Algal Research 32 (2018) 193–200

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

An integrated biorefinery process: Stepwise extraction of fucoxanthin, eicosapentaenoic acid and chrysolaminarin from the same *Phaeodactylum tricornutum* biomass

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ARTICLE INFO

Keywords:
Phaeodactylum tricornutum
Stepwise extraction
Fucoxanthin
Eicosapentaenoic acid
Chrysolaminarin

ABSTRACT

The cultivation of microalgae is a high energy consumption process, which consumes a large amount of water, nutrients, electric energy and manpower. Thus, comprehensive utilization of algal biomass is a key to achieving cost-effective industrial production of bioproducts. In this paper, an integrated biorefinery process was conducted on *Phaeodactylum tricornutum* biomass to produce three valuable bioactive compounds *via* stepwise extraction using different solvent systems. Fucoxanthin, highly concentrated eicosapentaenoic acid (EPA), and chrysolaminarin were successively purified, concentrated, and characterized from *P. tricornutum* with a series of separation and identification technologies, and the yield (the weight of the purified compounds/the absolute weight in algal biomass, %) of these active compounds were 34.03 ± 0.72%, 23.00 ± 0.29%, and 43.54 ± 0.91%, respectively. Moreover, the fucoxanthin extraction conditions were also optimized, and ethanol and microwave-assisted treatments of 1 min provided the best fucoxanthin yield. In conclusion, this study suggested an effective biorefinery process for the production of fucoxanthin, EPA, and chrysolaminarin from the same *P. tricornutum* biomass.

1. Introduction

Most microalgae are photoautotrophic growth. They convert nutrients and carbon dioxide into proteins, carbohydrates, and lipids *via* solar energy. They also biosynthesize pigments, polysaccharides, polyunsaturated fatty acids, phycobilins, tocopherols, and sterols that are widely used in food, cosmetics, medicine, and aquaculture due to their unique nutritional benefits [1,2]. Therefore, special attention has been paid to microalgal biomass in both the production of biofuels and value-added bioactive compounds. The microalgal biomass is an important biorefinery feedstock [3]. For instance, a biorefinery process was recently proposed to make biofuels production economically viable through comprehensive utilization of microalgal biomass [4].

As the name implies, a biorefinery converts biomass into various end products *via* different technologies while maximizing the value from different biomass components [2]. Williams and Laurens [5] emphasized that the production of microalgae biofuel is unlikely to be economical unless all of the biomass components are utilized. Consequently, many studies have designed a microalgae-based integrated process to make full use of the microalgae biomass to produce multiple end products—especially for some high-value bioactive compounds [6–8].

Phaeodactylum tricornutum is a microscopic marine diatom belonging to the class Bacillariophyceae of Heterokontophyta. It contains about 36.4% crude protein, 26.1% carbohydrates, 18.0% lipids, 15.9% ash, and 0.25% neutral detergent fiber on a dry weight basis under normal growth conditions [9,10]. Most importantly, it is rich in fucoxanthin, eicosapentaenoic acid (EPA), and chrysolaminarin. Many studies have shown that these three high-value natural bioactive compounds have extensive beneficial effects on human health; thus, they possess broad application prospects as pharmaceuticals [10,11].

Fucoxanthin is a major carotenoid in brown seaweeds, haptophytes, and diatoms [12]. It usually combines with chlorophyll *a*/*c* and apoprotein to form fucoxanthin-Chl*a*/*c*-protein complexes (FCP) in the thylakoid. This transfers light energy into chlorophyll-*a* of the photosynthetic reaction centers for photosynthesis [13]. Fucoxanthin shows extensive pharmacological bioactivities including antioxidant, anticancer, anti-diabetic, anti-obesity, anti-photoaging, anti-angiogenic, and anti-metastatic activity [14,15]; however, its low extraction efficiency and difficult synthesis limits its applications.

EPA is an ω-3 polyunsaturated fatty acid (PUFA); these fatty acids are important for human nutrition and disease prevention. They can

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https://doi.org/10.1016/j.algal.2018.04.002
Received 22 November 2017; Received in revised form 1 April 2018; Accepted 1 April 2018

