ENZYMATIC CELL DISRUPTION OF THE MICROALGAE *CHLORELLA SOROKINIANA*

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INTRODUCTION

Microalgae proteins as natural, plant-based, sustainable, alternative food sources and their components promise a bright future for their use as food and nutraceuticals. Microalgae, especially *Chlorella* species, are particularly promising due to their high protein content that can be found in cells, making them an attractive research target. In addition, although the use of microalgae biomass in food applications is a more economical and sustainable strategy, it is an important problem that the complex rigid cell wall structure of microalgae interferes with the accessibility of proteins. In this context, enzymatic lysis is an environmentally friendly and low-energy alternative degradation technique that is usually performed under mild conditions due to the ability of some enzymes to specifically degrade structural cell wall components (Phong et al. 2018, De Souza et al.2020).

Thus, it supports the recovery of intracellular proteins without being destroyed. The objectives of this study were: 1) to investigate and compare the effect of different pretreatments before disrupting the cell wall of *Chlorella sorokinina*; 2) to disrupt the cell wall structure by using different enzymes in the biomass 3) to compare the efficiency of the enzymes used in protein recovery rates 4) to define the protein fractions obtained after enzymatic degradation.

MATERIAL AND METHODS

This study was carried out in Ege University Food Engineering Nutrition Sciences Laboratory in Izmir, Türkiye. The production of *Chlorella sorokiniana* biomass grown under heterotrophic cultivation conditions was carried out by Marin Biotechnology Products and Food Company (Aydın, Turkey). The pure culture of SOROKIN-MYERS/TX 71105, Culture Collection of Autotrophic Organisms in Academy of Sciences, Trebon, Czech Republic was used. The enzymes (Alcalase, Protamex, and Celluclast) used in the study were obtained from Novozymes (Bagsværd, Denmark).

Different pretreatments (no pretreatment, 12-hour mixing, freeze-thawing, 24000 rpm, 20 min ultraturrax application, 20 min,108-watt ultrasound application) were applied to microalgae suspensions (10 g biomass/100 mL ultrapure water). After that, for enzymatic lysis, Alcalase and/or Celluclast, Protamex and/or Alcalase enzymes at different concentrations (0.5, 1, 5%) at 50°C, 5 h were applied to samples. The enzyme reaction was terminated by heat treatment applied at 90ºC for 10 minutes. Moisture and protein contents were determined (AOAC 2007). A nitrogen-to-protein conversion factor ranging from 4.58 to 5.60 has been proposed in the literature for microalgae and microalgae extracts, depending on non-protein nitrogenous components such as glucosamine, inorganic-nitrogen, nucleotides, and free amino acids (Lourenço, et al. 2004, Schwenzfeier et al. 2011). For this reason, an average value of 5.0 was used as a nitrogen-protein conversion factor (Groosman et al. 2018). The protein amount of the

samples after enzymatic lysis was determined by the biquinchoninic acid (BCA) assay using the Pierce™ BCA Protein Assay (Thermo Fisher Scientific Inc., Massachusetts, USA). SDS-PAGE (Polyacrylamide Gel Electrophoresis) analyzes were also performed for the molecular distribution of hydrolyzed proteins. SDS-PAGE analysis was performed according to Laemmli (1970) in microalgae biomass, supernatants obtained after enzymatic lysis for the identification of protein fractions. 5% stacking gel and 12% separation gel were prepared.

RESULTS AND DISSCUSSION

Moisture and protein content of microalgae biomass were found as 7.30 ± 0.07(g/100 g and 36.50 ± 0.04, respectively. After different pretreatments applied to microalgae suspensions, centrifugation (9000 rpm, 60 min) was applied, and BCA analysis was performed on the supernatants. No difference was observed between ultraturrax application and control, and between freeze-thaw and ultrasound procedures ($p>0.05$). It was found that the 12-hour mixing process was more effective than the ultrasound and freeze-thaw process ($p<0.05$). In terms of protein release, mixing and mixing + freeze-thaw pretreatments gave similar results ($p>0.05$), and mixing + ultrasound pretreatment was found to be the most effective application ($p<0.05$). It was observed that mixing the biomass with water at room temperature was effective in dissolving the proteins in the cell wall and releasing the proteins from the biomass (Figure 1).

