Small-scale batch technology for production of a natural food dye from green algae

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Abstract
This paper presents a small-scale, batch-type, technology for production of a natural extract which can be utilized as a food dye. This technology was designed based on the laboratory tests performed on *Mougeotia sp.* algae; it allows a simple way for processing the algal biomass into a valuable product for food industry, which is in the mean time an antioxidant and a food dye. The key steps involved in this process are saponification (with a solution of KOH in ethanol), extraction (with diethyl ether) and evaporation; the final product is an ethanolic extract rich in lutein, antheraxanthin and β-carotene, it's carotenoid composition being established by high performance liquid chromatography analysis.

Key words: Algae, food color, antioxidant, *Mougeotia*, extract, carotenoids.

Resumé
L'article présente une technologie discontinue pour produire un extrait utilisable comme colorant alimentaire. Cette technologie a été élaborée à la suite de certains tests de laboratoire qui ont utilisé comme matériau de départ l'algue *Mougeotia sp.* Ce processus assure une manière simple de conversion de la biomasse de l'algue dans un produit valeureux pour l'industrie alimentaire. Ce produit est en même temps colorant alimentaire et antioxydant. Les étapes principales de ce processus sont: la saponification directe de la biomasse de l'algue (avec une solution de KOH en éthanol), l'extraction (avec diethyle-ether) et l'évaporation. Le produit final est un extrait éthylique riche en lutéine, antheraxanthine et β-carotène, dont le contenu en caroténoïdes est déterminé par chromatographie de liquides de haute performance.

Mots cles: Algue, colorant alimentaire, antioxydant, *Mougeotia*, extracte, caroténoïdes.

Rezumat
Lucrarea prezintă o tehnologie discontinuă, la scară mică, de producere a unui extract utilizabil ca şi colorant alimentar. Această tehnologie a fost elaborată în urma unor teste de laborator în care s-a utilizat ca materie primă alga *Mougeotia sp.*, asigurând o modalitate simplă de procesare a biomasei algale într-un produs valoros pentru industria alimentară, care este în acelaşi timp colorant alimentar şi antioxidant.Etapele principale ale acestui proces sunt: saponificarea directă a biomasei algale (cu o soluţie etanolică de KOH), extracţia (cu dietileter) şi evaporarea; produsul final este un extract etanolic bogat in luteină, aneraxantină şi β-caroten, conţinutul de carotenoide al acestuia fiind determinat prin cromatografie de lichide de înaltă performanţă.

Cuvinte cheie: Alge, colorant alimentar, antioxidant, *Mougeotia*, extract, carotenoide.

1. Introduction

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Algae are able to synthesize at low costs many nutrients important for human beings such as vitamins, minerals and antioxidants. As raw materials for food industry, algae are advantageous as they contain high concentration of proteins (up to 70% reported to dry mass) and a relatively high concentration of natural colorants (chlorophylls and carotenoids). Another advantage is that the whole algal body can be used as a food supplement, as it contains also minerals, trace elements and other healthy ingredients. Algae can serve also for production of expensive fine chemicals for cosmetic industry, being additionally a potential source of biologically active substances for medicine and pharmacy. Besides, algae can clean exhaust gases and waste-water, converting contaminated wastes into valuable biomass. Algae can be an alternative to green herbs, useful for feeding animals or as fish feed in aquaculture. Some algae are a potential source of energy, by biofuel production.

Algae are not very demanding; they need only light, CO_2, water and small amounts of nutrients such as phosphates, nitrates and sulphates (Barbosa, 2001). However, the major limiting factor which is the cause that mass production of algae is not applied on large scale is a more efficient system to concentrate and to regulate sunlight (Barbosa, 2005).

Nowadays, most of the algal biomass finds its way as food supplement in western countries or as ingredient in cosmetics. Among carotenoids, astaxanthin is produced on a large scale using *Haematococcus pluvialis* while *Dunaliela salina* is well-recognised as a rich source of β-carotene. Apart of being pigments, these substances are also strong antioxidants. 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).

Whole algae *Spirulina* and *Chlorella* are commercially available as powders or pills in organic food shops. In fact, the major part of the market appreciate especially the whole algae; dried biomass or extracts produced from *Chlorella* (Lee, 1997), *Dunaliella* (Hejazi, 2004; 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).

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. However, for food production it is necessary to isolate the biologically active components, to extract them and to process into pure substances (Hejazi, 2004). This is possible using technologies such as membrane filtration, centrifugation, flocculation or extraction.

This paper presents an original small-scale, batch-type, technology for production of a natural extract which can be utilized as a food dye. This technology was designed based on the laboratory tests performed on *Mougeotia sp*. algae; it allows a simple way for processing the algal biomass into a valuable product for food industry, which is in the mean time an antioxidant and a food dye.

