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Detection in situ of carotenoid in microalgae by transmission spectroscopy

Yongni Shao [a], Jian Pan [a], Chang Zhang [a], Lulu Jiang [b], Yong He [a,*]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China
[b] Zhejiang Technology Institute of Economy, Hangzhou 310018, China

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ABSTRACT

Carotenoids, which can be part of the food additives and drug additive, are one of important internal quality indexes for living microalgae. In the present study, visible/near infrared (Vis/NIR) transmission spectra in situ of *Spirulina sp.* suspension were required using an Ocean Optics USB 4000 Spectrometer in the wavelength range of 346–1038 nm, and the loading weights (LW) analysis, uninformative variables elimination (UVE) and successive projections algorithm (SPA) were used to select important variables related to the carotenoids content (CC) for the *Spirulina sp.* suspension. Different concentrations of 100 samples of *Spirulina sp.* suspension were selected. The results showed the correlation coefficient (r), root mean square error (RMSE) and residual predictive deviation (RPD) in the prediction sets were 0.96, 0.23 mg/L, 3.40, 0.89, 0.39 mg/L, 1.59 and 0.96, 0.24 mg/L, 3.44 for x-LW-PLS, UVE-PLS and SPA-PLS model respectively. It indicated that SPA-PLS gave the best result, while x-LW-PLS was better than UVE-PLS. So, Vis/NIR transmission spectra combined with SPA method was feasible to assess CC of *Spirulina sp.* suspension. And SPA variable selection method can simplify the prediction model and improve the model prediction precision. Furthermore, the method can be used as a good example for the detection in situ of other pigment content in other microalgae.

1. Introduction

Algae are the subject of numerous research projects at an international level and this interest is due to their potential as a source of polyunsaturated fatty acids (PUFA) (Carvalho and Malcata, 2005; Guiheneuf et al., 2009), various carotenoids (Ben-Amotz et al., 1988; Garcia-Malea et al., 2005), proteins, chlorophyll, glycerol and saccharides (Becker, 2007; Fuentes et al., 2000; Yeh et al., 2010). *Spirulina sp.* is a microalgae that belongs to the class cyanobacterium, which can be consumed by humans and other animals. It used as a dietary supplement as well as a whole food, and also used as a feed supplement in the aquaculture, aquarium and poultry industries (Pulz and Gross, 2004). *Spirulina sp.* stands out as an important source of pigments of great commercial value. The major pigments present is β-Carotene. β-Carotene is a precursor to vitamin A and has many possible health benefits. In addition, β-Carotene is a powerful anti-oxidant and possible protector of cells, which can help the human body in many ways.

Conventional methods based on the solvent extraction of carotenoids from natural matrices are time consuming since they require multiple extraction steps and need large amounts of organic solvents, which are often expensive and potentially harmful. Therefore, there is a demand for rapid and cheap analytical techniques.

Near-infrared (NIR) spectroscopy is one of the most potential techniques for the realization of on-field monitoring of the growth and refined management of organism especially in agricultural fields (Liu et al., 2011). Gitelson et al. (2002) assessed the carotenoid content in plant leaves with three spectral bands, 510 ± 5 nm, either 550 ± 15 nm or 700 ± 7.5 nm and the near infrared range above 750 nm, and the RMSE of less than 1.75 nmol/cm^2 was obtained. Baranska et al. (2006) used NIR spectroscopy to determine β-carotene content in tomato fruits, and it showed determination coefficient of 0.85 and 0.80, SECV of 91.19 and 0.41 for lycopene and β-carotene, respectively. Brenna and Berardo (2004) reported the application of NIRS to determine the content of carotenoids in maize, and it indicated good correlations between HPLC values and NIRS estimates. However, there were few reports on using Vis/NIR to detect carotenoids content in aquatic organisms, and not to mention using transmission spectra in situ for microalgae. Wagner et al. (2010) used FTIR spectroscopy to assess quantitative changes in the biochemical composition of microalgae. They built prediction model for total protein, carbohydrate

* Corresponding author. Tel./fax: +86 571 88982143.
E-mail address: yhe@zju.edu.cn (Y. He).

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and lipid contents of microalgal cells based on the partial least square algorithm. Feng et al. (2013) reported the determination of lipid characterization and biomass composition for microalgal based on FT-IR and Nile Red methods. In the paper, it compared these two methods from the comprehensive assessments in both experimental requirement and methodological accuracy, and discussed the choices of methods for different sample sources and processing purposes.

The purpose of this paper were (1) to study the feasibility of Vis/NIR transmission spectra in situ to determine the carotenoids content (CC) in *Spirulina sp.* suspension, (2) to evaluate the relevant variable selection method of loading weights (LW) analysis, uninformative variables elimination (UVE) and successive projections algorithm (SPA), and (3) to compare the prediction performance of *x*-LW-PLS, UVE-PLS and SPA-PLS.