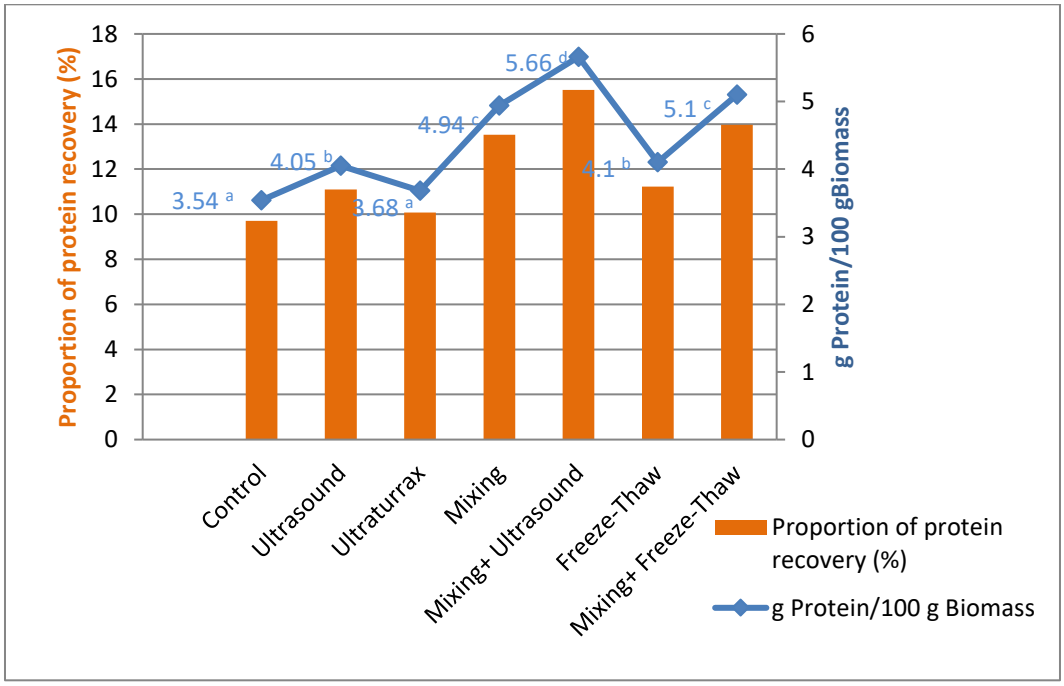

Figure 1. Protein content and protein recovery after pretreatments

Mixing and mixing+ultrasound were used as effective pretreatments in enzyme applications. The protein and protein amounts recovered after enzyme treatments are given in Figure 2. While there was no difference between the control and Celluclast (0.5% or 5%) application ($p>0.05$), other enzyme applications differed compared to the control ($p<0.05$). Cellulases are widely recommended enzymes for enzymatic lysis, as cellulose is thought to be one of the main components of the *Chlorella* cell wall. However, both the efficiency of this enzyme in breaking down the *Chlorella* cell wall and the existence of cellulose in this structure are controversial (de Souza vd., 2020). In our study, it was found that the Celluclast enzyme was not effective in breaking down the *C. sorokiniana* cell wall. Protamex enzyme was found to be less effective

compared to the Alcalase enzyme ($p<0.05$). The most effective protein release was obtained with the use of Alcalase (5% e/s) after 12 h of mixing in water (20.04 g protein/100 g biomass) and mixing + ultrasound pre-treatment (19.69 g protein/100 g biomass), with a proportion of protein recovery of 54.90% and 53.95% ($p<0.05$). It was found that the combined use of mixing + Alcalase (5% m/s) + Protamex (1% m/s) enzyme was less effective than mixing + Alcalase with 18.92 g protein/100 g biomass protein content and protein recovery (51.84%) ($p<0.05$).