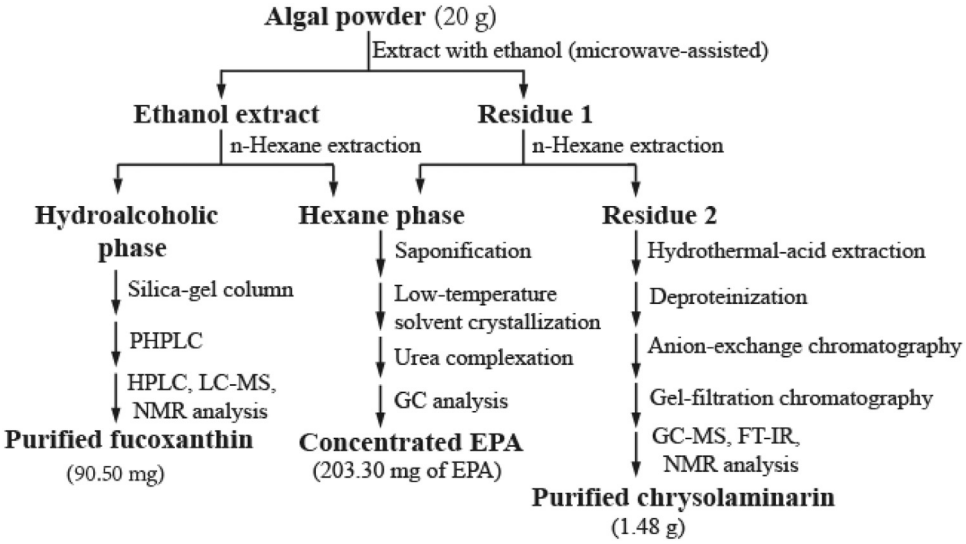

Fig. 1. Flowchart of extraction, isolation, purification and identification of three bioactive compounds from *P. tricornutum*. HPLC, high-performance liquid chromatography; PHPLC, preparative high-performance liquid chromatography; LC-MS, liquid chromatography/mass spectrometry; NMR, nuclear magnetic resonance; EPA, eicosapentaenoic acid; FAMEs, fatty acid methyl esters; GC–MS, gas chromatography/mass spectrometry; and FT-IR, Fourier transform-infrared spectroscopy.

lower serum triacylglycerol and cholesterol levels and increase membrane fluidity and have potential applications in cancer and AIDS [16]. EPA is found in fish oil, but collecting EPA from microalgae is renewable and odorless [17].

Chrysolaminarin (β-1,3-glucan) is the principal storage polysaccharide in the diatom [18]. It is a relatively short-chained, water-soluble (1 → 3)-linked β-D-glucopyranan (DP 20–60) with occasional branching through C-2 and/or C-6. It is relatively stable and is located in cellular vacuoles [19]. It also exhibits anti-tumor bioactivity and can inhibit the growth and reproduction of human colon cancer cells [20]. Although previous studies have isolated fucoxanthin, chrysolaminarin, and EPA from *P. tricornutum* biomass [10,21,22], none of them considered comprehensive utilization of microalgal biomass but rather focused on acquiring a single active fraction, while the valuable components remaining in the biomass were undervalued and lost.

Solvent extraction is a traditional extraction method and has been widely used in the isolation of bioactive compounds from microalgae [23,24], but it is time-consuming. In order to extract the target active compounds efficiently from natural sources, the solvent solubility parameter is the primary factor that needs to be considered. The Hansen solubility parameter (HSP) is an important numerical estimation used for evaluating the solubility of active compounds in different solvents [25]. This could provide the most suitable bio-based solvent, reducing the solvent selection process and waste generation. Sánchez-Camargo et al. [26] reported that ethanol was a suitable bio-based solvent for good recovery of fucoxanthin from *P. tricornutum* on the basis of HSP calculations. In addition, according to the reports by Castro-Puyana et al. [27] and Gilbert-López et al. [28], ethanol was also selected as the best bio-based solvent in extraction of carotenoids from algae. Therefore, ethanol was used as the extraction solvent in the first extraction step in this study. Meanwhile, microwave-assisted pretreatment was also conducted to reduce the extraction time and increase the extraction efficiency. In this study, an appropriate solvent system was designed, optimized, and applied for maximum extraction of more bioactive compounds from the same microalgal biomass. This paper describes the extraction of fucoxanthin, chrysolaminarin, and EPA from *P. tricornutum via* an integrated biorefinery approach with a set of successive and effective solvent systems, to find a feasible method for utilizing all of the microalgal biomass. This can broaden the potential applications of *P. tricornutum*.

2. Materials and methods

2.1. Organism and culture medium

The diatom *Phaeodactylum tricornutum* was obtained from Institute of Oceanography, Chinese Academy of Sciences, China. The stock culture was maintained in 250 mL flask with the modified L1 (mL1) medium [29] in our laboratory. The alga was first grown in column photobioreactors (6 cm inner diameter and 60 cm length) for seed culture at room temperature (25 ± 1 °C) with the unilateral illumination intensity of 70–100 μmol photons/m^2/s. This culture was used to inoculate a vertical flat-plate glass photobioreactor (240 cm length, 120 cm height and 6 cm width) for large volume culture with mL1 medium. Finally, the alga was harvested at the exponential phase with a biomass concentration of 2.35 ± 0.12 g/L using cylindrical centrifuge (Kubota, Japan) at 1940 × *g* for 10 min. This was lyophilized in a vacuum freezer-drier (Christ, Germany). The content (wt%) of fucoxanthin, EPA, chrysolaminarin in the algal powder were determined in the preliminary study, and they were 1.33 ± 0.03%,4.42 ± 0.06%, and 17.00 ± 0.35% of dry weight, respectively. The algal powder was stored at −20 °C prior to extraction.

2.2. Design of stepwise extraction and isolation procedures

A sequentially selective extraction method was designed for the extraction, isolation, and purification of fucoxanthin, chrysolaminarin, and EPA from *P. tricornutum*, and the detailed extraction processes are described below (Fig. 1).

2.3. Extraction, purification and identification of fucoxanthin

2.3.1. Optimization of fucoxanthin extraction

Some groups have reported that microwave-assisted treatment can improve the solvent extraction efficiency [30,31]. Thus, the microwave treatment was conducted on *P. tricornutum* biomass to enhance the yield of fucoxanthin. To avoid the loss of fucoxanthin caused by over-heating, each continuous interval microwave processing time was optimized. Freeze-dried powder of *P. tricornutum* (20 g) was first suspended into 400 mL of ethanol and subjected to microwave-assisted pretreatment on a EG720KG4-NA Midea microwave oven with a continuous microwave nonpulsed power supply (microwave power of 700 W, microwave frequency of 2450 MHz, Foshan, China). A 1000 mL beaker containing the mixtures of algae and ethanol was placed into microwave oven for different microwave treatments: time 0 min (control), 0.5 min, 1 min or