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# Evaluation of the antioxidant activity of three microalgal species for use as dietary supplements and in the preservation of foods

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**Abstract**

The antioxidant activity of the microalgal ethanolic extracts of *Porphyridium cruentum*, *Phaeodactylum tricornutum* and *Chlorella vulgaris* was determined by means of the β-carotene–linoleate model system. The results show that the activity of *C. vulgaris* extract was higher than those obtained for the other microalgal extracts tested and for the synthetics BHA (butylated hydroxyanisole), and BHT (butylated hydroxytoluene). In addition, the major constituents present in the ethanolic extracts of the three microalgae species were analyzed by means of GC and GC–mass spectrometry. The results showed that the tested microalgae may be an important source of natural antioxidants, as an alternative to higher plants or the production by chemical synthesis.
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*Keywords:* *Porphyridium cruentum*; *Phaeodactylum tricornutum*; *Chlorella vulgaris*; Antioxidant activity; β-Carotene–linoleate model system

## 1. Introduction

Plants in general and microalgae in particular are good sources of natural antioxidants. During the photosynthesis process they absorb solar light which is converted into chemical energy, later used in the conversion of $CO_2$ into carbohydrates, and at the same time, generating molecular oxygen, which can reach locally high concentration levels. As, oxygen is easily activated by ultraviolet radiation (UV) or heat from sunlight into toxic reactive oxygen species (ROS), plants and microalgae have developed a protective mechanism which consists in the preparation of antioxidant compounds able to minimize the concentration of these ROS (Lu & Foo, 1995).

*Porphyridium cruentum* presents light-harvesting polypeptides associated with photosystem I (LHCI) that bind chlorophyll *a*, and β-carotene (Tan, Cunningham, & Gantt, 1997), while *Chlorella vulgaris* is a rich source of proteins, eight kinds of essential amino acids, vitamins (B-complex, ascorbic acid), minerals (potassium, sodium, magnesium, iron, and calcium), β-carotene, chlorophyll, "C.G.F." (*Chlorella* growth factor) and other beneficial substances (Konishi et al., 1996).

Actually, the possible association between lipid peroxidation in living organisms and a wide range of degenerative diseases, as well as ageing, cancer, diabetes and cardiovascular diseases is receiving an increase of attention in the literature. Additionally, lipid peroxidation is one of the major reasons for deterioration of food components during processing and storage. Antioxidants are important inhibitors of lipid peroxidation, not only for food preservation but also as a defence mechanism of living cells against oxidative damage (Vimala, Norhanom, & Yadav, 1999). In addition, antioxidants have been proposed as important tools in the prevention and treatment of a wide range of degenerative diseases (Lorenz & Cysewski, 2000).

The knowledge of the potential antioxidant compounds present in an organism is not always a good indicator of its antioxidant capacity. The interaction between different antioxidant compounds (termed synergism) means that the total antioxidant effect may be greater than the

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addition of the individual antioxidant activities. Thus, the study of an isolated compound will not offer an accurate picture of the overall action ability (Jia, Zhou, Yang, Wu, & Liu, 1998; Poeggeler et al., 1995; Wu, Sugiyama, Zeng, Mickle, & Wu, 1998).

In addition, there are many different classes of antioxidant components in animal and plant tissues; and therefore, it is rather difficult to measure the activity of each antioxidant component separately. Thus, several analytical methods have been proposed to measure the total antioxidant activity of biological extracts so as to evaluate the total antioxidant capacity of biological samples (Cano, Hernandez-Ruiz, Garcia-Canovas, Acosta, & Arnao, 1998; Re et al., 1999; Wayner, Burton, Ingold, & Locke, 1985; Whitehead, Thorpe, & Maxwell, 1992).