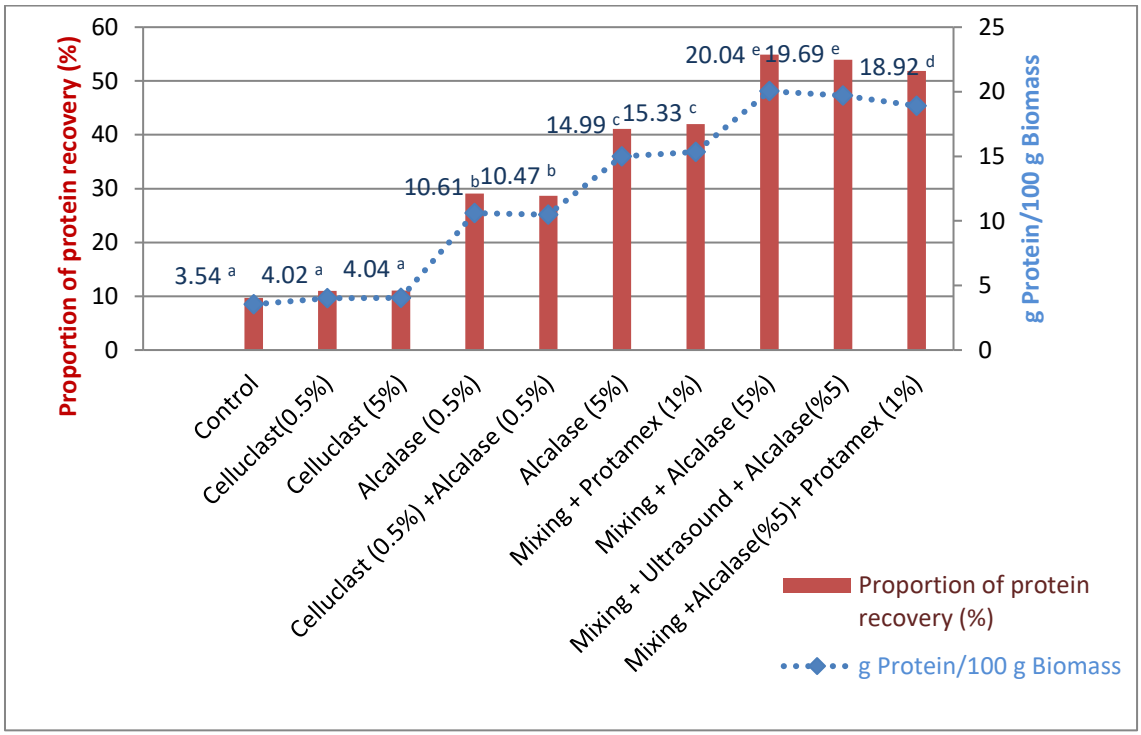

Figure 2. Protein content and protein recovery after enzyme applications

SDS PAGE analyzes were performed on microalgae biomass and samples treated with Alcalase, ultrasound+Alcalase, Protamex and Alkalase+Protamex after mixing for 12 hours (Figures 3 and 4). Considering Figure 3, the presence of water-soluble proteins in microalgae suspensions is observed (>15kDa). When SDS PAGE images are examined, the presence of peptide bands was observed by hydrolysis of proteins with the use of Alcalase, Protamex and Alcalase + Protamex enzymes (<15kDa). Enzymatic lysis with Alcalase and Alcalase + Protamex provided the highest peptide recovery from *Chlorella sorokiniana*, and peptide sizes were found to be less than 15 kDa. With the application of Protamex enzyme, lower peptide recoveries but larger peptide sizes were obtained. This is compatible with the literature (Rojo et al., 2021). In this context, enzymatic lysis is important as it allows valuable components to be obtained without degradation, in addition to being able to disrupt the cell wall in an alternative, environmentally friendly and low-energy way, usually under mild conditions. Further research should focus on improving protein recovery and digestibility without degradation of protein and peptide structures, as well as investigating the bioactivity of the resulting peptide structures.

