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Simulation of Absorption Spectrum of Photosynthetic Pigments of Chlorella Vulgaris B Algae

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ABSTRACT

Absorption spectrum of Chlorella vulgaris B algae was reconstructed by simulated the absorption spectrum of the mixture under examination by a linear combination of the absorption spectra of all its constituents. A C30 reversed high performance liquid chromatography equipped with photodiode array detector (HPLC-PAD) and absorption spectrophotometer were used to separate and identify photosynthetic pigments of Chlorella vulgaris B. Spectrum-reconstruction method (SRCM) was used to calculate concentrations of total carotenoids, chlorophylls a and b. Absorption spectrum of the total pigments extracted of Chlorella vulgaris B. was reconstructed from the sum of the spectrum of individual extracted pigments taken from (HPLC-PAD), and compared using normalization with absorption spectrum of the total pigments recorded by spectrophotometer. A total of seven Pigments were separated and identified

Keywords: Chlorella Vulgaris; Pigment Composition; Multi-Component Analysis; HPLC; Absorption Spectrum

1. Introduction

Chlorella is a unicellular green freshwater algae discovered by a Dutch microbiologist Martinus Beijernick in 1890. There are several species of chlorella, the most commonly used in nutritional are Chlorella pyrenoidosa and Chlorella vulgaris. This microalga is recognized as a rich in protein, chlorophylls, carotenoids, vitamins fatty acids, fiber and minerals (R.A., 1991),(Merchant R. E, 2000),(Merchant R.E, 2001) . Chlorella has been shown to improve the healing of stomach ulcers, duodenal ulcers, chronic gastritis, wounds, reduces the toxicity of chemotherapy in radiation therapy and inhibit tumor in animal models (Tanaka.K, 1998). Moreover Chlorella has been shown to reduce cholesterol, hypertension, prevent infection by boosting the immune system and protection from toxic metals (Yasukawa.K and Takido., 1996),(Simpson, 1989), and ,(Shiro Nakano, 2007) . Using photodiode array detector (PAD) with high performance liquid chromatography permits the immediate identification of the components of a mixture by their spectra characteristics and simultaneous recording of the chromatographic analysis at different wavelengths. The aim of this work is too reconstructed the absorption spectrum of Chlorella vulgaris B. algae from the sum of the spectrum of individual extracted pigments taken from (HPLC-PAD), and compared using normalization with absorption spectrum of the total pigments recorded by spectrophotometer

2. Materials and Methods

2.1 Chemicals

All solvents used were of HPLC grade and were obtained from Merck Chemicals. carotene, xanthophyll, chlorophylls a and b were purchased from Sigma Aldrich.

2.2 Extraction

Liquid culture of Chlorella Vulgaris Beijerinck was obtained from the culture collection of algae unit at the University of Malaya. Prior to extraction, algae suspensions were harvested on glass fiber membrane Whitman GF/F filters under vacuum. The filter was placed immediately in liquid nitrogen and grinding in acetone in dim light to minimize formation of induces trans-cis photoisomerization. The suspension was placing in an ultrasonic bath containing ice for 3 min and then stored at 28^oC for 8 h to achieve optimal extraction. After centrifugation for 2 min at 2000 rpm the supernatant was filter with $45\mu m$

syringe filter, injected into the HPLC system.

2.3 HPLC System

A Perkin-Elmer LC 200 series HPLC system equipped with an autosampler, quaternary pump, vacuum degasser, column oven, and diode array detector, controlled by TotalChrom 6.3 Workstation and Turboscan software. All separations have been performed with Carotenoid C30 column 250 mm 4.6 mm I.D, $5mum$ particle sizes including a 30-guard column (YMC, Japan). Column connections were made with PEEK (poly ether ether ketone) tubing to prevent damaging to carotenoids. The column temperature was controlled within 0.1^oC using Perkin-Elmer Series 200 peltier oven. The temperature of the column was kept at 23^oC. Sample injection was $70ul$, the flow rate of $1.0ml/min$ Separations of pigments were carried out using isocratic mobile phase of 70 methanol and 30 methyl tert-butyl ether (MTBE). Chlorophylls and carotenoids were detected at 431 nm (maximum absorbance for chlorophyll a. The absorption spectra of the eluted fractions were continuously monitored with photodiode array detector on wavelength range 190-700 nm.

