MICROALGAE BIOMASS: CHARACTERIZATION AND PREBIOTIC POTENTIAL BY CELLULOSIC OLIGOSACCHARIDES (COS) QUANTIFICATION

Luana A. Tavares[1], Marcos F. Silva[1], Priscila. H. Carvalho[1], Rosana. Goldbeck Coelho[1]

[1]*School of Food Engineering (FEA), University of Campinas (UNICAMP),Campinas, SP, Brazil.*
** Corresponding author's email address: luana_atavares@hotmail.com*

ABSTRACT

Microalgae, such as *Chlorella vulgaris*, are recognized for their potential as sustainable sources due to their ability to sequester carbon and produce biologically active compounds. This study aimed to evaluate the characterization of *C. vulgaris* biomass, focusing on the contents of proteins, carbohydrates, lipids, the prebiotic potential of cellulosic oligosaccharides (COS) derived from enzymatic hydrolysis of the biomass. Carbohydrates include starch (amylose and amylopectin), found in chloroplasts, and cellulose, a cell wall component, which can be converted into COS, beneficial for human health. The biomass was cultivated in BG-11 medium under controlled conditions. Centesimal analysis showed significant levels of proteins, carbohydrates, and lipids. The enzymatic hydrolysis reaction was performed by applying a commercial cellulase (Celluclast ®-Novozymes®), with prebiotic fractions of Cellotriose as the major products, with a conversion yield of 16.9 mg g-1 microalgal biomass.representing 82.8% of the produced COS.

Keywords: *Chlorella vulgaris,* microalgae, cello-oligosaccharides. cultivation, enzymatic hydrolysis.

1 INTRODUCTION

The increasing concerns about food demand have driven the search for alternative and sustainable biomass sources, including those derived from algae [1; 2]. In accordance with the principles of "green chemistry", which prioritize the use of biodegradable resources and the reduction of toxic waste generation for the environment [3], contemporary industry seeks environmentally responsible alternatives to meet the growing demands of society. This approach is in line with the 2030 Agenda for Sustainable Development, which establishes 17 goals to promote global sustainability. In this context, microalgae have stood out as a promising source of biomass, due to their ability to produce bioactive and phytochemical compounds, giving them the status of "functional foods" or "nutraceuticals" [4].

Among the various species of microalgae, *C. vulgaris* emerges as a notable candidate, classified in the plant kingdom as a green algae [5]. Its richness in nutrients, such as proteins, carbohydrates, lipids, vitamins and other bioactive compounds, makes it a valuable raw material for several industrial and pharmaceutical applications [6]. The cytoplasm of *C. vulgaris*, an aqueous matrix, is rich in soluble proteins and minerals, harboring several organelles essential for its biological functions. The chloroplast, for example, plays a crucial role in photosynthesis, with its internal membranes facilitating the transport of proteins and the synthesis of the cell wall contents like amylose and amylopectin and cellulose [7]. These carbohydrates can be converted into COS, beneficial for human health. COS are oligomers formed by 2 to 6 glucose units, linked by β-1,4 bonds, with several potential applications in the food, feed and bioenergy industries. In addition, the characterization of microalgae, including *C. vulgaris*, is influenced by external factors, such as the culture medium, which can modulate the concentration of different intracellular compounds [8].

Studies also demonstrate that *C. vulgaris* has an amino acid profile that meets the standards established by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), highlighting its potential as a nutritionally complete source [7]. Given this scenario, this study aimed to evaluate the composition of COS produced from *C. vulgaris* biomass, contributing to a better understanding of the nutritional and functional potential of this microalgae.

2 MATERIAL & METHODS

- **Cultivation of *Chlorella vulgaris*:**

The microalga *C. vulgaris* was kindly provided by the Phycology Laboratory of the Department of Botany at the Federal University of São Carlos (UFSCar), Brazil. The strain was cultivated in BG-11 medium, pH 7.5, in 500 mL flasks, with manual agitation every 24 hours, at 30°C, under continuous light at 100 µmol m^{-2} s^{-1} [9].

