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

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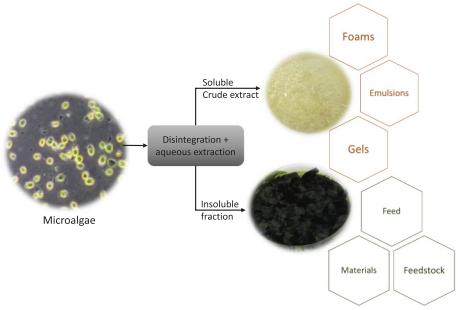
GRAPHICAL ABSTRACT



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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

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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.