

## Supplementary data

*Article*

# Potential of Coccolithophore Microalgae as Fillers in Starch-Based Films for Active and Sustainable Food Packaging

Ana S. P. Moreira <sup>1,2</sup>, Joana Gonçalves <sup>3</sup>, Francisco Sousa <sup>3</sup>, Inês Maia <sup>4</sup>, Hugo Pereira <sup>5</sup>, Joana Silva <sup>5</sup>, Manuel A. Coimbra <sup>2</sup>, Paula Ferreira <sup>3</sup> and Cláudia Nunes <sup>3,\*</sup>

<sup>1</sup> CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

<sup>2</sup> LAQV-REQUIMTE - Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

<sup>3</sup> CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

<sup>4</sup> CCMAR - Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

<sup>5</sup> GreenCoLab - Associação Oceano Verde, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

\* Correspondence: claudianunes@ua.pt

**Supplementary Table S1.** Elemental analysis and protein estimation from freeze-dried biomass of *E. huxleyi* (EHUX) and *C. pseudoroscoffensis* (CP) microalgae.

Samples	%C	%H	%N	%S	%CHNS	Protein (N × 4.78) <sup>c</sup>
<i>E. huxleyi</i> (EHUX)	27.3 ± 0.5	3.7 ± 0.1	3.4 ± 0.0	<i>t</i>	34.4 ± 0.6	16.1 ± 0.1
EHUX (other batch) <sup>a</sup>	20.3 ± 0.2	4.3 ± 0.1	3.2 ± 0.0	1.3 ± 0.3	29.0 ± 0.6	15.3 ± 0.1
<i>C. pseudoroscoffensis</i> (CP)	21.3 ± 0.2	2.9 ± 0.1	2.3 ± 0.1	<i>t</i>	26.5 ± 0.2	11.2 ± 0.3
CP (other batch) <sup>b</sup>	22.6 ± 0.3	3.4 ± 0.2	2.4 ± 0.1	<i>t</i>	28.5 ± 0.6	11.6 ± 0.4

*t*, traces (<0.5%); <sup>a</sup>Data from other batch of *E. huxleyi* biomass produced under the same culture conditions [1]; <sup>b</sup>Data from other batch of *C. pseudoroscoffensis* biomass produced under the same culture conditions [2]; <sup>c</sup>Nitrogen-to-protein conversion (4.78) proposed for microalgae [3].

**Supplementary Table S2.** TGA values at the second stage of thermal decomposition of starch films (control) and starch-based films containing 20% of commercial CaCO<sub>3</sub>, *E. huxleyi* (EHUX) or *C. pseudoroscoffensis* (CP)

Samples	T <sub>max</sub> (°C)	Remaining ash (%)
Starch (control)	295.8	8.0
Starch+CaCO <sub>3</sub> 20%	302.7	19.7
Starch+EHUX 20%	251.4	18.6
Starch+CP 20%	278.8	12.5

