E. Muntean, et all. Journal of Agroalimentary Processes and Technologies, Volume XIII, No.1 (2007), 143-148
Full Paper –Natural Food Extract and Additives

POTENTIAL USE OF *MOUGEOTIA SP.* ALGAE IN FOOD PRODUCTION, BASED ON ITS CAROTENOID CONTENT

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Abstract

Mougeotia sp. Agardt originating from the collection of the Institute of Biological Researches Cluj Napoca proved to be a valuable source of carotenoids. High performance liquid chromatography revealed that it contains five provitamin A carotenoids: 5,6-epoxy-β-carotene, α-carotene, β-carotene, 9Z-β-carotene and 15Z-β-carotene. The chromatographic profile of this algal strain is dominated by two major carotenoids: lutein and β-caroten. Chromatographic separations were achieved using a Nucleosil 120 - 5C_{18} column, with a gradient involving the following mobile phases: A - acetonitrile: water = 9: 1 and B - ethyl acetate), the total separation time being less than 20 minutes.
Keywords: *algae, Mougeotia, carotenoids, HPLC, chromatography*

Introduction

The properties of carotenoids as natural pigments have been industrially exploited for a long time. Astaxanthin and canthaxanthin are added to the feed of aquaculture grown salmon, trout and shrimp to provide the characteristic pink color, while lutein and zeaxanthin are used to enhance the yellow pigmentation of eggs and poultry.Carotenoids are also widely used in the cosmetics and pharmaceutical industries, besides other industrial applications as colorants for human food, such as the use of β-carotene in margarines (Fraser, 2004).

The increased interest in the biotechnology of carotenoids has been mainly generated by their health-related antioxidant properties.Dietary carotenoids fulfill essential requirements for human and animal nutrition (Demmig-Adams, 2002; Fraser, 2004); carotenoids with β-

ring end groups taken from the diet act as precursors for the production of retinoids in animal cells. The most effective dietary precursor of vitamin A is β-carotene; its deficiency leads to xerophthalmia, blindness and premature death. Controlling such deficiency in developing countries may require not only vitamin A supplementation but also the introduction of new plant-derived foods with increased provitamin A carotenoids levels (Fraser, 2004).

Carotenoids have the capacity of quenching singlet oxygen, acting as free radical scavengers and antioxidants in vivo, providing thus additional health benefits; an inverse relationship exists between the dietary intake of carotenoid-rich foods such as fruit and vegetables and the incidence of lung, breast, colon, and prostate cancers, UV-induced skin damage, coronary heart disease, cataracts, and macular degeneration (Demmig-Adams, 2002; Fraser, 2004; Handelman 2001; Johnson 2002; Stahl, 2005).

In algal biotechnology, many strains were studied for different purposes, nutritional supplements produced from microalgae being the primary focus of microalgal biotechnology for many years. Dried biomass or cell extracts produced from *Chlorella* (Lee, 1997), *Dunaliella* (Avron, 1992), and *Spirulina* (Vonshak, 1997) have dominated the commercial opportunities. *Spirulina platensis* has been recognized and used worldwide in the food industry; the uses of this valuable alga have risen substantially due to an increased understanding of its biological systems (Voshank, 1997). Later, scientific evidences clearly indicate that carotenoids from *Dunaliella* possess better hepatoprotection compared with those from *Spirulina* (Chidambara, 2005). High performance liquid chromatography of the carotenoids indicated that *Spirulina* contains only *β*-carotene, while *Dunaliella* contains also other carotenoids and xanthophylls. The increase in protection with *Dunaliella* indicates that mixed carotenoids exhibit better biological activity than *β*-carotene alone.

Experimental

The green algae *Mougeotia sp*. *Agardt* (AICB 560) originated from the collection of the Institute of Biological Researches Cluj Napoca. The AICB 560 strain was grown in a Bold nutritive solution that was mixed by introducing air containing 5% CO_2, under continuous

illumination (300 μmol.m^{-2}.s^{-1}, measured with a Hansatech Quantum Sensor QSPAR), at an average temperature of 20°C, for 15 days.

The algal suspension samples (5 ml) were saponified directly for ten hours, using 10 ml solution 30% KOH in methanol, at room temperature. Carotenoids were extracted using diethyl ether; the etheric layer was separated and washed repeatedly with brine, then with distilled water until free of alkali. The aqueous layers were re-extracted with small volumes of diethyl ether until colorless, then the organic layers were combined, washed several times with distilled water and evaporated to dryness under reduced pressure. The saponified extract was dissolved in 10 ml ethyl acetate, being then subjected to HPLC analysis.

High performance liquid chromatography analyses were conducted according to a previous published procedure (Muntean, 2006), using an Agilent 1100 system.

Results and Discussions

Determination of the total carotenoids' concentration in the algal suspension samples lead to a mean value 1.67 μg/ ml suspension, while the biomass concentration was of 0.573 g/l; the concentration of individual carotenoids is presented in the first column of table 1 and in figure 1a.

