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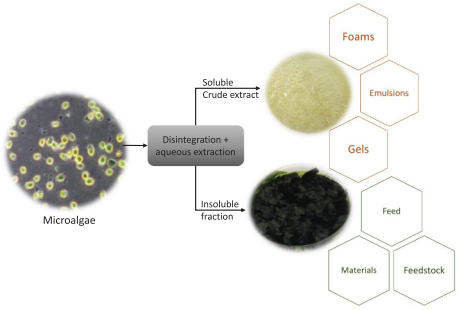
Selective and energy efficient extraction of functional proteins from microalgae for food applications

E. Suarez Garcia[a,*], J. van Leeuwen[b], C. Safi[b], L. Sijtsma[b], M.H.M. Eppink[a], R.H. Wijffels[a,c], C. van den Berg[a]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University and Research, PO Box 16, 6700 AA Wageningen, The Netherlands
[b] Wageningen Food & Biobased Research, Wageningen University and Research, PO Box 17, 6700 AA Wageningen, The Netherlands
[c] Nord University, Faculty of Biosciences and Aquaculture, N-8049 Bodø, Norway

GRAPHICAL ABSTRACT



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ABSTRACT

The use of a single controlled bead milling step of the microalga *Tetraselmis suecica* resulted in a soluble fraction, rich in functional proteins. This was achieved by fine-tuning the processing time, thereby exploiting the difference in rates of protein and carbohydrate release during milling. Soluble proteins were extracted under mild conditions -room temperature, no addition of chemicals, pH 6.5-, with a yield of 22.5% and a specific energy consumption of 0.6 kWh kg_{DW}^{-1}, which is within the recommended minimum energy for an extraction step in a biorefinery process. The resulting protein extract contained 50.4% (DW) of proteins and 26.4% carbohydrates, showed light green color and displayed superior surface activity and gelation behavior compared to whey protein isolate. The proposed process is simple (only one bead milling step), scalable, and allows the mild extraction of functional proteins, making it interesting for industrial applications in the food industry.

1. Introduction

Microalgae have been considered a promising feedstock for the feed and food industries due to their rich composition (broad range of biomolecules of diverse chemical nature), superior areal productivities compared to traditional crops and no dependence on fresh water and arable land (Draaisma et al., 2013). However, the implementation of algae fractions as functional ingredients in food products remains largely unexplored.

The fractionation and purification of biomolecules –in particular proteins- from algae is not trivial. The first step, for most algae strains, involves cell disruption in order to release intracellular components

* Corresponding author.
E-mail address: edgar.suarezgarcia@wur.nl (E. Suarez Garcia).

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into the bulk medium. For this, several technologies have been successfully employed, including bead milling, ultrasonication, enzymatic hydrolysis among others (Günerken et al., 2015; Phong et al., 2018). After cell disintegration, the resulting suspension is subjected to several separation steps which can be grouped into precipitation, filtration, extraction or combinations of these processes. Precipitation methods take advantage of the solubility and isoelectric point of the target molecules in order to induce selective precipitation. The method is commonly referred as pH-shifting and involves a broad range of pH adjustment to maximize protein solubility followed by precipitation at the isoelectric point (Ba et al., 2016; Benelhadj et al., 2016; Cavonius et al., 2015; Gerde et al., 2013). Filtration methods make use of differences in the polarity and molecular size of the components in suspension to obtain fractions rich in the molecules of interest (Safi et al., 2017a,b, 2014a,b,c). More elaborated processes involving three-phase partitioning (Waghmare et al., 2016), extraction + precipitation + filtration (Chronakis, 2001; Ursu et al., 2014) or precipitation + dialysis + adsorption (Schwenzfeier et al., 2011) have also been investigated to purify proteins from algae. In these cases, higher purities are obtained at expenses of intricate and costly processing steps.

As indicated by Ruiz Gonzalez et al. (2016), algae products for the food market will be profitable within the next decade if further cost reductions in both cultivation and downstream processing are achieved. This could be attained by reducing the number of unit operations (process integration) while keeping the final products in a high-end market segment (functional ingredients) (Cuellar-Bermudez et al., 2015). In spite of the several studies dealing with protein fractionation and purification, little attention has been paid to simple processing and to product functionality for food applications. In previous research (Postma et al., 2016a,b) it was observed that the release of soluble proteins from microalgae already reaches a maximum at early stages of bead milling, and that the rates of protein release are significantly superior compared to the rates of carbohydrate release. It appears that controlling the residence time during bead milling allows the selective fractionation of proteins from carbohydrates.