2. Materials and methods

Laboratory scale experiments were conducted using the green algae *Mougeotia sp*. *Agardt* (AICB 560) originated from the collection of the Institute of Biological Researches Cluj Napoca. This strain was grown in a Bold nutritive solution that was mixed by introducing air containing 5% CO_2, under continuous illumination (4500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 colourless, then the organic layers were combined, washed several times with distilled water and evaporated to dryness under reduced pressure. The saponified extract was dissolved

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in 10 mL ethanol, being then subjected to carotenoid analysis.

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

3. Results and discussion

The total carotenoids' concentration in the algal suspension samples lead to a mean value of 1.67 μg/mL suspension, while the biomass concentration was of 0.573 g/L; the concentration of individual carotenoids is presented in table 1, while the corresponding HPLC chromatogram is listed in figure 1.

The chromatographic profile of *Mougeotia* is a simple one (figure 1), dominated by only two major carotenoids: lutein and antheraxanthin, these being accompanied by the xanthophylls neoxanthin and zeaxanthin, and by the carotenes α-carotene, β-carotene, 9Z-β-carotene and 15Z-β-carotene.

Table 1. The carotenoid concentrations in algal samples

Carotenoids	(μg/ mL algal suspension)
Neoxanthin	0.01
Antheraxanthin	0.60
Lutein	0.56
Zeaxanthin	0.05
A-carotene	0.01
β-carotene	0.03
9Z-β-carotene	0.01
15Z-β-carotene	0.01

The obtained extract is dark yellow and has a strong tinctorial power.

Based on the results obtained in laboratory trials, the following technology was designed (figure 2): the algal suspension is feed into a decanting centrifuge (1), where the water content is decreased up to 50%; the resulted sludge is then transferred into a reaction vessel (2), in which saponification occurs in contact with an alcoholic solution of KOH (30%). The reaction last twelve hours, at 20^0C, after which the reaction mixture is transferred into a separator (3); the saponified extract is mixed with diethyl ether, then washed several times using brine and finally with small amounts of water, until the final pH of the hypophase is neutral. The etheric extract is evaporated to dryness into the evaporator (4), the residue being redisolved in ethanol.

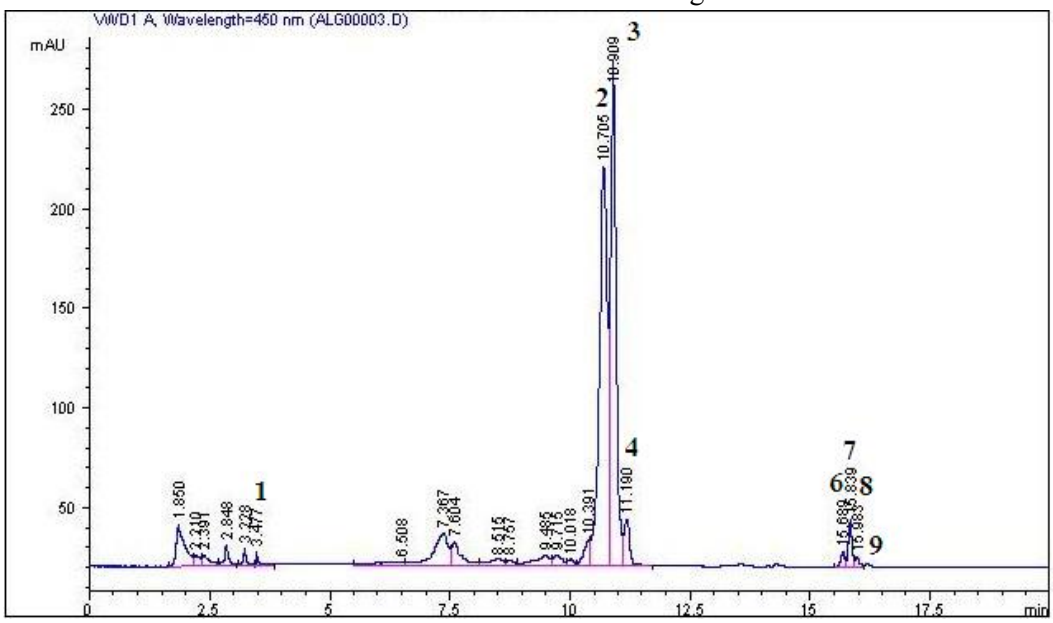

Figure 1. HPLC chromatogram of the total saponified extract obtained from *Mougeotia*.
Peak identities are: 1 – neoxanthin; 2 – anteraxanthin; 3 – lutein; 4 – zeaxanthin; 6 – α-carotene; 7 – β,β-carotene; 8 – 9Z-β,β-carotene, 9 – 15Z-β,β-carotene.