The aim of this study was to evaluate the antioxidant activity of ethanolic extracts of three microalgae species cultivated in Almería (Spain): *P. cruentum*, *Phaeodactylum tricornutum* and *C. vulgaris*. For this purpose, the antioxidant activity assay applied was the β-carotene breaching method proposed by (Miller, 1971). In addition, the major products in the ethanolic extracts of the three microalgae species were analyzed by means of GC and GC–Mass Spectrometry.

## 2. Materials and methods

### 2.1. General

*P. cruentum*, *P. tricornutum* and *C. vulgaris* were collected from lab cultures in the University of Almería (Spain). After harvesting, the biomasses were lyophilised and stored at –20 °C. Then, the lyophilised microalgae were separated and stored in airtight envelopes. β-Carotene, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA).

The extraction of each sample was accomplished by using ethanol as solvent. In each extraction, the sample (2 g) was mixed with ethanol (25 ml), placed in a hermetic flask and stirred for 24 h at room temperature in darkness. After that, the solution was vacuum filtered, and the residual powder was extracted twice again using the same solvent. Finally, the microalgal extract was lyophilised and stored at –20 °C.

To measure the antioxidant activity, different concentrations of ethanol solutions of each extract were prepared at room temperature.

#### 2.1.1. Determination of the antioxidant activity with the β-carotene–linoleate system

Miller's spectrophotometric method (Miller, 1971), based on the ability of the different extracts to decrease oxidative losses of β-carotene in a (β-carotene/linoleic acid) emulsion, was used. Crystalline β-carotene (2 mg) was dissolved in chloroform (10 ml). One milliliter of this solution was then added to a round-bottomed flask containing a mixture of purified linoleic acid (20 mg) and of Tween 40 (200 mg). The solvent was removed *in vacuo*, and oxygenated distilled water (50 ml) (prepared by bubbling of pure oxygen through triple distilled water for 0.5 h) was added to the flask with vigorous stirring. Five milliliter aliquot parts of the resulting aqueous emulsion were added to several tubes containing the different ethanolic antioxidant solutions (50 ml).

A zero reading was taken at 470 nm on the reaction mixture in each tube immediately after addition of the emulsion to the antioxidant solution. A control sample with distilled water instead of extract was also analyzed for antioxidant activity. The tubes were then stopped and stored at 50 °C. Subsequent readings were taken at regular intervals until the carotene had been decolourised (about 2 h). The antioxidant activity was measured by the AAC (antioxidant activity coefficient), which is an estimation of the relative oxidation in the presence or absence of extracts.

$$\mathrm{ACC} = \frac{\text{Abs. Ext. } 120' - \text{Abs. Control } 120'}{\text{Abs. Control } 0' - \text{Abs. Control } 120'} \times 1000$$

#### 2.1.2. Composition of the ethanolic extracts of three microalgae species

Gas chromatography–mass spectroscopy was performed in a system equipped with an electron ionization device (Hewlett–Packard 5890/II GC-5971/A MSD) and a Supelcowax 10 column (60 × 0.25 mm, film thickness 0.25 μm). Carrier gas was helium. Mass spectra were taken at 70 eV.

The identification of compounds was performed through the comparison of their mass spectra with those in Wiley libraries.

#### 2.1.3. Statistical analysis

Experimental results were obtained as mean ± S.D. of five parallel measurements. A multifactorial analysis of the variance (ANOVA) was effected using the data obtained from each extract. $p$ values $< 0.05$ were regarded as significant.

## 3. Results and discussion

### 3.1. Extraction yield

Prior to this work, several authors have optimized the extraction procedure and solvent type to maximize the yield of the antioxidant compounds extraction. Thus, ethanol was selected as the most effective solvent for microalgal biomass extraction with antioxidant compounds extraction purposes (Bazykina, Nikolaevskii, Filippenko, & Kaloerova, 2002).

As a source of natural antioxidants, we selected the three microalgae described previously, on the basis of their high content in phenolic components (phenolcarboxylic acids and their derivatives, catechols, flavonoids, carotenoids, ...).

