

Protein Extraction from *Arthrospira platensis* for Use in Food Processing [†]

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Abstract: Algae protein has emerged as a sustainable and non-allergenic alternative to animal protein as the market seeks to reduce reliance on traditional animal protein sources. To effectively utilize algae protein isolates, particularly from *Arthrospira platensis*, it is essential to develop an efficient method for protein extraction and isolation that can be scaled up. This work aims to optimize the extraction conditions to obtain high-purity protein extracts. HPLC-DAD was used to determine the total and free amino acid profiles, while SDS-PAGE and HPLC-MS/MS were used for the protein characterization. An optimized extraction method was selected based on achieving the highest protein content and purity.

Keywords: food industry; food processing; protein extraction; non-animal protein; non-allergic protein; algae; *Arthrospira platensis*; sustainability



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1. Introduction

Arthrospira platensis, a cyanobacterium, is gaining recognition as a sustainable and environmentally friendly protein source [1–3]. With a protein content ranging from 60% to 70% of its dry weight and the presence of phycobiliproteins, it holds great promise for various applications [4]. As a non-allergenic and non-animal protein source, it offers a viable alternative to conventional proteins in several industries [5,6]. However, to realize its full potential, the development of efficient protein extraction and purification methods is essential [7,8]. Therefore, this study aimed to evaluate different protein extraction methods - such as agitation, bead milling, and ultrasound - and protein isolation via precipitation using ethanol or ammonium sulfate to optimize the protein yield and purity.

2. Materials and Methods

2.1. Sample and Protein Extraction

The *Arthrospira platensis* powder, with a protein content of 63–67% as determined using the Kjeldahl method, was obtained from a local producer (Allmicroalgae—Natural Products S.A., Portugal). However, HPLC analysis showed a total protein content of $50.79\% \pm 4.22$. Figure 1 provides an overview of the protein extraction methods used in this study. These methods include agitation, bead milling, and ultrasound, each applied under different conditions in terms of the incubation time, pH levels, and specific chemical solutions. The extracted proteins were subsequently isolated via precipitation using ethanol

or ammonium sulfate. Dialysis (MW cut-off 12–14 kDa) was the final purification step. In total, 45 different extraction conditions were tested.

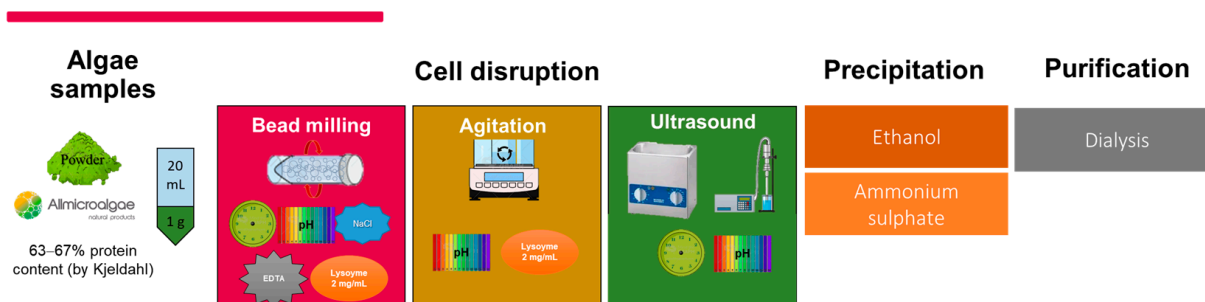


Figure 1. Representation of the various protein extraction conditions tested.

2.2. Amino Acid Quantification

The amino acids were quantified via HPLC-DAD using a C18 column (Acclaim™ 120, 4.6 × 250 mm, particle size 5 µm) on a Vanquish system (Thermo Fisher Scientific, Waltham, MA, USA). The procedure included derivatization with *o*-phthaldialdehyde in borate buffer, 2-mercaptoethanol (OPA-2MCE), and 9-fluorenylmethylchloroformate (FMOC), based on the method described by Herbert et al. [9] with some modifications. To analyze the total amino acids, 10 mg of algal biomass was hydrolyzed with 6 M HCl (1 mL) at 110 °C for 24 h. Further, 200 µL of β-alanine at 2.5 mg/mL was used as the internal standard, and an 8-point standard curve for the L-amino acids was constructed. The mobile phases consisted of eluent A, ultrapure water (100%); eluent B, methanol (MeOH) (100%); eluent C, sodium acetate buffer (0.36 M, pH 8); and eluent D, acetonitrile (100%). Proline and hydroxyproline were detected at 262 nm, while the other amino acids were detected at 337 nm.

2.3. Protein Characterization

The algae proteins were resuspended in a sample buffer comprising 2% (w/v) SDS, 40% (v/v) glycerol, 0.02% (w/v) bromophenol blue, 0.08 M Tris-HCl at a pH of 8.0, and 10% (v/v) Bolt Sample Reducing Agent (Thermo Fisher Scientific). This mixture was then heated at 65 °C for 30 min and separated on precast Bolt Bis-Tris Plus gels (4–12% gradient polyacrylamide concentration) using MES SDS Running Buffer (Thermo Fisher Scientific). Coomassie-stained protein bands were excised and subjected to in-gel trypsin digestion. The resulting peptides were analyzed via LC-MS/MS using an LTQ XL Linear Ion Trap Mass Spectrometer from Thermo Fisher Scientific [10].

2.4. Statistical Analysis

One-way ANOVA with a Scheffé post hoc test was employed to determine significant differences in the total amino acid profile among the extraction methods.