The aim of this study was to demonstrate that with a simple process strategy (one unit operation, low energy consumption) it is possible to selectively concentrate proteins from green microalgae in a crude extract. Furthermore, the techno-functional properties (foaming, emulsification, gelation) of the resulting crude extract were determined and compared to the commercial standard whey protein isolate.

2. Material and methods

2.1. Algae cultivation, harvesting and fractionation

2.1.1. Cultivation

Tetraselmis suecica (UTEX LB2286, University of Texas Culture Collection of Algae, USA) was cultivated in 25 L flat panel photobioreactors (AlgaePARC, Wageningen - The Netherlands) located in a greenhouse. The cultures were supplied with artificial light, CO_2 and nutrients as described by Postma et al. (2016a,b). The biomass was harvested via continuous centrifugation (E10, Evodos, NL) at 80 Hz and concentrated to ~20% dry weight (dw). This suspension was stored at 4 °C in the dark for up to 7 days until disruption experiments, in order to limit the extent of biomass decay due to bacterial growth.

2.1.2. Fractionation

A fresh algal suspension (biomass) is fed to a bead milling where both cell disintegration and aqueous extraction are taking place. The bead milled suspension is centrifuged (14000 rpm, 30 min, 20 °C) and the resulting fractions regarded as soluble crude extract and insoluble fraction.

2.2. Algae disruption

2.2.1. Bead milling

Algae suspensions containing about 100 $g_{DW}\,L^{-1}$ were prepared in distilled water and used as feed for the disruption experiments. Disruption was conducted in a horizontal 0.075 L bead mill (Dyno-Mill Research Lab, Willy A. Bachofen AF Maschinenfabrik, Switzerland) containing 0.4 mm Y_2O_3 stabilized ZrO_2 beads at 65% filling percentage. The system was operated in batch recirculation mode, with a constant agitation speed of 2039 rpm. The temperature of the suspension was controlled at ~25 °C with an external cooler (FP40-HE, Julabo® GmbH).

2.2.2. Sample collection

Samples from the feed chamber were collected at different time intervals and used for the estimation of the disintegration rates and for the quantification of component release. For the latter, the samples were centrifuged and the supernatants and pellets analyzed separately.

2.3. Analytical methods

2.3.1. Cell disintegration

Cell disintegration was quantified in a flow cytometer (BD Accuri C6®). In this technique, forward scattering data was used to estimate the number of intact cells remaining at every time step compared to the initial amount of intact cells (Postma et al., 2016a,b).

2.3.2. Biomass characterization

Dry weight and total ash were estimated gravimetrically after drying in an oven at 100 °C for 24 h and burning in a furnace at 575 °C respectively. Proteins were measured with the method of Lowry (Lowry et al., 1951), total carbohydrates with the method of Dubois (Dubois et al., 1956) and total lipids with the method of Folch (Folch et al., 1957). Starch content was estimated with a commercial kit (Total Starch, Megazyme® International, Ireland).

2.3.3. 2.3.3 Pigment release

The release of pigments was determined by measuring the UV-spectra of supernatants at several times using a UV–Vis spectrophotometer DR 6000 (Hatch Lange, The Netherlands). The wavelengths 430 nm, 450 nm and 660 nm were selected as representative for total pigments (Chlorophyll *a* and *b*).

2.3.4. Mass yields

Mass yields per component (Y_i) were estimated according to:

$$\mathbf{Y_i}\% = \frac{\boldsymbol{m_{i,j}}}{\boldsymbol{m_{i,b}}} \times 100 \quad (1)$$

where m_i is the mass of component i (protein, carbohydrates, etc.). Subscripts j and b refer to each fraction evaluated (supernatant, pellet) and initial biomass, respectively.

2.3.5. Acrylamide native gel electrophoresis

Protein samples were diluted with native buffer (Biorad) at a ratio 1:0.8 v/v. 25 µL of the resulting solution was loaded per lane in a 4–20% Criterion TGX gel (Biorad). Electrophoresis was run at 125 V for 75 min using Tris-Glycine (Biorad) as running buffer. Gels were stained overnight with Bio-Safe Coomassie blue (Biorad).