- **Characterization:**

Moisture content was measured by the difference in weight before and after drying at 105°C. Ash content was determined by the difference in weight before and after incineration at 550°C for 1 h (10). Protein extraction followed the methodology described by DUMAS (11). Lipid content was determined using the method of Bligh and Dyer (1959) [12], and carbohydrate determination was calculated by the method of difference [13].

- **Enzymatic hydrolysis an COS determination:**

Enzymatic hydrolysis was carried out in 2.0 mL microtubes using a concentration of 10 g L^{-1} of microalgal biomass in acetate buffer, 0.05 mol L^{-1}, pH 5.0. Approximately 1 mL of microalgae biomass solution was added from Celluclast ® 1.5 L at a concentration of 20 FPU mL^{-1}. The tubes were incubated for 72 hours at 50 °C and 1000 rpm in a dry bath (DB-HS, Loccus, Brazil). Samples were collected every 12 hours to monitor the concentration of glucose and cello-oligosaccharides. COS were quantified according to (Ávila et al., 2020) by high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD), using Dionex® chromatograph equipment (Sunnyvale, CA, USA) equipped with a Carbopac PA column -1 (4×250 mm) and Carbopac PA-1 protection pre-column (4×50 mm), GP50 pump and ED40 electrochemical detector. The running conditions applied were flow of 1 mL min^{-1}, 25 °C and total running time of 25 min [14].

3 RESULTS & DISCUSSION

Table 1 .Composition of *C. vulgaris* biomass.

ANALYSES	%
CARBOHYDRATES	20.4 ± 1.5
PROTEIN	48.2 ± 1.4
LIPIDS	17.5 ± 0.2
MOISTURE	12.3 ± 0.4
ASH	1.64 ± 0.2

The results are expressed as the mean followed by the standard deviation.

The centesimal composition of *C. vulgaris* biomass revealed a proportion of 48.2% proteins, 17.5% lipids and 20.4% carbohydrates, values that are in agreement with previous studies on the chemical composition of microalgae, evidencing the intrinsic variability of this species [7].

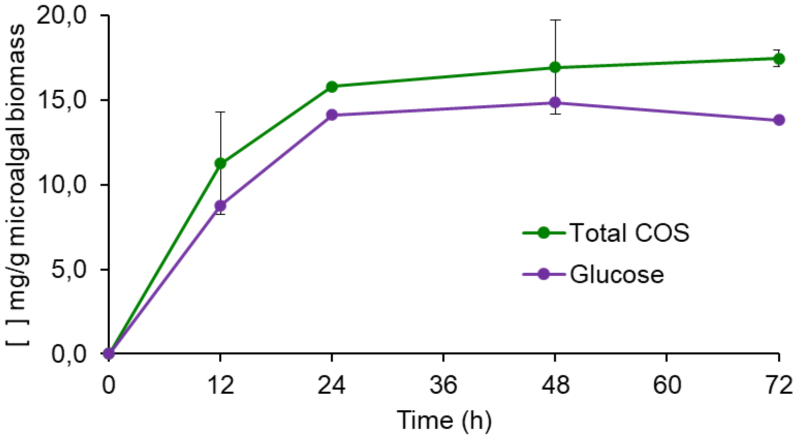

Figure 1 Total concentration of cello-oligosaccharides

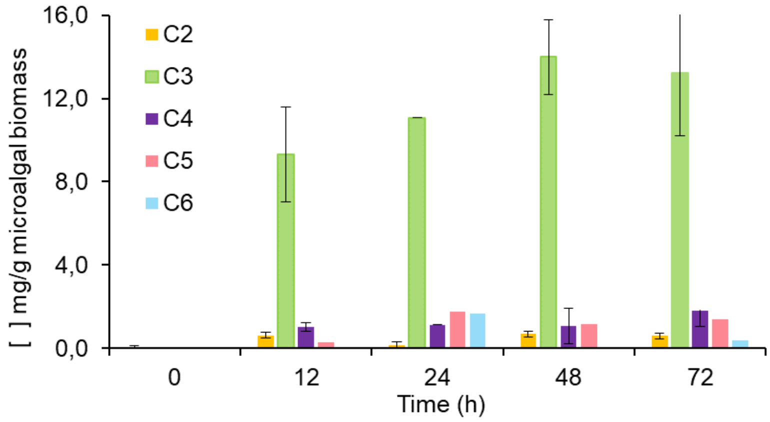

Figure 2 Composition total COS.