Table 1. The carotenoid concentrations in algal samples (μg/ ml algal suspension)

Carotenoids	Control sample	Irradiation with 4500 μmol.m^{-2}.s^{-1}	Irradiation with 1500 μmol.m^{-2}.s^{-1}
Violaxanthin	0.02	0.01	0.04
Antheraxanthin	0.10	0.60	1.60
Lutein	1.00	0.56	1.79
Zeaxanthin	0.00	0.05	0.11
5,6-epoxy-β-carotene	0.04	0.00	0.02
α-carotene	0.04	0.01	0.15
β - carotene	0.19	0.03	0.71
9Z - β - carotene	0.04	0.01	0.13
15Z - β - carotene	0.01	traces	0.05

The chromatographic profile of *Mougeotia sp* is a simple one (figure 1a), dominated by only two major carotenoids: lutein and β-

carotene, these being accompanied by the xanthophylls violaxanthin, antheraxanthin and 5,6-epoxy-β-carotene and by the carotenes α - carotene, 9Z - β - carotene and 15Z - β - carotene.

a.

b.

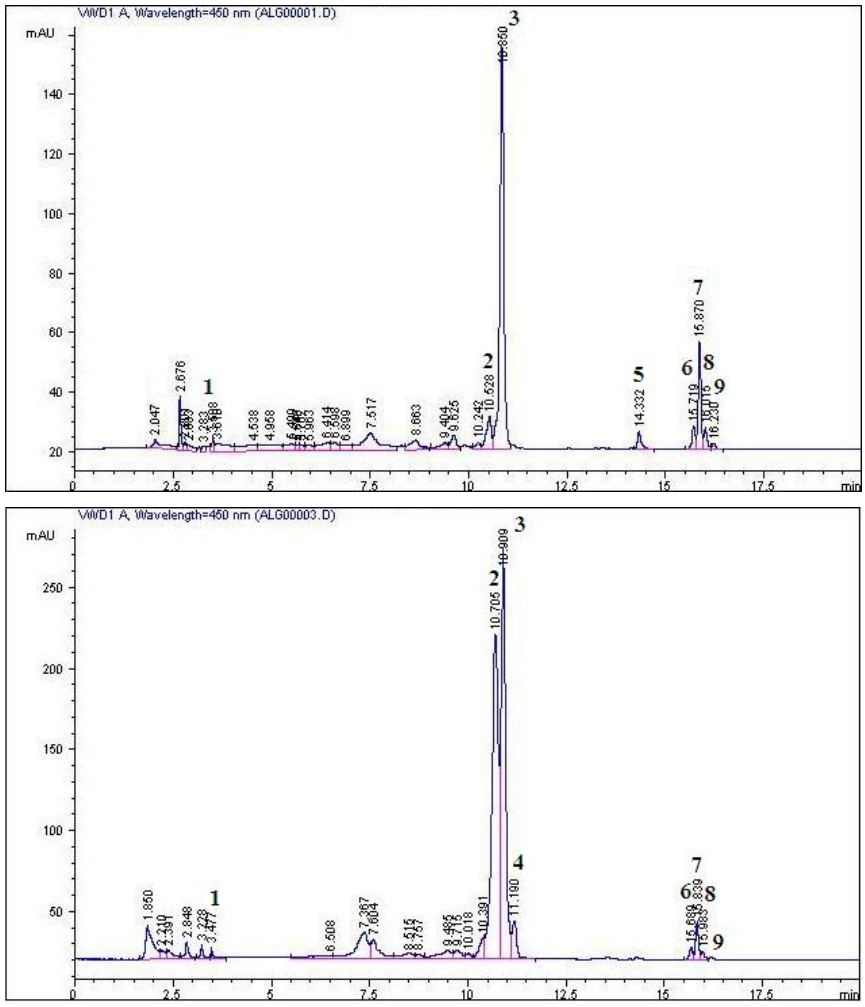

Figure 1. HPLC chromatograms of carotenoids from the *Mougeotia sp.*
a.) control sample; b.) illumination with 4500 μmol.m^{-2}.s^{-1}; Peak identities: 1 - violaxanthin, 2 - antheraxanthin, 3 - lutein, 4 - zeaxanthin, 5 - 5,6-epoxy-β-carotene, 6 - α-carotene, 7- β-carotene, 8 - 9Z-β-carotene, 9 - 15Z-β-carotene.

During the growing experiments it was observed that the carotenoid content of *Mougeotia* is highly dependent by illumination

conditions. When the algal culture was exposed to a high light irradiation (4500 μmol.m^{-2}.s^{-1}), the content of antheraxanthin increased strongly (figure 1b, table 1). The chromatographic pattern shows two major carotenoids: lutein and antheraxanthin, while among minor carotenoids 5, 6-epoxy-β-carotene disappeared. In a second series of tests conducted with irradiation with 1500 μmol.m^{-2}.s^{-1}, the violaxanthin level decreased again and the antheraxanthin concentration is close to that of lutein, while the increase in zeaxanthin concentration is quite small (table 1).

Conclusions

The obtained data demonstrates that the studied *Mougeotia* strain contains important levels of carotenoids to be considered as an ingredient for food production. It contains five provitamin A carotenoids: 5,6-epoxy-β-carotene, α-carotene, β-carotene, 9Z-β-carotene and 15Z-β-carotene, but their levels are low. However, the concentration of lutein was high in all experiments, lutein being a potent antioxidant and an important carotenoid in visual mechanism. The best way of using these algae is in a dehydrated form, obtained preferably by liophilisation – this procedure conserving the biological activity. However, this study is not enough for using *Mougeotia* in food production; further researches will be necessary to prove that this matrix is beneficial for this purpose.

Acknowledgements

This work was possible as a result of funding through 2-CEx06-11-54/26.07.2006 research grant.

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