2.4. Techno functional properties

Prior to the evaluation of the techno-functional properties, samples were freeze-dried during 24 h in a Sublimator 2 × 3 × 3, Zirbus Technology® GmbH, and stored at 4 °C in sealed bags. Unless otherwise noticed, all experiments were conducted at room temperature (~23 °C); all runs were performed in duplicate.

2.4.1. Whey Protein Isolate (WPI)

Whey protein isolate (BiPRO, Davisco Foods international) with a purity of 97.6% was used as commercial reference protein.

2.4.2. Surfactant activity

Surface activity of samples containing alga proteins was determined via static drop experiments in an Automated Drop Tensiometer (ADT Tracker®, Teclis Scientific, France). With this technique, the surface tension of individual drops created automatically is monitored over time. Two distinct setups were used to assess foaming and emulsification activity.

To investigate foaming activity, algae samples were dissolved in MilliQ® water to obtain solutions containing 0.1% (w/v) protein at pH 7. A single drop containing 11 µL of protein solution is formed and held hanging, while subjected to a stream of saturated air flowing vertically in a 5 ml cuvette. Emulsification studies were conducted on a 20 µL drop of hexadecane (Anhydrous, > 99%, Sigma Aldrich) submerged in 5 ml of a 0.1% (w/v) protein solution.

2.4.3. Gelation

Gelation tests were conducted according to Martin et al. (2014), using an Anton Paar MCR 302® (Modular Compact Rheometer). Solutions containing 10% protein (w/v) were prepared in MilliQ® water and adjusted to pH ~ 7. Gel strength was measured in terms of the storage modulus of the sample (G' [=] Pa), which represents its elastic behavior. G' was recorded along a heating–cooling profile in the range 25–95 °C, using a heating rate of 5 °C min^{-1}.

3. Results and discussion

3.1. Bead milling and component release

The kinetics of cell disintegration and component release during bead milling of microalgae was investigated by Postma et al. (2016a,b) at different bead sizes (0.3–1 mm). In the case of *T. suecica*, it was observed that the same kinetic rates were obtained regardless of the bead size. In addition, the rates of protein release were over 6 times higher than those of carbohydrate release. The authors argued that the slow release of sugars is due to the solubilization of saccharides from the cell wall and starch granules. To further confirm this reasoning, a complete analysis of component release during bead milling was conducted for a period of 3 h. The results for disintegration, proteins, carbohydrates and starch are presented in Fig. 1A. In accordance with our previous findings, proteins are quickly released into the bulk medium, reaching a plateau phase after ~10 min. Cell disintegration takes place at lower rates, but the disintegration profile flattens out after ~30 min. This can be explained considering a two-steps disintegration process: Cell bursting, where the intracellular content is released without tearing the cells, and comminution, where the cells are fragmented over time into smaller debris. The method used to quantify intact cells (Section 2.3) is based on particle size and thus, cells are counted as intact even if the cell wall has received damage without fragmentation.

The rates of carbohydrate release (i.e., total sugars in the soluble phase) and the fraction of starch loss (i.e., total starch in the insoluble phase) were also quantified (Fig. 1A). In both cases, the experimental data follow a first-order model ($r^2 > 0.96$) with corresponding kinetic constants of $5.9 \times 10^{-3} \pm 2.7 \times 10^{-4}\,s^{-1}$ and $3.6 \times 10^{-3} \pm 1.6 \times 10^{-4}\,s^{-1}$ for carbohydrates and starch respectively. This suggests that the enrichment of sugars in the soluble phase can be explained in part by the loss of starch from the insoluble phase. In fact, starch dextrinization, which is the process of partial depolymerization, can occur under intense shear conditions (Sarifudin and Assiry, 2014) as occurring in the bead mill. Additional tests were conducted in order to rule out the possibility of starch degradation due to intracellular enzymes (data not shown). In line with the kinetic data published for *T. suecica*, our observations confirmed that ~8 min processing time is sufficient to release nearly 95% of the maximum achievable amount of proteins in the soluble phase. Although not confirmed experimentally, it is expected that a shorter bead milling time leads to larger fragments of cell debris which are less stable in the soluble phase and thus, their removal becomes easier.