When investigating the enzymatic hydrolysis of cellulose present in *C. vulgaris* biomass, the progressive production of cello-oligosaccharides (COS) was observed over the reaction time, as shown in Figure 2. The COS concentration values increased consistently, reaching 17.5 mg g^{-1} after 72 hours, with a corresponding amount of cellotriose of 13.23 mg g^{-1}. In parallel, the analysis of glucose concentration during the enzymatic hydrolysis process revealed significant variations over time, as shown in Figure 1. Initially, the glucose concentration was zero (0 mg g^{-1}) at the beginning of the process (0 hours), increasing to 14.86 mg g^{-1} after 48 hours and then decreasing to 13.81 mg g^{-1}after 72 hours. This dynamic suggests an effective degradation of the cellulose present in *C. vulgaris*, resulting in the release of glucose as a byproduct.

Previous studies reinforce the relevance of cellobiose and cellotriose as substrates for the growth of probiotic strains [15;16], and this emphasizes the functional potential of COS derived from microalgae such as *C. vulgaris.*

These results demonstrate the effectiveness of enzymatic hydrolysis of cellulose in *C. vulgaris* for the production of COS, highlighting the progressive increase in the concentration of these compounds over time. Furthermore, the stabilization of glucose concentration suggests adequate control of the enzymatic hydrolysis process, which is crucial to ensure the efficiency and quality of the obtained products. These findings expand the potential of *C. vulgaris* as a promising source of COS with applicability in several industries, including functional foods and nutraceuticals.

4 CONCLUSION

This research elucidated the nutritional profile of *Chlorella vulgaris* biomass, characterized by high levels of proteins, carbohydrates, and lipids and its potential as a functional food ingredient. Furthermore, enzymatic hydrolysis produced significant amounts of COS, with cellotriose emerging as the predominant component, comprising 82.8% of the total COS produced. These COS exhibit prebiotic properties, and therefore offer potential applications in the food and health industry.

REFERENCES

1. BIBI, R., et al. 2017. Renew. Sustain. Energy Rev. 71. 976–985.
2. PEKKOH, J., et al. 2023. Biomass Convers. Biorefin. 13 (7). 6299–6315.
3. NGOME, M.T., et al. 2019. Hig. Aliment. 525-529.
4. WELLS, M.L., et al. 2017. J. Appl. Phycol. 29 (2). 949–982.
5. BICUDO, C.E.M. & MENEZES, M. 2006. Gêneros de algas de águas continentais do Brasil: chave para identificação e descrições. RiMa, São Carlos. 502 p.
6. CORONADO-REYES, J.A., et al. 2022. Food Sci. Technol. (Brazil) 42. 1–11.
7. SAFI, C., et al. 2013. J. Appl. Phycol. 25 (2). 523–529. Disponível em: http://link.springer.com/10.1007/s10811-012-9886-1.
8. ZAMALLOA, C., et al. 2011. Bioresour. Technol. 102 (2). 1149–1158.
9. RIPPKA, R., et al. 1979. Microbiology 111 (1). 1–61.
10. SHOKRKAR, H., Ebrahimi, S., Zamani, M. 2017. Commun. Eng. Chem. 204 (11). 1246–1257.
11. DUMAS, JBA 1831. Process organic analysis, Ann. Chim. Phys. 247: 198-213.
12. AOAC (Association of Official Analytical Chemists). Official methods of analysis AOAC. 17.ed. Maryland, 2000.
13. BLIGH, E.G., DYER, W.J. 1959. Can. J. Biochem. Physiol. 37 (8). 911–917.
14. GOLDBECK, R., et al. 2016. J. Mol. Catal. B: Enzymatic 36-46.
15. LJUNGH, A; WADSTROM, T.l. Curr. Issues Intestinal Microbio , v. 7, n. 2, pág. 73-90, 2006.
16. ÁVILA, P. F.; DE MÉLO, A.H.F; GOLDBECK, R. Innovative Food Science & Emerging Technologies, v. 85, p. 103335, 2023.

ACKNOWLEDGEMENTS

We thank the agencies CAPES, CNPq, and FAPESP for their financial support.