEF extracts of Ulva, in comparison with the extracts by osmotic shock (Figure 4c). Overall, the antioxidant activity of the PEF-extracted Ulva protein concentrates supports the application of these proteins in human nutrition and in the food industry.

We found that the antioxidative activity of the PEF-extracted macroalgae protein concentrates was between 10 to 20 times higher than that of several standard food protein isolates (β-Lg, BSA, and potato protein), Figure 5b, c. In the existing literature, it was found that these commercially available protein isolates do exhibit antioxidant activity in the tested mechanisms, which increases with protein concentration. For example, β-lactoglobulin, potato protein, and their hydrolysates were found to have antioxidant activity, with the hydrolysates being more effective.54–56 Moreover, protein isolates from plants often comprise nonprotein residues, including phenolic compounds, which are a major fraction of those nonprotein residues, due to the tendency of many polyphenols to adsorb to proteins. For example, a significant polyphenol fraction has been reported for soybean isolates. Phenolic compounds are present in algae, as in most plant materials, and can significantly contribute to the antioxidant activity.58

It has been proposed59–61 that the antioxidant activity of macroalgae, and especially macroalgae extracts, results not only from the presence of bioactive compounds such as polyphenols but also from bioactive proteins, peptides, and free amino acids.

However, it is important to mention that, in this work, the protein content in the tested PEF-extract was 20%. Therefore, the nonprotein fraction is expected to contribute a significant part of the detected antioxidant activity.


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Figure 5. (a) Digital image of the freeze-dried PEF-extracted macroalgae protein concentrate (20% protein). (b) Antioxidant activity by the hydrogen atom transfer (HAT) mechanism of PEF-extracted Ulva macroalgae protein concentrate, and of three common food protein isolates (BSA, β-Lg and potato protein). All samples contained 0.01 mg/mL protein thus 0.05 mg/mL of extract. Error bars represent standard error of two repeats, each performed in triplicate. (c) Antioxidant activity by the single electron transfer mechanism (SET) of PEF-extracted macroalgae Ulva protein concentrate and of common protein isolates (BSA, β-Lg and potato protein). All samples contained 1 mg/mL protein, thus 5 mg/mL of extract. Results presented were of two repeats, each performed in triplicate. Error bars represent ± SE.
of this phenomenon can be mitigated by selecting safe materials for the electrode (e.g., titanium, carbon), by proper chamber design and by tuning of process parameters. Importantly, PEF processes are already approved by health authorities for food processing, e.g., in the US and the EU.27,67

■ CONCLUSIONS

Growing and harvesting macroalgae in offshore facilities should reduce the pressures on terrestrial agricultural systems. Subsequent fractionation and valorization of the macroalgal components could turn seaweeds into new and renewable food sources to feed the growing global population, and potentially mitigate the adverse impacts of current practices on the environment, such as waste disposal from algal hydrocolloid production. Furthermore, macroalgal biomass is a promising and sustainable feedstock for biorefineries; however, it is challenging to extract and fractionate. In this work we reported a PEF-based technology that enables extraction of proteins from algal biomass, providing two important products: Dry algal protein concentrates with strong antioxidant properties and residual (dry press cake) biomass with less ash and a higher protein concentration than the initial biomass. We showed that by using PEFs we achieved an approximately 7-fold higher total protein extraction compared to the conventional osmotic shock method, although the yield of extraction remained under 5% of the initial protein content. The extracted protein concentrates showed a 10–20-fold higher antioxidant capacity than β-Lg, BSA, and potato protein isolates. The results of this study provide novel missing information and technologies for the use of macroalgae as a protein source for promoting sustainable human nutrition and health.

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Notes
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