Seagriculture
21 - 22 June 2023
Trondheim, Norway

12th International Seaweed Conference EU

Exploring the antioxidant potential of *Lobosphaera* sp. and *Odontella* sp. biomasses under different extraction and preservation conditions

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INTRODUCTION

Microalgae has aroused academic and industrial interest due to the great variety of biomolecules present in their biomasses, in addition to other products released to the extracellular medium during cultivation [1]. *Lobosphaera sp.* is a freshwater green alga known to accumulate large amounts of polyunsaturated fatty acids (PUFAs), especially arachidonic acid (ARA, ω-6) [2]. In contrast, *Odontella* sp. is a marine diatom that has been reported as a potential source of fucoxanthin and eicosapentaenoic acid (EPA, ω-3). Also producing chrysolaminarin (β-1,3-glucan) as a storage polysaccharide, which has a high capacity to scavenge hydroxyl radicals [3]. Nevertheless, other antioxidants can be obtained through ethanolic extraction of some polar compounds (e.g., phenols, flavonoids, chlorophylls) [4]. Under specific conditions *Lobosphaera* sp. and *Odontella* sp. were able to produce extracts with considerable amounts of phenolic compounds, 11.5 and 20.9 mg GAE·g^{-1}, respectively. Interestingly, studies have suggested that phenolic compounds have the main effect on total antioxidant activity when compared to carotenoids and tocopherols, for example [5,6]. Finally, the ferrous-ion chelating ability (FICA) and DPPH• radical scavenging activity (RSA) of ethanolic extracts obtained from these genera were also investigated, varying from 43 to 60 % and 1 to 9 %, respectively. This work is part of the EXTRATOTECA project, which aims to create a 'bank of microalgae and cyanobacterial extracts', initially focusing on 30 genera. Furthermore, this project is coordinated by ©A4F Algae for future in partnership with five Portuguese institutions.

AIMS

- ❑ Evaluate the antioxidant potential of ethanolic extracts of *Lobosphaera* sp. and *Odontella* sp.
- ❑ Analyze how the extraction and preservation conditions affect the antioxidant potential of the final product.

MATERIALS AND METHODS

The biomasses used in this study were cultivated and provided by © A4F Algae for future to partners from the University of Aveiro, who proceeded with the extraction by High Pressure Processing (HPP) of part of them. The crude and deconstructed biomass were then preserved by freezing or freeze-drying to obtain ethanolic extracts for further analysis, according to the following schemes (Figures 1 and 2).

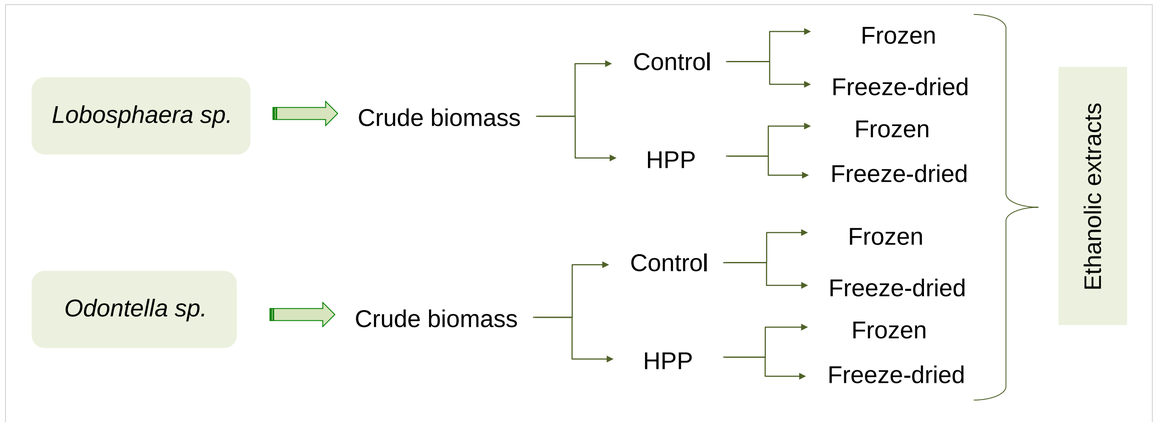

Figure 1. Graphical representation of the extraction procedure and the preservation condition. HPP conditions: 500 MPa/15 min at room temperature.