2.4 Absorption Spectrum

The absorption spectra of pigments extract of Chlorella Vulgaris, individual extracted pigments taken from (HPLC-PAD), carotene, xanthophyll, chlorophylls a and b in methanol were taken in a spectra rang between 350 and 750 nm with a spectral resolution of 1 nm and the absorbance values were sampled at every 1 nm in standard cuvette ($l = 1cm$) with a Hitachi$UV - 3010$ spectrophotometer interfaced to a personal computer.

2.5 Identification of Pigments

The identification of carotenoids was based on their retention time, the spectral data provided by PAD and absorption spectrum of individual pigment collected from HPLC-PAD compared with standards and the data in literatures.

3. Result and Discussion

Figs. 1 and 2 show chromatogram of ıChlorella Vulgaris pigments extracted in acetone detected at $431nm$, temperature 23^oC and absorption spectra of the peaks of the chromatogram recorded by PAD respectively. The absorption spectra of peaks $1, 2, 4$ of the chromatogram were matched well with violaxanthin, neoxanthin and lutein respectively as in literatures data(Li.H.P, 2002),(Razi Naqvi.K, 1997) ,(Lichtentaler.H.K., 1987) . Retention times and absorption spectra of peaks $3, 5, 6, 7$ agreed well with standard samples of Chl_b, Chl_a, xanthophyll, $\beta - carotene$ respectively. The extracted pigments fromıChlorella Vulgaris and their relative occurrence were shown in table 1. Chl_a was the major pigment present in these extraction followed by lutein, Chl_b, violaxanthin, neoxanthin, xanthophyll,$\beta - carotene$. The concentrations of chlorophylls a, b and total carotenoids C_{Chla},C_{Chlb}, and C_{car} respectively were determined by spectrum-reconstruction method (SRCM) (Razi Naqvi.K, 2004), (Razi Naqvi.K, 1997). SRCM very briefly simulates the absorption of the test sample by a linear combination of the absorption spectra of all its constituents recorded on the same instrument (to avoid errors in the wavelength inaccuracy of the spectrometer). Figure 4 shows the application of SRCM to *Chlorella vulgaris*, the absorption spectrum of the pigment extract of Chlorella vulgaris in acetone and diluted in methanol labeled$A_p(\lambda)$. Absorption spectra of Chl_a and Chl_b prepared in methanol and recorded on the same instrument are denoted by $f_a(\lambda)$ and $f_b(\lambda)$, respectively. Then multiple linear regression analysis is used to find the regulating coefficients in the simulated spectrum $A_s(\lambda) = b_0 + b_1\lambda + b_a f_a(\lambda) + b_b f_b(\lambda)$ to origin spectrum in the $550 - 700nm$ region. Once the best-fit found values of b_a and b_b are used to build the functions $A_Chl(\lambda) = b_a f_a(\lambda)$,$A_{Chlb}(\lambda) = b_b f_b(\lambda)$ and $A_{car}(\lambda) = A_p(\lambda) - A_S(\lambda)$ (carotenoids spectrum). Using the values of the specific coefficients of Chl_a, Chl_b and total carotenoids in methanol from literature [15] we found the ratio of$C_{Chla}/C_{Chlb} = 3.13$ and ratio$C_{Chla}/C_{car} = 3.8$. Spectrum of the extracted pigments of ıChlorella Vulgaris was reconstructed by adding the spectrum of each individual pigment recorded by HPLC-PAD and normalizes at the Soret (blue) peak to absorption spectrum recorded by the spectrophotometer. In Fig4. the two curves looks very similar and the differences can ascribe to the following reasons. First two different solvent used (methanol and mobile phase), and different instruments (PAD and spectrophotometer). Second the sample may contain Chl_c pigments in addition to carotenoids, Chl_a and Chl_b, or degradation of chlorophyll a, which may co-elute with Chl_a. Third package effect, this effect account for the fact that pigments are not in solution, but are packaged within chloroplasts and the relationship between pigment concentrations and absorption coefficients of algae is nonlinear (.A and Bricaud., 1981).