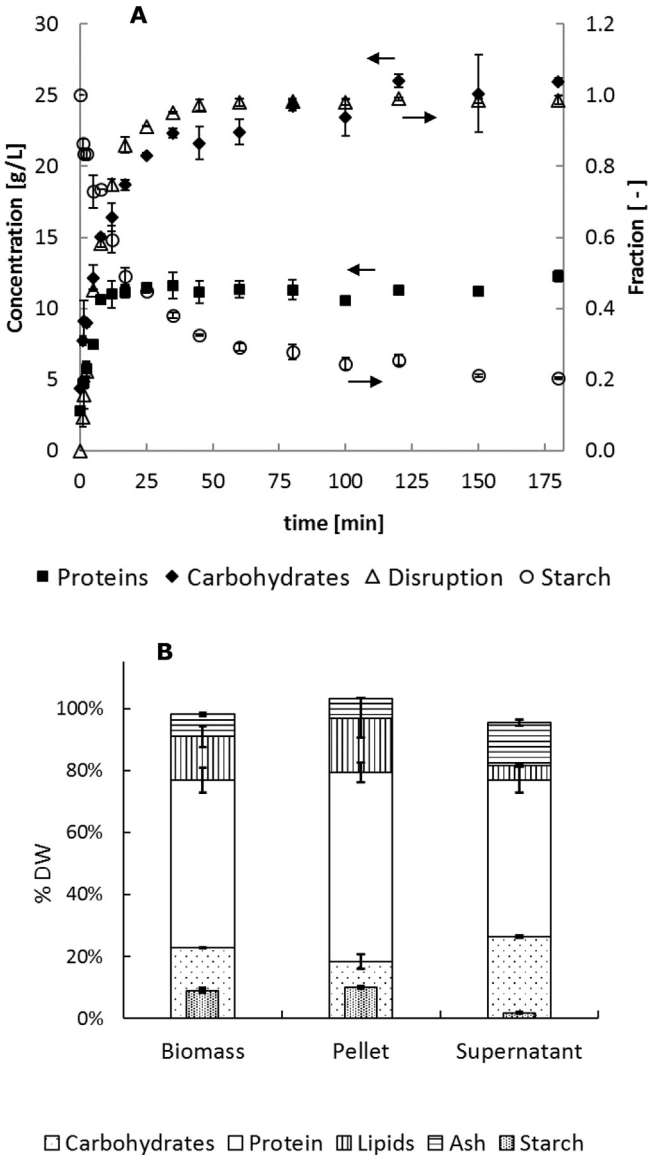

Fig. 1. A. Overview of cell disruption (fraction of disintegrated cells), protein and carbohydrate concentration (in the soluble phase), and starch content (fraction remaining in the insoluble phase compared to initial biomass content) during 3 h bead milling of *T. suecica*. Arrows point to the corresponding axis for each curve. **B.** dry weight compositions of fractions after 8 min bead milling and centrifugation.

In Fig. 1B, the corresponding compositions of the insoluble (pellet) and soluble fractions (supernatant) after bead milling are presented. The protein content in the biomass is 54.0 ± 4.0%, significantly higher compared with ~30–43% (dw) published for the same strain (Postma et al., 2016a,b) and ~35% (dw) for *Tetraselmis sp.* (Schwenzfeier et al., 2011). This can be due to variations in light supply as the alga cultures were kept in a greenhouse. Michels et al. (2014) reported values of protein content fluctuating from 35 to 55% (dw) for *T. suecica* in the period from February to March, for cultures maintained in greenhouses.

As displayed in Fig. 1B, the content of proteins and carbohydrates changes marginally from biomass to pellet and supernatant. The pellet phase, nonetheless, is richer in lipids (17.6% ± 6.5%) and contains a

small fraction of ash (6.3 ± 0.1%), making it an interesting feedstock for feed formulations. Although whole microalgae cells have long been used as feed in aquaculture (Shields and Lupatsch, 2012), the pellet phase represents a more attractive alternative for various reasons. First, its high protein and lipid content and the low amount of ash resembling typical diets in fish trials (Sorensen et al., 2016). Second, *T. suecica* has been reported to contain polyunsaturated fatty acids, in particular Eicosapentanoic acid (EPA) (Abiusi et al., 2014), which is a crucial ingredient to develop feed formulations devoid of fish meals. Moreover, since the pellet phase contains mostly cell debris, it is expected that nutrient accessibility is superior, leading to enhanced digestibility (Teuling et al., 2017). Other applications of the insoluble fraction include bio-based materials or feedstock for other industries.