2. Materials and methods

2.1. Algae cultivation and sample preparation

Spirulina sp. was obtained from the freshwater algae culture collection at the Institute of Hydrobiology (FACHB-collection) in China. *Spirulina sp.* was cultivated in 1000 mL Erlenmeyer flasks in *spirulina* medium at room temperature (25 °C), the illumination level was set between 2500 and 3500 Lux for the microalgae culturing platform so as to provide suitable illumination and magnetic stirring. The samples of algae were collected at stationary phase, the OD560 nm of which was 1.487.

The samples with different volumes of 2, 4, 6, 8, 10 (ml) were filled into five 15 ml centrifugal tubes, and then they were diluted with distilled water with volume of 8, 6, 4, 2, 0 (ml) respectively to get 10 ml samples at 5 concentration levels. In our investigation, each concentration level was duplicated multiple times and eventually there were 20 samples prepared for each concentration level, and in total there were 100 samples investigated. For each sample, the spectrum was collected, and then each algal suspension sample was allocated into 3 centrifugal tubes with volume of 3 ml for carotenoids content (CC) measurement, and the measurement of CC was described in detail on chapter 2.2.

2.2. Measurement method for carotenoids content

The carotenoids content (CC) was measured with the solution extracted from the algal suspension which was conditioned by ethanol (95%, v). The specific measurement procedure was as following (Zhu et al., 2010):

a. In order to improve the centrifugal effect on algal suspension, add 7 ml water into each prepared sample to make the suspension volume reach 10 ml, then this mixture was centrifuged at 9000 r/min for 10 min (Neofuge 15R, Heal Force, Shanghai, China), and eliminate the supernatant from the tube after the sample settled to steady state.
b. 3 ml heated ethanol (95%, v) (under 75 °C) was added to each sample from previous procedure, then ultrasonic technology was used to break algal cells for 30 min, and finally bring tubes into the dark and let it stand for 12 h.
c. Centrifuge these samples again at 5000 r/min for 5 min to separate the extracted solution from the algal cells.
d. The absorbance of extracted solution was measured at 450 nm with Spectrophotometer (UV2450, Shimadzu, Japan) (Katrangi et al., 1984).

Eventually, the CC was estimated using the equations of Higby (1962) and expressed on a suspension volume basis (mg/L).

$$CC = \frac{A \times 100}{25 \times L \times W} \quad (1)$$

Where CC (mg/L) is the concentration of carotenes in original sample, A is the absorbance value, L is cuvette length in cm, and W is the original sample per ml of the final dilution measured.

2.3. Spectral acquisition

A spectral acquisition system was build for this investigation, and it consists of a portable spectrometer (USB4000, Ocean optics INC, USA) which has transmitting and receiving fiber bundles connected by a fiber optic probe, a halogen light source, and a computer. Because of the non homogeneous of algal suspension, we used transmission mode instead of reflectance mode to collect the spectral data. At the same time, there was also another benefit to use transmission mode, it can help to minimize the influence due to the background of light. When using the transmission mode, the depth of the probe into the algal suspension can be adjusted, which is preferable for the objective with non homogeneous. We also used window curtains to reduce the influence caused by environmental light source. And 5 mm optic distance was selected for the spectral collection. During the experiments, the depth of the probe into the algal suspension kept exactly the same. And all the algal suspension samples were shook up to be well distributed before spectral data collection and carotenoids content measurement. Before collecting the spectral data, the instrument was calibrated by transmission through distilled water according to the manufacturer's instruction, which help to eliminate the influence of the background light. The transmission spectral data were collected at ambient temperature between 23 °C and 26 °C and in the range from 346 nm to 1038 nm at intervals of 0.3 nm. Each spectrum was recorded as average of 5 spectra with the integration time of 13 ms, and they were measured successively by immersing the probe in *Spirulina sp.* suspension with fixed depth, as shown in Fig. 1. The transmission of *Spirulina sp.* suspension was automatically adjusted for the distilled water reference and processed instantly in a laptop computer by RS3TM Spectral Acquisition Software (v. 5.6, Analytical Spectral Devices Inc., Boulder, CO, USA) attached to the USB4000 instrument, and saved for future retrieval using Unscrambler®9.8 (CAMO AS, Oslo, Norway). Before the calibration stage, the Vis/NIR spectra data were preprocessed. Moving average smoothing was used to reduce noise with a segment size (for averaging) of 35.

2.4. Selection of the effective wavelengths (EWs)

Normally, the full spectra might contain hundreds of variables; therefore, removing uninformative variables is an important approach and strategy to get better prediction and simple but effective models. Using optimum wavelengths might be more efficient than using full wavelengths in multivariate analysis (Wold et al., 1996), and in our study, UVE and SPA were used to select the EWs. UVE-PLS is a method for variable selection, and to eliminate the variables that have no more information for modeling than noise. This method evaluates the reliability of each variable in the model based on analysis of regression coefficients of PLS and selection criterion (leave-one-out validation). It has been widely applied in analytical chemistry for removing the low-frequency varying background and the high-frequency noise (Chen et al., 2005). Details of the UVE-PLS algorithm could be found in the literature (Center et al., 1996). SPA is a forward selection method which comprises three phases (Araujo et al., 2001). It starts with one wavelength, and then incorporates a new one for each following iteration until a specified number of wavelengths are reached (Bao et al., 2012). With SPA, the informative variables