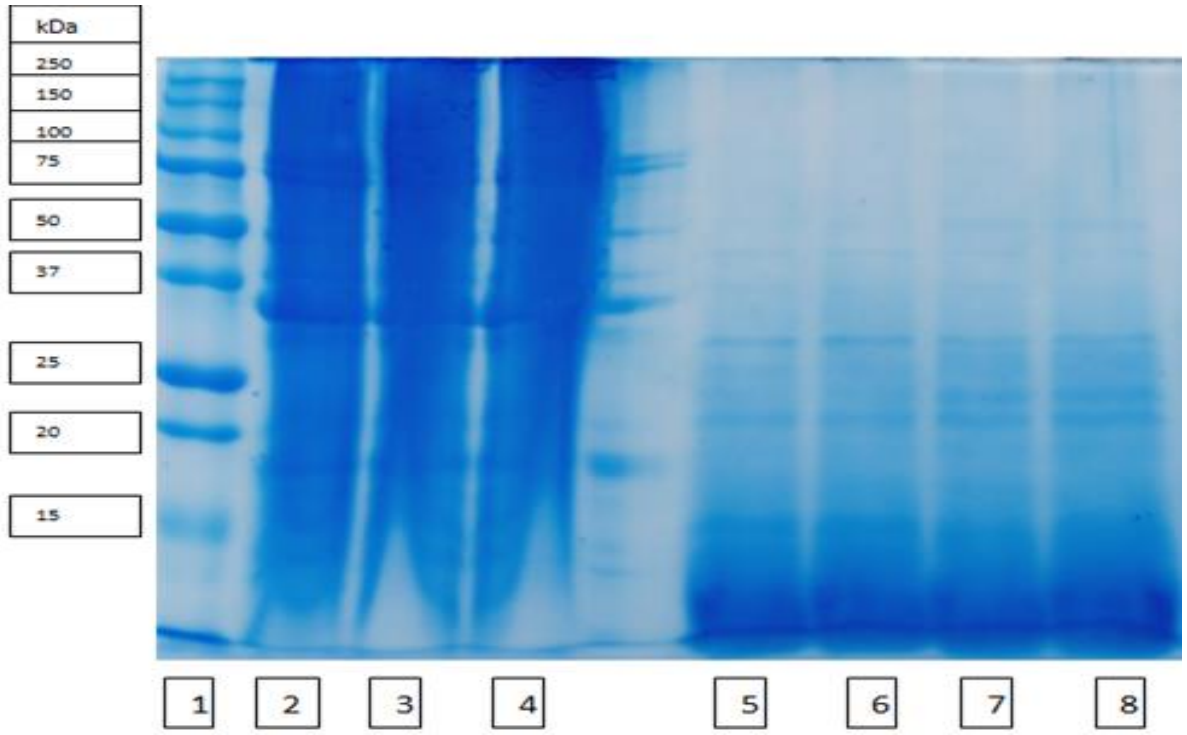

Figure 3. SDS PAGE 1: marker, 2, 3, 4:Microalgae biomass, 5, 6: Alcalase, 7, 8: Ultrasound +Alcalase

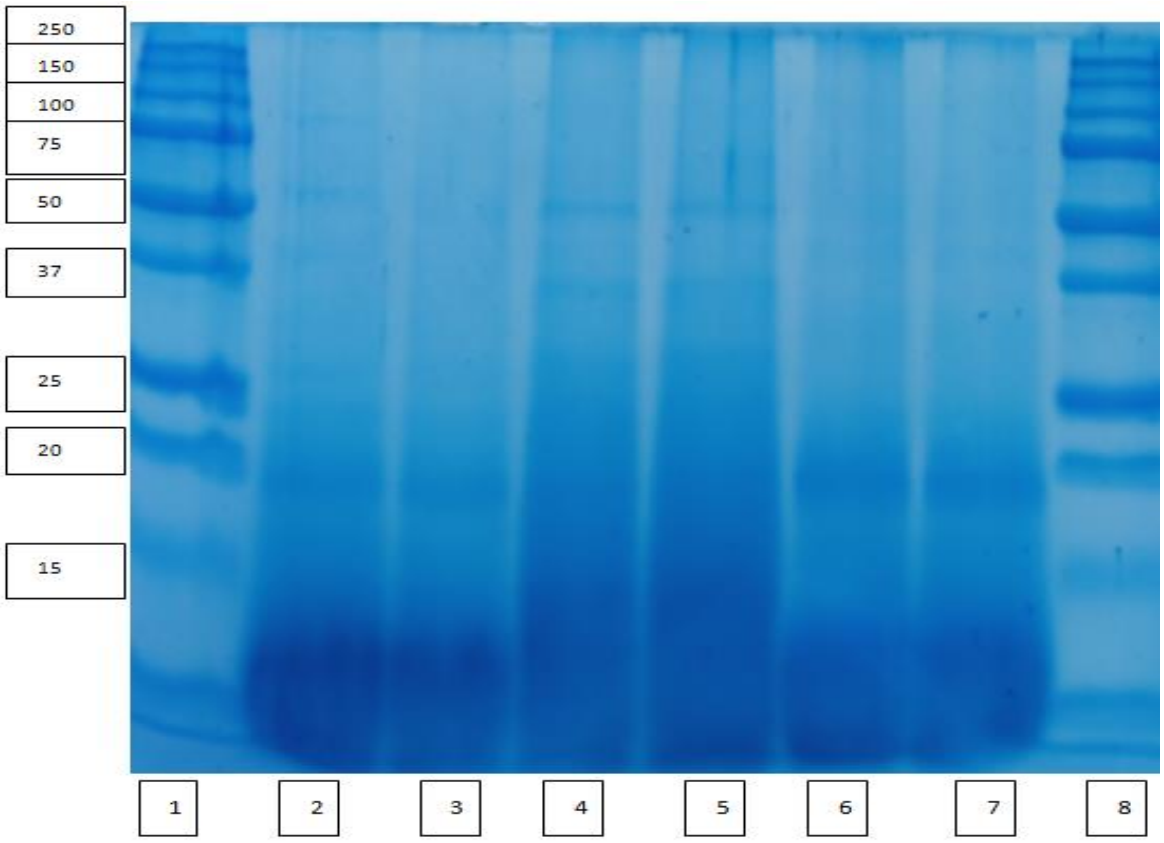

Figure 4. SDS PAGE 1, 8: marker, 2, 3:Alcalase, 4, 5: Protamex, 6,7: Alcalase+Protamex

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