3.2. Protein yields and energy consumption

The kinetics of protein and carbohydrate release for *T. suecica* during bead milling (Fig. 1A) was exploited in order to limit the enrichment of carbohydrates and pigments in the soluble phase. After short bead milling (8 min), the ratio of proteins to carbohydrates was 3.31 g g^{-1}, significantly higher compared to 0.45 g g^{-1} when disintegration is run for 3 h (Fig. 1). Also, the amount of released pigments is reduced. This was confirmed by visual inspection of the samples and by determining the amount of pigment released in the soluble phase (Fig. 2A). Pigments migrate to the soluble phase at slower rates compared to the rates of disintegration and protein release (Fig. 1A) as also observed by Postma et al. (2015). This is expected as pigments are usually located in the chloroplast as part of the light harvesting mechanism of the cells (Knoetzel et al. 1988) and therefore not freely available upon cell disintegration. We have found that the kinetics of pigment release does not clearly follow a first-order model ($r^2 > 0.85$). Fig. 2A shows that beyond 120 min bead milling time, the amount of released pigments follows a linear trend, implying a two-steps mechanism. An overall kinetic constant of $8.1 \times 10^{-4} \pm 3.9 \times 10^{-4}$ s^{-1} was estimated as representative for all pigments. This value is up to 2 orders of magnitude inferior to the kinetic constants reported by Postma et al. (2015) for pigments from the microalgae *C. vulgaris*, which can be the result of physiological differences among the two algal species.

The corresponding mass yields in the supernatant fraction are 22.5% for proteins, 27.7% for carbohydrates, 8.1% for lipids and 45.5% for ash. The bead milling process can be considered as an aqueous extraction process and thus, the largest fraction of lipids remains in the pellet phase due to their hydrophobic nature and density. The small amount of total lipids in the soluble phase corresponds to phospholipids, prosthetic groups (e.g., porphyrins) (Schwenzfeier et al., 2013), lipoproteins and pigments. Similarly, the largest fractions of carbohydrates and proteins remain in the insoluble phase. Starch, which accounts for almost 40% of the total carbohydrates, is insoluble at room temperature. The rest of the carbohydrates in the pellet phase correspond to cell wall fragments (Becker et al., 1998) and other insoluble cell debris.

Regarding proteins, previous studies have found yields in the soluble phase of 21–24% for *Tetraselmis* species after mechanical disintegration (Postma et al., 2016a,b; Schwenzfeier et al., 2011). Such low yields can be explained considering the characteristics of the extraction process (aqueous buffer, native pH ~ 6.5, room temperate) and the nature of the proteins in green microalgae. We hypothesize that for *T. suecica* proteins are present in pools (Fig. 2B). A first pool (Aqueous) corresponds to proteins that exist in the cytosol and in internal organelles like the pyrenoid (González et al., 2015). Upon cell rupture, those proteins migrate rapidly to the bulk fluid due to a concentration gradient. A second pool (Structure) are proteins which have a more structural role, for instance, proteins in the cell wall and membrane, and in the flagella. The extraction of such proteins has been reported using detergents (Becker et al., 1998; Gödel et al., 2000). Another major pool (Function) corresponds to proteins which are present in the chloroplast and are involved in the light-harvesting system of the cells. Such proteins are non-covalently bound to an intricate assembly of pigments and have been shown to be soluble in detergents (Knoetzel et al., 1988). It becomes evident that the proteins present in the pellet phase (72% of the total) could only be extracted under detergent conditions, or using methods in which the insoluble structures, in which proteins are entangled, are solubilized. For the present case, mechanical shear caused by bead milling is insufficient to induce such solubilization.

