IMPACT OF FERMENTATION ON THE ANTIOXIDANT ACTIVITY AND COLOUR OF *HAEMATOCOCCUS PLUVIALIS* AND *PORPHYRIDIUM CRUENTUM*

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Introduction

Microalgae are emerging as a promising ingredient for the food industry due to their sustainable cultivation and production of a variety of bioactive compounds, including proteins, lipids, carbohydrates, antioxidant compounds, and pigments [1]. Among the many cataloged species of microalgae, two stand out in research due to their powerful applications in the pharmaceutical, nutraceutical, cosmetic, and food and beverage industries. The first is *Haematococcus pluvialis*, a unicellular microalga known for its high content of astaxanthin (3,3-dihydroxy-β, β-carotene-4,4-dione), one of the most important natural antioxidants [2]. The second is *Porphyridium cruentum*, a red marine microalgae that serves as a reservoir of high-value compounds such as carotenoids, sulfated exopolysaccharides (EPSs), β-phycoerythrin (PE), and lipids [3]. Carotenoids and astaxanthins are well-known antioxidants with numerous applications in the food and pharmaceutical industries. However, the application of microalgae in food faces challenges related to their intense flavour and odour. Fermentation of microalgae biomass with bacteria has emerged as a promising strategy to mitigate these problems [4]. However, the fermentation process can modify other characteristics of the biomass.

Objective

The objective of this work was to evaluate the colour, total phenolics and antioxidant activity of *H. pluvialis* and *P. cruentum* biomasses before and after fermentation.

Material and Methods

The biomasses provided by GreenCoLab were individually fermented with *Lactobacillus plantarum* at 30 °C for 48 hours without agitation (ratio of 0.1 LAB:1 biomass, 10^6 CFU/mL). To evaluate the antioxidant activity and total phenolic content (TPC), methanolic extracts (0.6:10 m/v) were prepared and agitated at 60 rpm for 16 hours, using a roller shaker. TPC was quantified using the Folin-Ciocalteu method, with absorbance measured at 765 nm, and results were expressed in mg GAE/100 mg DW.

The antioxidant activity was assessed using the ABTS, DPPH, and ORAC methods. Absorbance readings were taken in 96-well microplates (Synergy H1, Biotek), at 734 nm for ABTS, and 515 nm for DPPH, and fluorescence for ORAC. Results were expressed as µmol TE/100 mg DW.

The colour of fermented and non-fermented samples was measured (n=10) using a CR400 colorimeter (Konica, USA) following the CIELab system, *L** (lightness), *a** (green to red), and *b** (blue to yellow), and calculating Chroma (*C**) and hue (*h*).

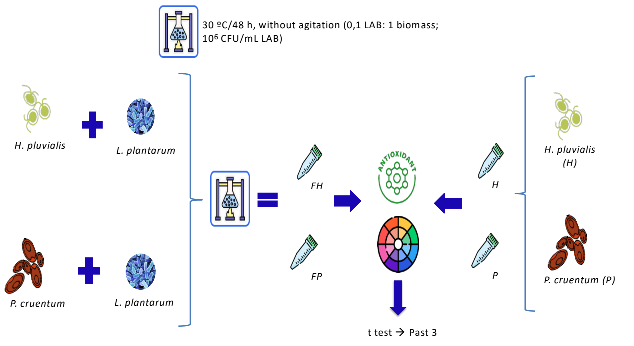

Figure 1. Workflow for the determination of the colour and antioxidant activity of fermented and non-fermented biomasses.

Results

Significant differences ($p < 0.05$) were observed in the *a**, *b**, *L**, *C**, and *h* parameters before and after fermentation, indicating that fermentation had a substantial impact on biomass colour (Table 1).

Table 1. Colour evaluation in both fermented and non-fermented biomasses of *H. pluvialis* and *P. cruentum.*

	Haematococcus pluvialis		*Porphyridium cruentum*	
	Non-fermented	Fermented	Non-fermented	Fermented
L*	9.51 ± 0.51	12.32 ± 0.38	19.11 ± 1.29	16.77 ± 1.34
Hue (°)	27.80 ± 1.10	35.30 ± 2.20	40.00 ± 1.10	34.30 ± 0.60
C*	5.00 ± 0.22	3.50 ± 0.15	10.13 ± 1.01	7.74 ± 0.81

Mean results and standard deviation (n=10)

Jin *et al.* [5] also reported colour alterations in the microalga *Chlamydomonas reinhardtii* following fermentation with *L. plantarum*.
The results obtained for TPC and antioxidant activity in both fermented and non-fermented biomasses of *H. pluvialis* and *P. cruentum* are presented in Table 2. It was not possible to determine DPPH for *H. pluvialis* biomass due to colour interference in the extracts. The findings indicate that fermentation with *L. plantarum* enhances the antioxidant activity and TPC of the biomasses.
An increase in the antioxidant capacity of TPC during fermentation was also reported by Tomassi et al. [6] in *Chlorella vulgaris* and *Aphanizomenon flos-aquae* fermented with *Saccharomyces cerevisiae* and *L. plantarum*.

Table 2. TPC and antioxidant activity in both fermented and non-fermented biomasses of *H. pluvialis* and *P. cruentum.*

	Haematococcus pluvialis		*Porphyridium cruentum*	
	Non-fermented	Fermented	Non-fermented	Fermented
TPC	0.1474 ± 0.0056	1.0795 ± 0.2299	0.0816 ± 0.0032	0.1802 ± 0.0257
ABTS	2.3967± 0.1299	3.2185 ± 0.3526	0.3251 ± 0.0285	0.2644 ± 0.0005
DPPH	-	-	0.1368 ± 0.0026	0.0836 ± 0.0037
ORAC	43.3276 ± 2.9053	54.3239 ± 1.7887	1.3806 ± 0.1215	3.1111 ± 0.1273

Mean results and standard deviation (n=3)

Conclusions

The results demonstrate that the fermentation of *Haematococcus pluvialis* and *Porphyridium cruentum* with *Lactobacillus plantarum* for 48 hours is a promising strategy for enhancing antioxidant activity and phenolic content, as well as changing biomass colour. This reinforces the potential of fermented microalgae as functional ingredients in food products for health benefits. The sensory impact of these modifications and consumer acceptance will be explored in future studies.

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