Starch



Starch + CaCO<sub>3</sub> 2.5%



Starch + CaCO<sub>3</sub> 5%



Starch + CaCO<sub>3</sub> 10%



Starch + EHUX 2.5%



Starch + EHUX 5%



Starch + EHUX 10%



Starch + CP 2.5%



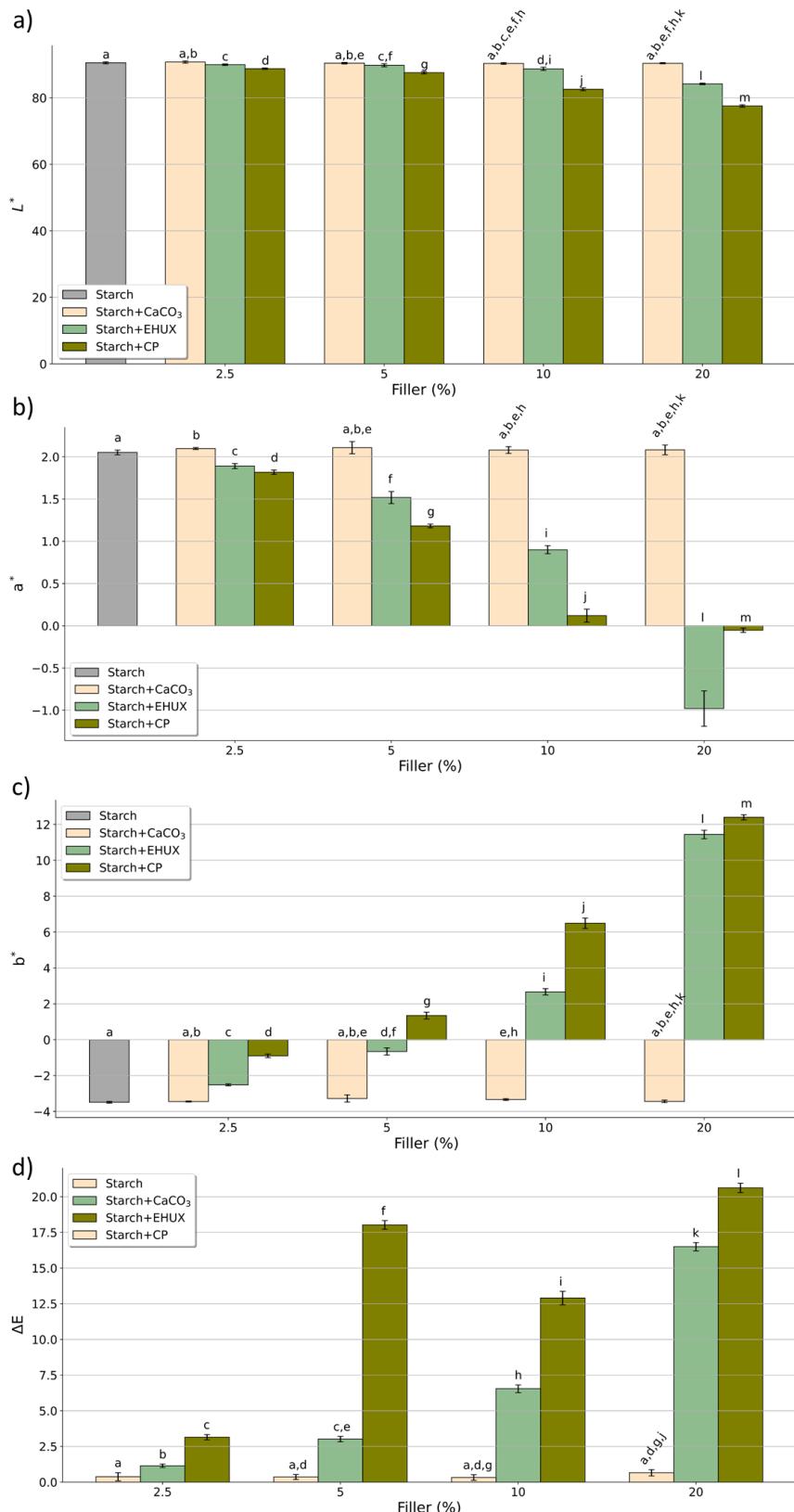
Starch + CP 5%



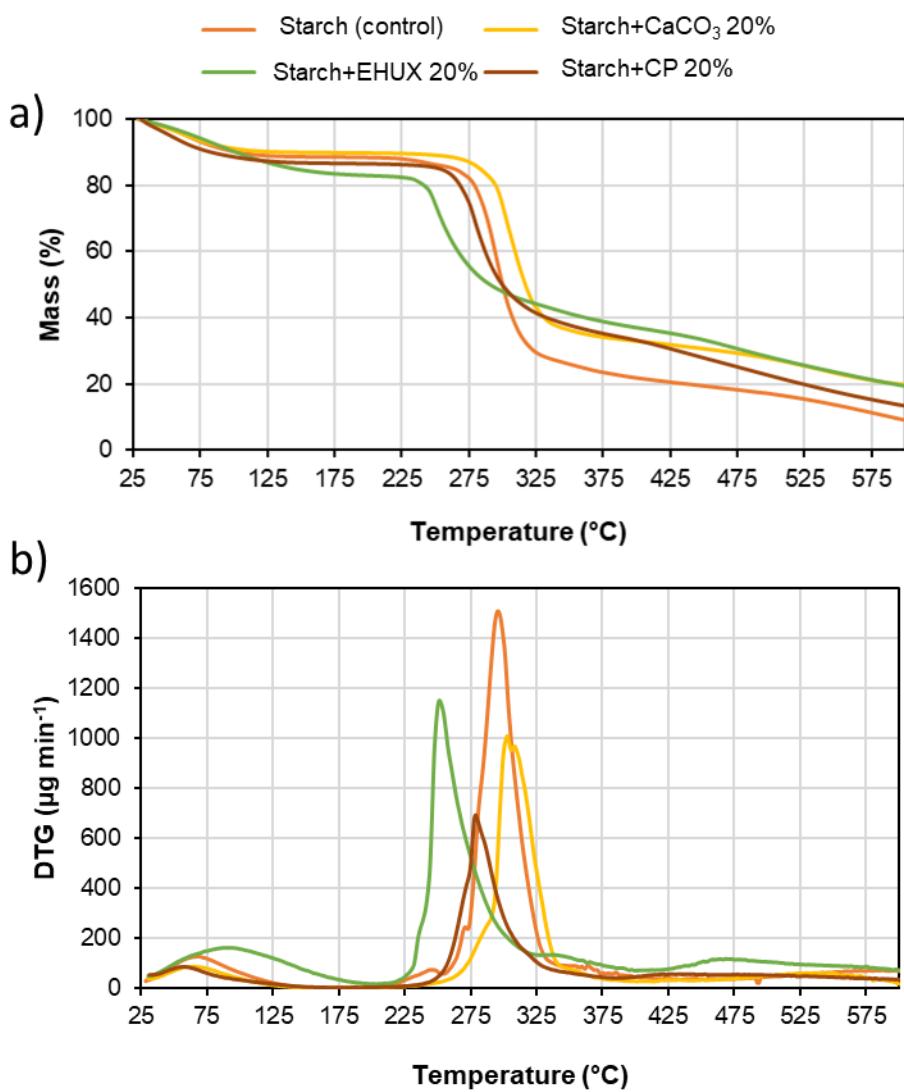
Starch + CP 10%



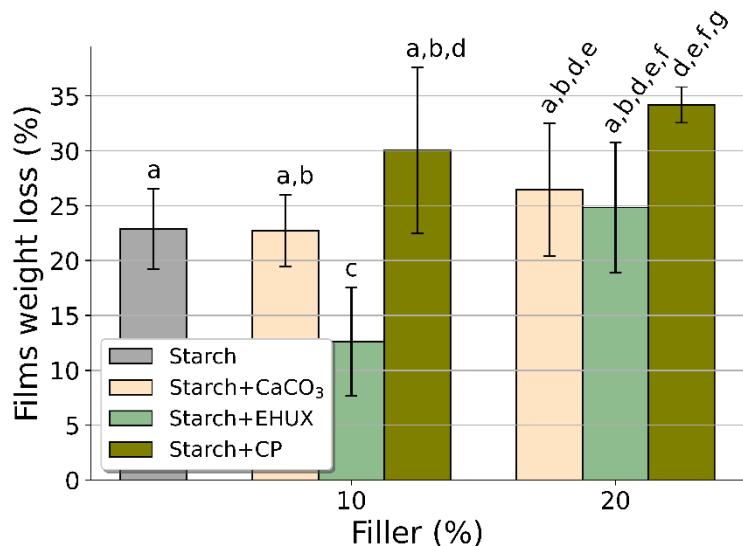
**Supplementary Figure S1.** Real images of starch-based films without and with 2.5, 5, and 10% (w/w of dry starch weight) of commercial CaCO<sub>3</sub>, *E. huxleyi* (EHUX) and *C. pseudoroscoffensis* (CP) biomass.



**Supplementary Figure S2.** Values (mean  $\pm$  standard deviation) for lightness ( $L^*$ ), red-green ( $a^*$ ), yellow-blue ( $b^*$ ), and total colour variation ( $\Delta E$ ) of pristine starch films (control) and starch-based films containing commercial  $CaCO_3$ , *E. huxleyi* (EHUX) or *C. pseudoroscoffensis* (CP) at different percentages (2.5, 5, 10 and 20% w/w). Different letters between each condition indicate significant differences (Student's t-test;  $p < 0.05$ ).



**Supplementary Figure S3.** a) TGA curves and b) first derivatives of starch films (control) and starch-based films containing 20% of commercial CaCO<sub>3</sub>, *E. huxleyi* (EHUX) or *C. pseudoroscoffensis* (CP).