Simulation of Absorption Spectrum

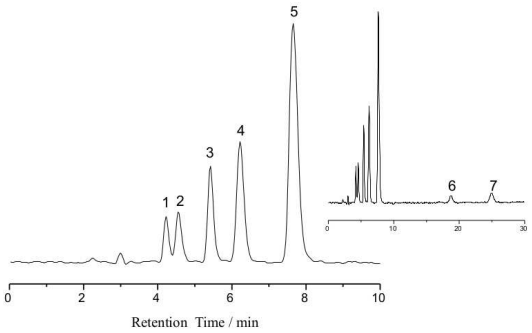

Figure 1: Chromatograms of pigments extract of Chlorella Vulgaris ($\lambda = 431nm, T = 23^oC$).

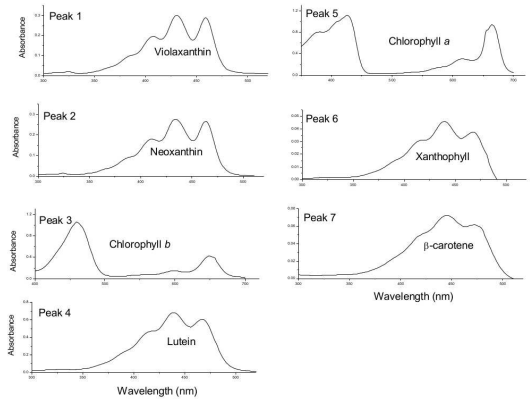

Figure 2: Absorption spectra of pigments extract of Chlorella Vulgaris ($\lambda = 431nm, T = 23^oC$).

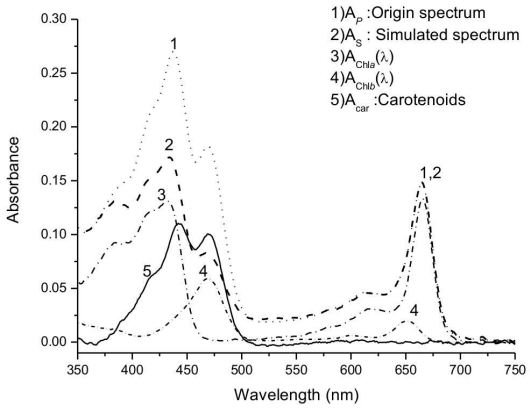

Figure 3: . Absorption spectra of the total pigment extract of Chlorella Vulgaris algae (1), the simulated spectrum (2),Achla (3), Achlb (4), and ACar (5).

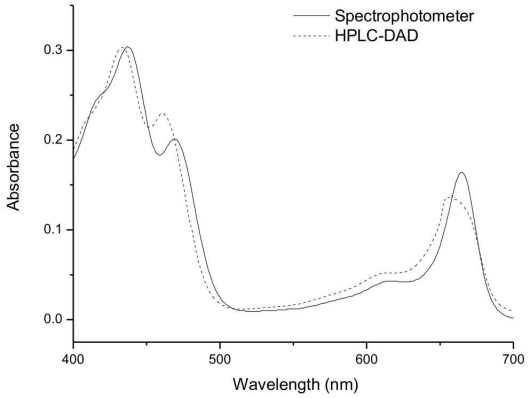

Figure 4: . Comparison of the absorption spectra of extract pigments of Chlorella Vulgaris (solid line) recoded by spectrophotometer and the sum of absorption spectra of extract pigments obtained by HPLC-DAD.

Acknowledgments

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