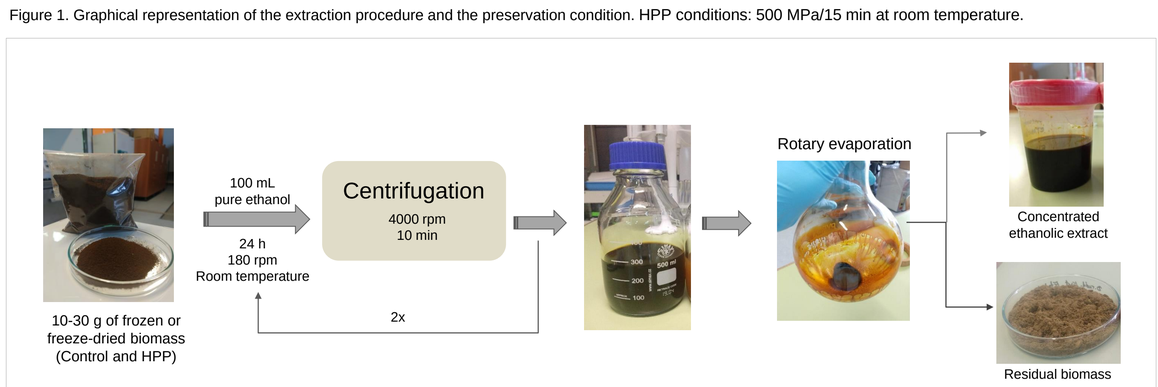

Figure 2. Graphic representation of the process for obtaining ethanolic extracts. Corrêa et al. [6] with slight modifications.

Solutions of concentration 1,0 mg·g^{-1} were prepared from the concentrated ethanolic extracts (after drying) for subsequent quantification of phenolic content and antioxidant analyses. Total phenolic content was analyzed according to Haoujar et al. [7], ferrous-ion chelating ability (FICA, %) and DPPH radical scavenging activity (RSA, %) were evaluated as described by Corrêa et al. [6] through Equations (1) and (2), using EDTA-Na_2 and gallic acid (GA), respectively, as references.

$$FICA\ (\%) = \left[\frac{A_0-(A-A_b)}{A_0}\right] * 100;\ \lambda = 562\ \text{nm}. \quad (1) \qquad RSA\ (\%) = \left[\frac{A_0-(A-A_b)}{A_0}\right] * 100;\ \lambda = 517\ \text{nm}. \quad (2)$$

Where A_0 is the absorbance of the control; A the absorbance of the sample or standard references and A_b is the absorbance of the blank.

Statistical analysis was performed by IBM® SPSS® Statistics v. 27 through analysis of variance (ANOVA) and Tukey's test with 95 % of confidence, being statistically significant when $p < 0.05$.

ACKNOWLEDGMENTS

EXTRATOTECA
a4f algae for future
universidade de aveiro
cieti centro de inovação em engenharia e tecnologia industrial
ISEP INSTITUTO SUPERIOR DE ENGENHARIA DO PORTO
FCT Fundação para a Ciência e a Tecnologia
Cofinanciado por:
COMPETE 2020 PROGRAMA OPERACIONAL COMPETITIVIDADE E INTERNACIONALIZAÇÃO
PORTUGAL 2020
UNIÃO EUROPEIA Fundo Europeu de Desenvolvimento Regional

RESULTS AND DISCUSSIONS

Table 1. Dry biomass:solvent ratio, extraction yield and total phenolic compounds content (mg GAE·g^{-1}) of *Lobosphaera* sp. and *Odontella* sp. samples obtained by different extraction and preservation procedures.

Species	Extraction process	Preservation condition	Dry biomass:solvent ratio (dw/v)	Extraction yield (%)	Total Phenolic Compounds (mg GAE·g^{-1})
Lobosphaera sp.	Control	Freezing	7:1	28.77	4.98 ± 0.17^{b,c}
		Freeze-drying	35:1	12.53	6.12 ± 0.07^{b,c}
	HPP	Freezing	15:1	32.01	11.50 ± 0.21^{d}
		Freeze-drying	34:1	8.40	4.61± 0.06^{a,b}
Odontella sp.	Control	Freezing	5:1	71.08	20.92 ± 1.19^{f}
		Freeze-drying	33:1	18.95	6.50 ± 0.14^{c}
	HPP	Freezing	10:1	61.86	16.52 ± 1.05^{e}
		Freeze-drying	34:1	16.16	3.14 ± 0.31^{a}

Control and HPP Frozen samples

Freeze-dried samples

GAE: Gallic Acid Equivalents. Same superscript letter means no significant difference between values, with 95% of confidence level.

✓ Freezing as a preservation procedure seems to improve the extraction of phenolic compounds.

✓ Highest phenolics content was found in control frozen samples of *Odontella* sp. (20.9 mg GAE·g^{-1}).

✓ HPP combined with ethanol extraction improved phenolics extraction in *Lobosphaera* sp., resulting in 11.5 mg GAE·g^{-1}.

✘ Freeze-drying seems to impair the extraction of phenolic compounds, the contents varied from 3.1 to 6.5 mg GAE·g^{-1}.

Due to the different processing techniques (HPP, freezing and freeze-drying), the proportions of dry biomass to solvent varied in the preparation of the ethanolic extracts.

This affected not only the extraction yield, but possibly also the composition of the extracts.