3. Results

3.1. Optimization of the Protein Extraction Method

The results show that out of the 45 conditions tested, the most successful protein extraction method was method 3. This method involved bead milling for 24 h, the addition of 1 M NaCl, and pH adjustment to 7, followed by precipitation with 75% ethanol, as shown in Figure 2. This method yielded a significantly higher protein content of $58.19\% \pm 6.23$, with an extraction yield of 23.66%. These results provide compelling evidence of the economic viability and suitability of the selected method for large-scale industrial implementation.

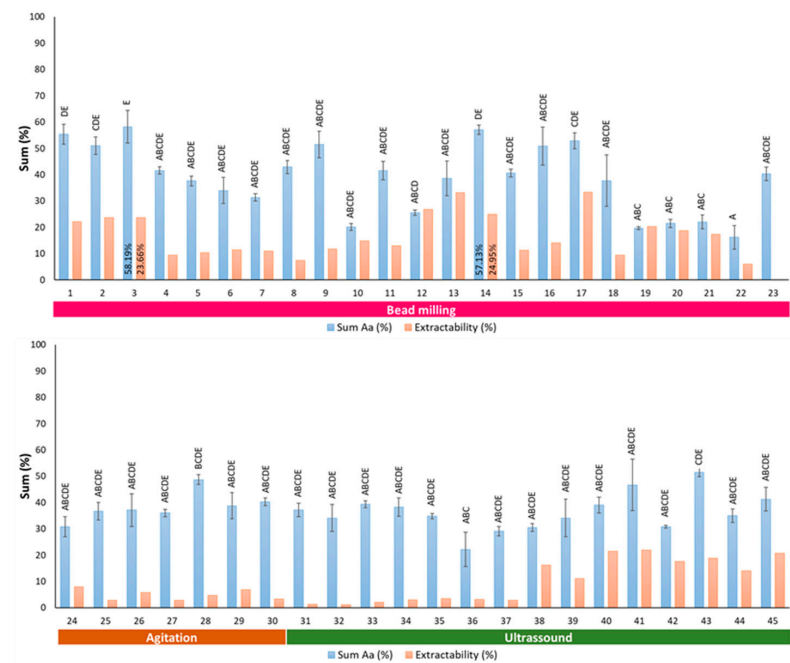


Figure 2. Total amino acid content and extractability were obtained using the diverse methods tested. For each sum of amino acids (%), bars with the same letter are not significantly different (Scheffé test, $p < 0.05$).

3.2. Quantification of Amino Acid in Extracted Proteins Using the Optimized Method

Method 3 exhibited significantly higher levels of aspartic acid (5.8 ± 0.2), glutamic acid (4.8 ± 0.3), and proline (15.0 ± 4.4). However, when the same procedure was used with a shorter extraction time (20 h, method 14), proteins with significantly higher leucine (7.9 ± 0.1), phenylalanine (7.8 ± 0.1), and isoleucine (5.1 ± 0.1) levels were obtained. This highlights the importance of carefully optimizing protein extraction protocols to achieve the desired amino acid profile. For example, proline is a critical amino acid found in conventional protein sources, such as wine-finishing agents. Therefore, the selection of the optimized method is based on the highest total protein and proline content.

3.3. Protein Characterization

The SDS-PAGE profile showed a more concentrated and representative band in the 14–28 kDa range, consistent with the expected presence of phycobiliproteins (Figure 3a). For protein identification, a database search was performed using the OMSSA search algorithm (Figure 3b). MS/MS spectra were searched against a database containing 118,562 *Arthrospira* sequences retrieved from UniProt (<https://www.uniprot.org/> accessed on 7 November 2023).

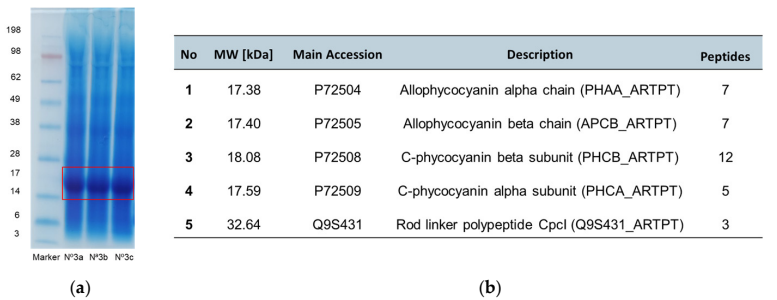


Figure 3. (a) SDS-PAGE profile of the protein extract from method 3 (in triplicate). Sizes (in kilodaltons) of protein molecular weight markers are shown on the left (Precision Plus Protein All Blue Prestained Protein Standards, Bio-Rad, CA, USA); (b) peptide mass fingerprinting results of the most representative bands (highlighted with a red rectangle in (a)).

4. Conclusions

In this study, we have developed an efficient method for extracting and isolating proteins from *Arthrospira platensis*. The extraction method involved 24 h of bead milling at a pH of 7 with 1 M NaCl, followed by 75% ethanol precipitation and subsequent dialysis, resulting in a total amino acid content of 58.13%. Proline, aspartic acid, glutamic acid, and leucine were identified as the predominant amino acids in the extracted protein. Furthermore, SDS-PAGE analysis showed a prominent protein band in the 14–28 kDa range, confirming the presence of phycobiliproteins associated with *Arthrospira platensis*. Additionally, mass spectrometry enabled the identification and validation of five different proteins in the protein extract. These results highlight the potential of *Arthrospira platensis* as a protein source.

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