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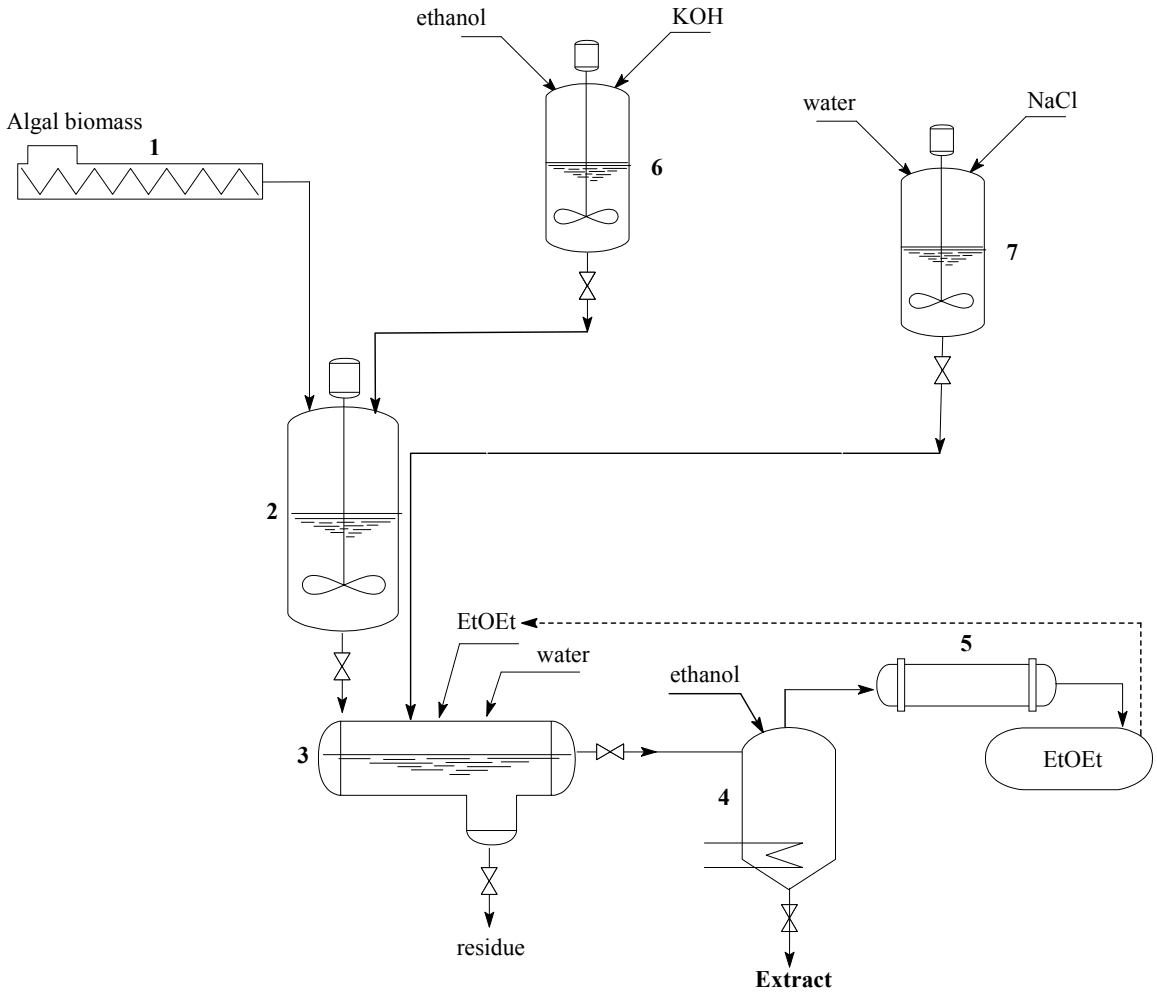

Figure 2. The simplified scheme of a small-scale batch plant for producing natural food dyes from algae

The diethyl ether vapours are condensed in a condenser (5), being then recirculated. The ethanolic KOH solution is obtained in small reactor with a mixing device (6) and in the same manner is obtained the necessary brine (in 7).

4. Conclusions

Nowadays, more and more concern is directed toward natural resources; this project follows the same line, offering a simple way for producing healthy ingredients from algae. The resulted product is in the mean time a food coloring agent and an antioxidant, due to the biological activity of the carotenoids contained. Although the proposed technology was designed based on the laboratory tests performed on *Mougeotia sp*. algae, it can be applied also on other filamentous green algae as it is.

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