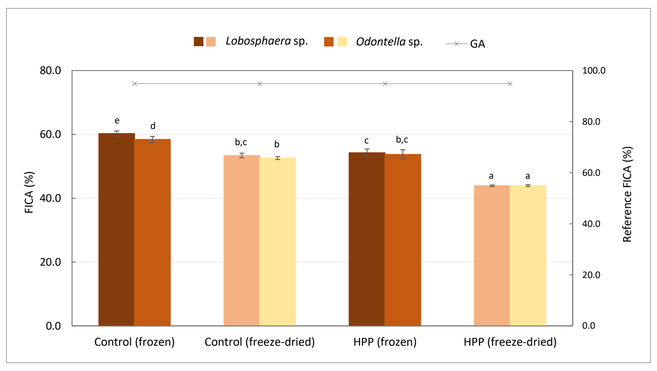

Figure 3. Ferrous-ion chelating ability (FICA) of *Lobosphaera* sp. and *Odontella* sp. extracts (1.0 mg·mL^{-1}) obtained from different treatments. Gallic Acid.

Both *Lobosphaera sp.* and *Odontella sp*. showed high FICA in control (frozen) samples, 58-60%.

The results obtained in control (freeze-dried) and HPP (frozen) samples were statically similar.

Lowest FICA were observed in HPP (freeze-dried) samples for both genera.

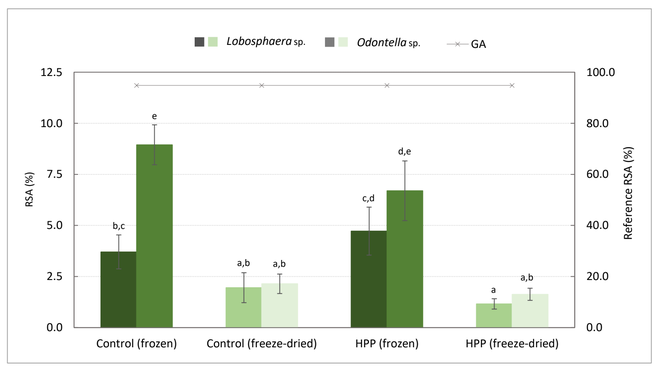

Figure 4. DPPH· radical scavenging ability (RSA) of *Lobosphaera* sp. and *Odontella* sp. extracts (1.0 mg·mL^{-1}) obtained from different treatments. GA: Gallic Acid.

Highest DPPH were achieved in *Odontella* sp. control- and HPP- frozen samples, 9.0 and 6.7 %, respectively.

Lowest RSA were observed in freeze-dried samples for both genera, varying from 1.2 to 2.1 %.

✓ In general, higher antioxidant activities were found in samples with higher amounts of phenolic compounds.

✓ *Lobosphaera* sp. control (frozen) showed a surprisingly high FICA, as the amount of phenolic content was low (4.98 mg GAE·g^{-1}) compared to the other samples.

✘ Freeze-dried samples seems to impair not only phenolics extraction, but also DPPH• RSA. However, FICA varied from 43 to 53% in these samples.

CONCLUSIONS AND PERSPECTIVES

✓ Freezing as a preservation procedure led to the highest amounts of phenolic content in *Odontella* sp., 20.9 and 16.5 mg GAE·g^{-1}, control and HPP, respectively.

✓ HPP improved the extraction of phenolic compounds in frozen samples of *Lobosphaera* sp., increasing from 4.98 to 11.5 mg GAE·g^{-1}, control and HPP, respectively.

✓ The impact of phenolic content on DPPH• RSA is apparently more substantial than on FICA.

✓ As the results presented are preliminary, the antioxidant analyses are still inconclusive. The composition of the extracts, as well as other analyses related to antioxidant properties should be performed to elucidate the real antioxidant potential of the extracts.

❑ Repeat the antioxidant potential analyses, also performing other analytical methods.

❑ Analyze the composition of the extracts in terms of carotenoid, lipid and phenolic profiles.

❑ Investigate the stability of the composition and the antioxidant properties of the extracts over time.

REFERENCES

[1] P.S. Corrêa, W.G. Morais Júnior, A.A. Martins, N.S. Caetano, T.M. Mata. Processes. 9(1) (2021) 1-43.
[2] S. Vasilieva, K. Shibzukhova, A. Solovchenko, O. Chivkunova, C. Antipova, A. Morozov, E. Lobakova. J. Mar. Sci. Eng. 11 (2023).
[3] W. Zhao, R. Yao, X.-S. He, Z.-H. Liao, Y.-T. Liu, B.-Y. Gao, C.-W. Zhang, J. Niu.. Aquaculture. 555 (2022), 738206.
[4] M.R. Gauthier, G.N.A. Senhorinho, N. Basiliko, S. Desjardins, J.A. Scott. Ind. Technol. 18 (3) (2022), 168-175.
[5] H. Safafar, J. van Wagenen, P. Møller, C. Jacobsen. Mar. Drugs. 13 (2015), 7339-7356.
[6] P.S. Corrêa, W. G. Morais Júnior, N.S. Caetano. Algal Res. 67 (2022), 102859.
[7] I. Haoujar, F. Cacciola, J. Abrini, D. Mangraviti, D. Giuffrida, Y.O. El Majdoub, A. Kounnoun, N. Miceli, M.F. Taviano, L. Mondello, F. Rigano, N.S. Senhaji. Molecules. 24 (2019), 4037.