**Supplementary Figure S4.** Weight loss percentage observed after immersion in water for 8 days of pristine starch films (control) and starch-based films containing commercial CaCO<sub>3</sub>, *E. huxleyi* (EHUX) or *C. pseudoroscoffensis* (CP) at different percentages (10 and 20% w/w). Different letters between each condition indicate significant differences (Student's t-test; p < 0.05).

## References:

- [1] S.S. Aveiro, T. Melo, A. Figueiredo, P. Domingues, H. Pereira, I.B. Maia, J. Silva, M.R. Domingues, C. Nunes, A.S.P. Moreira, The polar lipidome of cultured *Emiliania huxleyi*: A source of bioactive lipids with relevance for biotechnological applications, *Biomolecules* 10 (2020) 1434.
- [2] A.S.P. Moreira, J. Gonçalves, T.A. Conde, D. Couto, T. Melo, I.B. Maia, H. Pereira, J. Silva, M.R. Domingues, C. Nunes, *Chrysotila pseudoroscoffensis* as a source of high-value polar lipids with antioxidant activity: A lipidomic approach, *Algal Res.* 66 (2022) 102756.
- [3] S.O. Lourenço, E. Barbarino, P.L. Lavín, U.M. Lanfer Marquez, E. Aidar, Distribution of intracellular nitrogen in marine microalgae: Calculation of new nitrogen-to-protein conversion factors, *Eur. J. Phycol.* 39 (2004) 17-32.