The yields obtained in three different assays by using ethanol acting on the three biomasses are shown in Fig. 1. No statistical differences were found among the yields of the different microalgal extracts at $p < 0.05$.

### 3.1.1. Antioxidant activity measured by the β-carotene method

The antioxidant activity of carotenoids is based on the formation of radical adducts of carotenoids with free radicals derived from linoleic acid. The linoleic acid free radicals attack the highly unsaturated β-carotene models. In the presence of carotenoids, not only the free radical concentration becomes significantly lower, but there is also a reduction of $Fe^{3+}$ to $Fe^{2+}$ by carotenoids. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralising the linoleate-free radical and other free radicals formed in the system (Jayaprakasha, Selvi, & Sakariah, 2003).

Fig. 2 shows the antioxidant activity of pure compounds (BHT and BHA) and the three microalgal extracts as measured by the bleaching of β-carotene. The AAC was measured at different concentrations 0.5, 1, 1.5, 2 and 2.5 mg $ml^{-1}$. An analysis of the variance suggest that the use of different concentration extracts to measure the antioxidant activity has statistical significance on results ($F$-ratio = 4.65; $p = 0.0081$).

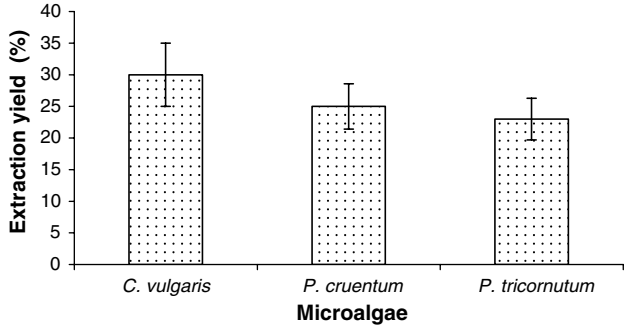


Fig. 1. Ethanolic extraction yields of three microalgae.

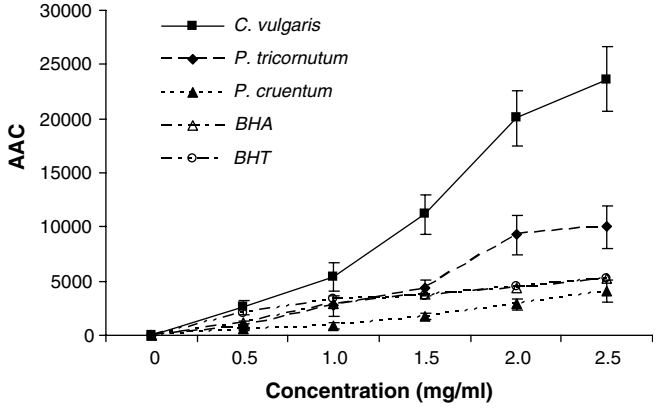


Fig. 2. β-Carotene bleaching assay for BHT, BHA and microalgal solutions at different concentrations (AAC values in ---).

The antioxidant activity coefficient was measured at the same concentrations tested for the compounds above mentioned. The ANOVA statistical analysis indicates that *C. vulgaris* extract AAC differs significantly from the *P. cruemtum* extract AAC ($p < 0.05$).

The applied assay indicates an AAC in the microalgal extracts of *C. vulgaris* higher than the AAC values obtained for BHA, BHT, *P. tricornutum* and *P. cruentum* ($p < 0.0$). In fact, *C. vulgaris* AAC values seems to be among the highest AAC reported up to date in the literature for any biological extract or pure compound tested.

Table 1 shows the composition of the three microalgae ethanolic extracts analyzed by GC–MS. The major components in the extracts of *C. vulgaris* were methyl linolenate, ethyl-9,12-octadecadienoate, ethyl-9,12,15-octadecatrienoate and ethyl linoleolate in the ester fraction, phytol in the alcohol fraction, heptadecane and 1-heneicosene in the hydrocarbon fraction, and hexadecanoic acid and oleic acid in the acidic fraction. *P. cruentum* and *P. tricornutum* showed a similar profile; however it is found a higher quantity of hexadecanoic acid and oleic acid in the acidic fraction and ergosta-4,6,22-triene than in the *Chlorella* spp. fraction.

