# Microalgae as sustainable bio-factories of healthy lipids: evaluating fatty acid content and antioxidant activity

**Tiago A. Conde[1,2,3†], Bruna F. Neves[1,2†], Daniela Couto[1,2], Tânia Melo[1,2], Bruno Neves[3], Margarida Costa[4], Joana Silva[4], Pedro Domingues[1] and M. Rosário Domingues[1,2]**

[1]Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal

[2]CESAM - Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal

[3]Department of Medical Sciences and Institute of Biomedicine – iBiMED, University of Aveiro, 3810-193 Aveiro, Portugal

[4]Allmicroalgae Natural Products S.A., R&D Department, Rua 25 de Abril 19, 2445-287 Pataias, Portugal

[†]both authors contributed equally for the manuscript

Corresponding author: mrd@ua.pt

**Table S1.** Lipid content of *Chlorella vulgaris, Chlorococcum amblystomatis, Scenedesmus obliquus, Tetraselmis chui, Phaeodactylum tricornutum, Spirulina* sp. and *Nannochloropsis oceanica.* Kruskal–Wallis test followed by Dunn's post-hoc comparisons).

| Microalgae | *Chlorophyta* | | | | *Bacillariophyta* | *Cyanobacteria* | *Ochrophyta* |
|---|---|---|---|---|---|---|---|
| | *Chlorella vulgaris* | *Chlorococcum amblystomatis* | *Scenedesmus obliquus* | *Tetraselmis chui* | *Phaeodactylum tricornutum* | *Spirulina* sp. | *Nannochloropsis oceanica* |
| Lipid content (%) | 8.8 ±0.7 [a,b] | 16.6 ±0.9 [a,c] | 11.1 ±1.1 | 6.5 ±0.4 [c,d,e] | 12.6 ±1.2 [e] | 10.7 ±1.0 [f] | **20.9 ±3.4 [b,d,f]** |

**Table S2.** – Evaluation of the antioxidant activity for the extracts of different microalgae. Concentration of lipid extract ($\mu g.mL^{-1}$) providing 50% inhibition of the ABTS•+ radical and 20% inhibition of the DPPH• radical, and their respective Trolox equivalents (TE; $\mu mol.g^{-1}$). The values are displayed as the mean (n = 3) ± standard deviation. Matching letters (a-f) indicate statistically significant differences between microalgae species, i.e. the same letter represents significant differences (q < 0.05, Kruskal–Wallis test followed by Dunn's post-hoc comparisons).

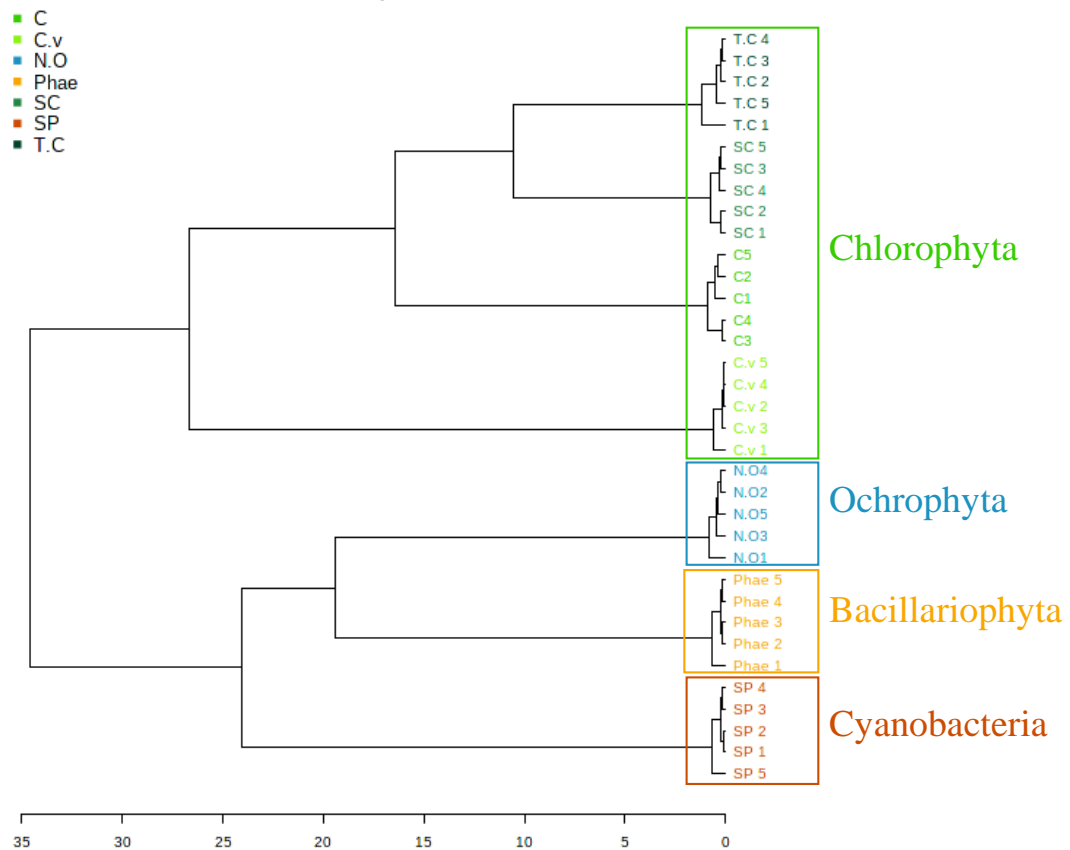| | | *Chlor.ophyta* | | | | *Bacillariophyta* | *Cyanobacteria* | *Ochrophyta* |
|---|---|---|---|---|---|---|---|---|
| | | *C. vulgaris* | *C. amblystomatis* | *S. obliquus* | *T. chuui* | *P. tricornutum* | *Spirulina* sp. | *N. oceanica* |
| **ABTS•+** | IC50 ($\mu g.mL^{-1}$) | 51.1 ±3.7 | 52.6 ±4.6 | 29.4 ±1.2 [a,b] | 40.9 ±4.7 | 57.3 ±4.5 [b] | 38.7 ±1.6 [c] | 101.9 ±1.7 [a,c] |
| | TE ($\mu mol.g^{-1}$) | 368.4 ±27.0 | 358.8 ±32.4 | 637.5 ±27.4 [a] | 432.9 ±51.3 | 306.6 ±23.5 | 452.2 ±9.6 | 184.0 ±3.2 [a] |
| **DPPH+** | IC20 ($\mu g.mL^{-1}$) | 50.5 ±12.3 [d,e] | 58.4 ±10.7 [f] | 89.1 ±6.6 | 225.7 ±6.9 [d,f] | 75.4 ±4.6 | 96.9 ±9.7 | 175.6 ±8.7 [e] |
| | TE ($\mu mol.g^{-1}$) | 191.8 ±40.0 [d,e] | 143.0 ±25.1 [f] | 114.5 ±8.9 | 45.0 ±1.4 [d,f] | 122.3 ±7.2 | 84.3 ±9.1 | 52.5 ±2.7 [e] |