Several extraction methods have been proposed in the literature for the extraction of soluble proteins from microalgae. Those methods can be divided into physical and chemical. Physical methods involve mechanical shear (bead milling, high-pressure homogenization, ultrasonication, explosive decompression, microfluidization), electric fields and thermal treatments (thermal shock, microwaves). Chemical methods include solvents, ionic liquids, pH shifts and enzymatic hydrolysis (Günerken et al., 2015; Phong et al., 2018). The extent of cell damage and consequently the resulting protein yields depend greatly on the algal strain and on the amount of energy that can be effectively transferred to the cells. In Fig. 3 several physical processes reported for the extraction of proteins from microalgae are compared in terms of yield and specific energy consumption (*Em* [kWh kg_{DW}^{-1}]). Two reference

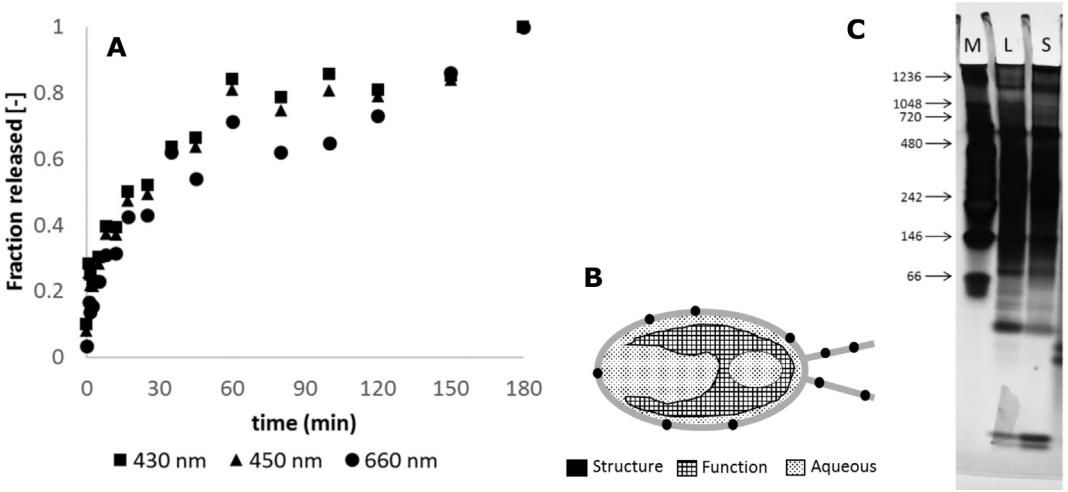

Fig. 2. A. Absorbance of soluble phases during bead milling. **B.** Schematic representation of hypothetical protein pools in *T. suecica.* **C.** Native gel electrophoresis (M, marker) of samples after long (L, 3 h) and short (S, 8 min) bead milling.

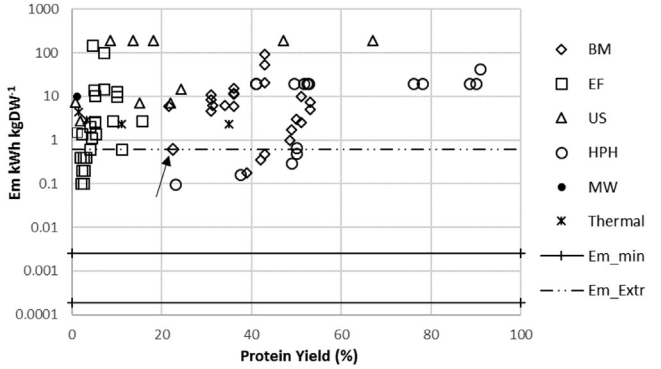

Fig. 3. Overview of specific energy consumption (***Em*** [kWh kg_{DW}^{-1}]) for algal disintegration and corresponding protein yields. **BM:** Bead milling (arrow: present study, (Postma et al., 2016a, 2015; Safi et al., 2017a)), **EF:** Electric Fields ('t Lam et al., 2017a,b; Grimi et al., 2014; Parniakov et al., 2015; Postma et al., 2016a; Safi et al., 2017a), **US:** Sonication (Grimi et al., 2014; Parniakov et al., 2015; Passos et al., 2015; Safi et al., 2014c; Wenjuan et al., 2013), **HPH:** High Pressure Homogenization (Grimi et al., 2014; Safi et al., 2017a, 2014a,c), **MW:** Microwaves (Passos et al., 2015), **Thermal** processing (Jazrawi et al., 2015; Passos et al., 2015), ***Em_{min}*:** Minimum specific energy for cell rupture (Günther et al., 2016; Lee et al., 2013), ***Em_{Ext}*:** Target maximum specific energy for extraction (Illman et al., 2000).