The high antioxidant activity of *C. vulgaris* could be due to its chemical composition. However, although other

Table 1
Composition of the ethanolic extracts of three microalgae species

| Components | RT[b] | Percentage in the ethanolic extracts[a] | | |
|---|---|---|---|---|
| | | *C. vulgaris* | *P. tricornutum* | *P. cruentum* |
| 1-Heptadecane | 27.4 | 4.5 | 2.8 | 2.1 |
| Neophytadiene | 35.4 | 7.8 | 3.8 | 2.7 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 36.4 | 1.4 | 0.8 | 1.0 |
| Methyl palmitoleinate | 45.3 | – | 3.4 | – |
| Methyl hexadecatrienoate | 48.1 | – | 2.0 | – |
| Methyl eicosa-5,8,11,14,17-pentaenoate | 48.6 | – | 2.1 | – |
| Ethyl-(*Z*,*Z*)-9,12-Octadecadienoate | 53.4 | 1.0 | – | 1.0 |
| Methyl-9,12,15-Octadecatrienoate | 54.3 | 0.7 | – | 1.4 |
| Ethyl-(*Z*,*Z*,*Z*)-9,12,15-octadecatrienoate | 55.2 | 1.6 | – | 1.1 |
| Phytol | 55.7 | 5.5 | 8.4 | 3.1 |
| Methyl tridecanoate | 59.3 | – | 8.2 | – |
| 1-Heneicosene | 59.5 | 7.3 | – | – |
| Ethyl araquidonate | 59.9 | – | – | 9.4 |
| (*E*,*E*,*E*)-1,4,8-dodecatriene | 60.9 | | 7.3 | – |
| Hexadecanoic acid | 63.3 | 6.1 | 19.0 | 7.8 |
| Linoleic acid | 65.3 | 4.1 | – | 1.9 |
| Ethyl linoleolate | 66.9 | 4.8 | – | 3.5 |
| 3-Tetradecen-5-yne | 68.4 | 2.1 | – | – |
| Oleic acid | 68.9 | 6.4 | 20.2 | 12.6 |
| Linolenic acid | 70.4 | 15.5 | 5.0 | 6.7 |
| Methyl linolenate | 72.7 | 23.1 | – | 7.1 |
| Ergosta-4,6,22-triene | 76.3 | – | 5.4 | 18.6 |
| Total identified (%) | | 91.0 | 88.4 | 80.0 |

[a] % Area on total area (100%).
[b] Retention time.

authors have described a high content in sterols in *C. vulgaris* (Rzama, Dufourc, & Arreguv, 1994) our analysis did not show the presence of any of them, which probably could be due to the high polarity of the extracting system that was used in this work. On the other hand, we attribute the high antioxidant activity detected in *C. vulgaris* to its high content of polyunsaturated fatty acids. In this sense, other authors (Cercaci, Passalacqua, Poerio, Rodriguez-Estrada, & Lercker, 2007) have found that no correlation can be established between the antioxidant activity of olive oil and the amount of sterols present in the same one.

Possibly, the additional use of a less polar extracting solvent such as hexane or ethyl acetate would improve the antioxidant activity of the extract, as this could lead to the extraction of some phenolic compounds would be extracted, such as has been previously effected in several *Chlorella* species (Li et al., 2007). Nevertheless, for use in the alimentary industry, biocompatible ethanol seems to be the better option for antioxidant compounds extraction.

## 4. Conclusion

The results of the present study indicate that the microalgal extracts tested in this work possess a higher antioxidant activity than other antioxidants of common use. These microalgae have potential important applications for the pharmaceutical and food industries, especially *C. vulgaris* extracts.

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