## A. Hierarchical clustering



*Figure S1.* (**A**) Hierarchical cluster analysis using relative abundance after Glog normalization of all fatty acids identified in *Chlorella vulgaris, Chlorococcum amblystomatis, Scenedesmus obliquus, Tetraselmis chui, Phaeodactylum tricornutum., Spirulina* sp. and *Nannochloropsis oceanica*. The green, blue, yellow, and orange boxes show microalgae from the phylum Chlorophyta, Ochrophyta, Bacillariophyta and Cyanobacteria, respectively. Abbreviations: C, *Chlorococcum amblystomatis*; C.v, *Chlorella vulgaris*; SC, *Scenedesmus obliquus*; T.C, *Tetraselmis chui*; Phae, *Phaeodactylum tricornutum*; SP, *Spirulina* sp.; and N.O, *Nannochloropsis oceanica*.
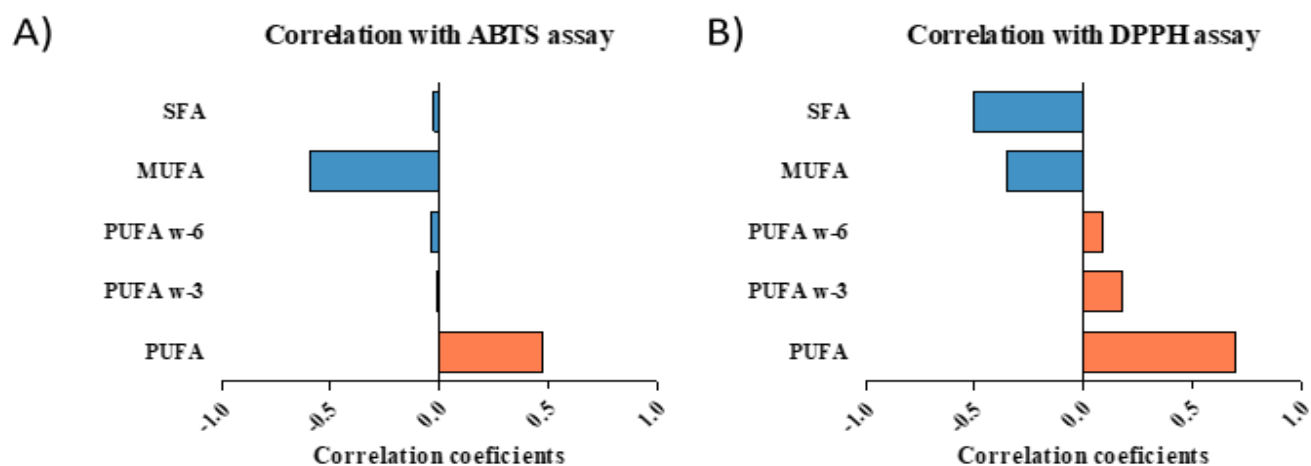
*Figure S2.* Correlation coefficients analysis using the sum of relative abundances from saturated fatty acids (SFA), mono-unsaturated FA (MUFA), polyunsaturated FA (PUFA), omega-3 PUFA and omega-6 PUFA, and antioxidant activity. (**A**) Correlation coefficients with ABTS assay. (**B**) Correlation coefficients with DPPH assay.