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Microalgae biomass colourings. 1. Evaluation of antioxidant activity

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INTRODUCTION

Microalgae are an excellent source of phenolic compounds and natural pigments, such as carotenoids, chlorophylls and phycobiliproteins, presenting great industrial potential to be used as colouring agents. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects. Their function as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans (*1*). In fact, microalgae are photoautotrophic organisms that are exposed to high oxygen and radical stresses, and consequently have developed several efficient protective systems against reactive oxygen species and free radicals (*2*). The content and type of antioxidant compounds depends on the microalgae species and growing conditions.
The aim of the present work was to select effective extraction conditions of antioxidant compounds from three microalgae (*Chlorella vulgaris, Isochrysis galbana* and *Diacronema vlkianum*) and to evaluate their antioxidant potential through free radical scavenging capacity assays – DPPH (2,2-diphenyl-1picrylhydrazyl) and to relate with its total phenolic compounds (Folin-Ciocalteau method).

MATERIALS AND METHODS

Microalgal biomass production

C. vulgaris (INETI 58) used in this study was cultivated in appropriated growth medium (*3*) and growing in airlift bioreactors, bubbling air, at 25 ºC temperature and at low light conditions (150 µE m^{-2} s−1). *I. galbana* and *D. vlkianum* (IPIMAR) were grown in Wallerstein & Miquel medium (3:1) in airlift bioreactors at 18 ºC, according to the method described by Bandarra *et al.* (4) and Donato *et al.* (*5*). Microalgal biomass harvesting was produced without flocculation by simply removing agitation, concentration by centrifugation and freeze drying.

Extraction methods

The following conditions were used to prepare the methanolic extracts from *C. vulgaris* (15 mg/ml): (i) stirring with glass beads during 0.5 min (trial A), 1 min (trial B) and 2 min (trial C) in a Vortex mixer at room temperature; (ii) keeping at -80 ºC for 3 hours (trial D) or 24 hours (trial E) or two cycles at -80 ºC (1.5 h each) (trial F); and (iii) autoclaving at 125 ºC (trial G) and 140 ºC (trial H) for 25 min. These extraction conditions were used in two consecutive extractions.

Analytical methods

The radical scavenging activity of the extracts was evaluated using DPPH (2,2-diphenyl-1picrylhydrazyl) method as described by Ferreira *et al* (6). The determination of phenolic content was done by the Folin and Ciocalteu method and the results were expressed as mg of gallic acid (Ferreira *et al* (6). Based on the DPPH values the Radical Scavenging Capacity (RSC) was calculated in mg DPPH oxidized/g microalgae and also as mg DPPH oxidized/mg phenolic compounds.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the RSC and phenolic content results. Normality and homogeneity of variances were verified by Kolmogorov–Smirnov and Bartlett tests, respectively.

Results and Discussion

In Table 1 are presented the RSC values and the phenolic content of the methanolic extracts prepared by the different methods.

Table 1 – Radical Scavenging Capacity (mg DPPH/g microalgae and mg DPPH/mg phenolic compounds) and phenolic content (mg of gallic acid/g microalgae) in different methanolic extracts of *C. vulgaris*. Each value is the mean of three replicates (±standard deviation).

	RSC (mg DPPH/g microalgae)		Phenolic content (mg of gallic acid/g microalgae)		RSC (mg DPPH/mg gallic acid)	
	1st extraction	2nd extraction	1st extraction	2nd extraction	1st extraction	2nd extraction
A	3.50^{a} (±0.04)	1.12^{a} (±0.03)	2.38^{a} (±0.04)	0.78^{a} (±0.17)	1.47^{a} (±0.04)	1.45^{a} (±0.34)
B	3.89^{b} (±0.14)	1.45^{b} (±0.06)	3.31^{b} (±0.23)	1.31^{b} (±0.10)	1.16^{b} (±0.05)	1.10^{a} (±0.07)
C	3.82^{b} (±0.32)	1.43^{b} (±0.06)	3.72^{b} (±0.33)	1.42^{c} (±0.01)	1.03^{b} (±0.21)	1.01^{b} (±0.01)
D	3.88^{b} (±0.19)	0.85^{c} (±0.05)	2.58^{ac} (±0.21)	0.70^{a} (±0.08)	1.51^{a} (±0.07)	1.18^{a} (±0.08)
E	3.78^{b} (±0.17)	1.35^{b} (±0.16)	3.01^{bc} (±0.40)	0.98^{a} (±0.13)	1.25^{b} (±0.17)	1.38^{a} (±0.10)
F	3.95^{b} (±0.15)	1.25^{b} (±0.13)	3.06^{b} (±0.14)	1.09^{d} (±0.13)	1.28^{b} (±0.10)	1.14^{a} (±0.08)
G	4.46^{b} (±0.43)	0.49^{d} (±0.12)	5.61^{d} (±0.75)	1.62^{c} (±0.34)	0.80^{b} (±0.05)	0.30^{c} (±0.02)
H	3.74^{b} (±0.17)	1.30^{b} (±0.31)	6.67^{e} (±0.56)	1.53^{c} (±0.21)	0.56^{c} (±0.04)	0.85^{d} (±0.09)

Different letters in the same column indicate significantly different values ($p<0.05$).

The extracts obtained in the first extraction under the reported conditions did not present significant differences in their radical scavenging activity with the exception of trial A where the stirring time was only 0.5 min. The second extract showed also radical scavenging activity much lower than the first one. In what concerns phenolic compounds the highest amount was obtained in trials where autoclaving conditions were used in both extractions. The lowest RSC (mg DPPH/mg gallic acid) obtained in the extracts prepared by autoclaving may be due to the highest phenolic concentration in these extracts. These results led to choosing the most feasible procedure i.e. stirring with a Vortex for 1-2 min. The autoclaving seems to promote the disruption of cell walls but it requires drastic conditions and involves higher costs.

D. vlkianum exhibited the highest radical scavenging capacity (RSC) followed by *I. galbana* and *C. vulgaris* (Table 2). The same order was observed for the phenolic content. These results suggest that phenolic compounds are responsible for the antioxidant activity of these microalgae. When the actioxidant activity (AA) is expressed in terms of phenolic compounds a different order is obtained where the AA of *D. vlkianum* and *C. vulgaris* was not significantly different but higher than *I. galbana*. These results suggest that phenolic compounds of *I. galbana* have lower AA.

Table 2 – RSC value (mg DPPH/g microalgae) and phenolic content (mg of gallic acid/g microalgae) in the three microalgae extracts. Each value is the mean of three replicates (±standard deviation).

	C. vulgaris	*I. galbana*	*D. vlkianum*
RSC (mg DPPH/g microalgae)	3.8^{a} (±0.14)	12.8^{b} (±0.52)	50.8^{c} (±0.72)
Phenolic compounds (mg gallic acid/g microalgae)	3.3^{a} (±0.23)	15.8^{b} (±3.90)	23.3^{b} (±8.04)
mg DPPH/mg gallic acid	1.17^{a} (±0.05)	0.81^{b} (±0.21)	2.18^{a} (±0.99)

Conclusions

The results obtained may draw the following conclusions:

- Stirring of microalgae with methanol and glass beads for 1-2 minutes is adequate for the extraction of antioxidant compounds.
- *D. vlkianum* exhibited the highest antioxidant activity as well as the highest phenolic content.
- *I. galbana* phenolic compounds showed the lowest antioxidant activity.

References

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