levels are indicated: the minimum experimental specific energy (Em_{min}) required to break a cell of *T. suecica* (Lee et al., 2013) and *C. vulgaris* (Günther et al., 2016) which varies from 1.9×10^{-4} to 2.5×10^{-3} kWh kg_{DW}^{-1} depending on the strength of the cell wall and on the osmolality, and the maximum specific energy (Em_{ext}) recommended for the extraction step within a biorefinery, which is estimated at 0.6 kWh kg_{DW}^{-1} and equivalent to ~10% of the energy content in microalgae (Illman et al., 2000). It is remarkable that all the reported methods require at least 100 times more energy than Em_{min}. This is due to energy losses and dissipation to the bulk media, in other words, energy that is not effectively applied to the cells. From Fig. 3, only mechanical methods namely bead milling and high-pressure homogenization resulted in acceptable yields (> 20%) and energy consumptions below the target Em_{ext}. In the present study, we measured a protein yield of 22.5% and an E_m of 0.607 ± 0.002 kWh kg_{DW}^{-1}. Safi et al. (2017a) reported yields from 23 to 51% and corresponding energy consumptions of 0.1–0.6 kWh kg_{DW}^{-1} for the microalgae *Nannochloropsis gaditana* using bead milling and high-pressure homogenization. Such high yields reflect structural differences between the two algae strains, since both works implemented comparable operation conditions for the case of the bead milling (BM) process.

In general, the application of electric fields leads to low yields even at high energy consumptions. This is due to the presence of a cell wall, which remains practically unaffected by electric fields ('t Lam et al., 2017a,b). Parniakov et al. (2015) reported protein yields of nearly 15%, but the process involved pulsed electric fields at pH 8.5, thus a synergistic effect is likely. Ultrasound, which mechanism of action on the cells involves shear caused by cavitation, can lead to appreciable protein yield at expenses of a high energy consumption. This is the case of the work of Safi et al. (2014c) who reported protein yields ranging from 8 to 67% for several algae strains under the same processing with a consequent specific energy consumption of *Em* ~ 187 kWh kg_{DW}^{-1}. Worth noticing, several works in which thermal treatments were implemented reported energy consumptions of the order of 3 kWh kg_{DW}^{-1} with protein yields ranging from 1.5 to 35%. It is not surprising to obtain low yields of soluble proteins under thermal processing, due to denaturation and consequently thermal coagulation. However, a 35% yield reported by Jazrawi et al. (2015) after processing *Chlorella vulgaris* at 200 °C suggests instead a high degree of protein denaturation via hydrolysis, rendering the extract phase rich in small protein fragments. The effect of the extraction process on the protein conformation is rarely studied. As shown in Fig. 2C, all the protein bands expected for *T. suecica* (Postma et al., 2016a,b) are preserved, confirming the mildness of the present extraction process.

3.3. Techno-functional properties

Despite its importance, the techno-functionality of algae proteins is often overlooked. Most studies focus on presenting yields or extraction efficiencies, without addressing potential applications beyond a mere amino acid profile analysis. The issue of purity is also frequently neglected. In general, for a given separation process, purity and yield are inversely related. In other words, higher yields can be achieved at expenses of low purities. Cavonius et al. (2015) and Ba et al. (2016) reported a process of high-pressure homogenization and pH-shifting which resulted in protein yields of 68–84% with purities of 26–44% (DW). On the contrary, high purities can be obtained by implementing several unit operations, but having as consequence low yields. This is the case of the process presented by Schwenzfeier et al. (2011), in which bead milling, adsorption, precipitation and dialysis yield 7% of the total protein in the soluble phase with a purity of over 64% (DW). Similarly, two processes leading to protein purities above 78% (Chronakis, 2001; Waghmare et al., 2016) are complex, requiring multiple and expensive unit operations, thereby unsuitable for large -scale applications. It is also clear that pH-shifting is the most common method to extract proteins (Benelhadj et al., 2016; Gerde et al., 2013; Ursu et al., 2014). However, this requires the addition of chemicals, which is economically undesirable and can lead to protein denaturation. The present research involves a single unit operation and leads to a protein yield of 22.5% and a purity of 50.6% (DW).

The functional activity of the crude protein extracted after short-term bead milling was further investigated in terms of surface activity and gelation behavior. Samples were compared with whey protein isolate. Dynamic surface activity for air-water interfaces (foaming) and oil-water interfaces (emulsification) showed the expected tendencies (Fig. 4A): a sharp decline in surface tension followed by a slow decrease to reach an equilibrium value. This behavior reveals three basic mechanisms namely diffusion towards the surface, adsorption and molecular reorientation (Serrien et al., 1992). Hence, superior surface activity is reflected in a curve with a higher slope and a lower value of surface tension at equilibrium. From Fig. 4A it is clear that the crude protein fraction presented a higher surface activity than whey protein isolate (WPI) for foaming and emulsification. This superior performance can be due to the presence of charged carbohydrates and glycoproteins as hypothesized by Schwenzfeier et al. (2014). In addition, the presence of lipids (8% by weight) in the crude extract could have contributed to the development of more stable interactions around the surfaces (Gerde et al., 2013).

The gelation behavior was studied by measuring the storage modulus (G' [Pa]) during a defined heating-cooling profile (Fig. 4B). During the heating phase (25–90 °C) proteins unfold, exposing their functional groups. This makes possible the formation of covalent bonds with neighboring molecules, which result in the development of a film-like structure. During the cooling phase (90–25 °C) the gel further hardens due to the formation of non-covalent interactions such as hydrogen bonds and hydrophobic interactions (Martin et al., 2014). WPI did not show gel-like behavior at 10% protein content. WPI is composed of small globular proteins and thus, steric repulsion may prevent the formation of a stable network during the heating-cooling treatment. On the contrary, the algae extract contains proteins of a broad range of sizes (Fig. 2C) in addition to sugars, lipids and ash, which may be contributing not only to forming new bonds during the heating phase but to enhancing the rigidity of the gel network. Chronakis (2001) suggested that hydrophobic interactions are mainly responsible for molecular association and aggregation. It was also argued that, for the case of proteins extracted from cyanobacteria, gelation properties are controlled from protein complexes rather than from individual proteins.

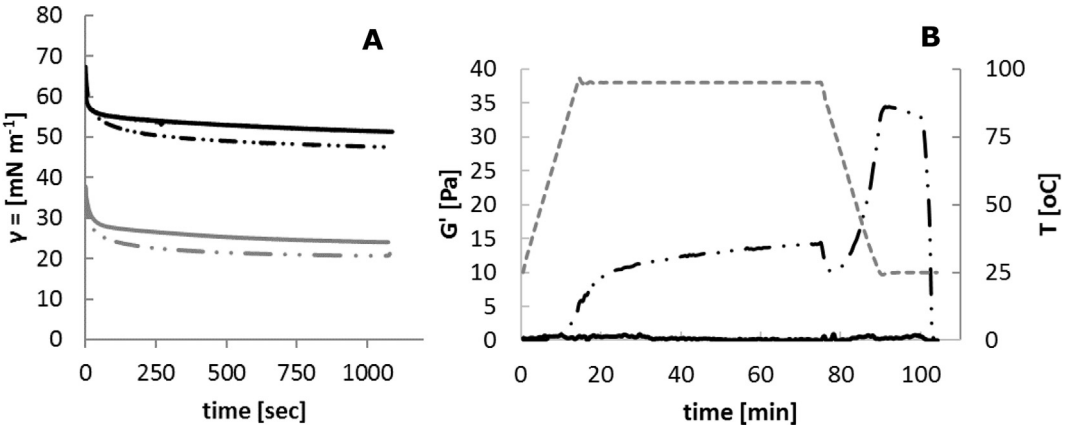

Fig 4. A. Surface activity for algae samples ·· ─ and Whey Protein Isolate WPI (–) for foaming (black) and emulsification (grey). **B.** Gelation of alga samples and corresponding temperature ─ ─.

4. Conclusions

In this study it was shown that the soluble extract from green microalgae can be significantly enriched with proteins by performing cell disintegration –via a single bead milling step- for short times. This also ensures low specific energy consumptions, well below other disintegration methods like ultrasonication and electric fields. The resulting crude protein extract displayed excellent surface activity and gelation behavior, superior to whey protein isolate. The proposed process is easily scalable, does not require the addition of chemicals or expensive unit operations and lead to a product with potential application as functional ingredients in foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biortech.2018.07.131.

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