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Research Article

Nutritional Evaluation of Edible Freshwater Green Macroalga *Spirogyra varians*

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Abstract

Freshwater macroalgae are important sources of bioactive natural substances. Macroalgae accumulate specific secondary metabolites which are high value products that have applications in the cosmetic, food and pharmaceutical industries. Macroalgae *Spirogyra* is being used as nutrient dense foods and sources of fine chemicals. The freshwater alga *Spirogyra varians*, a filamentous charophyte, collected from the slow running stream, was identified on the basis of morpho-anatomical characters. In this study, a novel strategy that employs a single macroalgae *S. varians* grown in a natural habitat as a platform to produce pigments, protein, rich carbohydrate and lipid biomass, as desired, was introduced. The pigments, phenolic content and mineral composition showed the possibility of using them as food supplement for human. The total content of protein (% dry weight) ranged from 12.0 % to 24.4%; carbohydrate from 42.8% to 62.0% and lipid from 14.8% to 21.0%. Therefore, the study results suggested that *S. varians*, could be used for nutritional, pharmaceutical and cosmetic products.

Keywords phenolic content, pigments, minerals, nutrition, *Spirogyra varians*

Introduction

Algae have critical functions of energy cycle in nature ecosystem as well as in human society, and their biomass is widely applied for the production of pharmaceutics, food, bioactive compounds and bioenergy application (Ramaraj et al. 2010; Ramaraj et al. 2014a; 2014b). Since algae can be one of the most important crops with efficient energy conversion and a significant niche of food web structure in ponds, lakes and reservoir (Ramaraj et al. 2015a; 2015b). Research on this macroalga has reported several biotechnological applications. They are a significant source of human food, especially in Asian countries. Macroalgae can be classified as red algae (Rhodophyta), brown algae (Phaeophyta) or green algae (Chlorophyta), depending on their nutrient and chemical composition (Dawczynski et al. 2007).

Many macroalgae species have been used in the industry, principally for the extraction of phycocolloids (algin, carrageenan, and agar) and as a source of pharmaceutical substances. They have been used as ingredients in both medicinal and food preparations, traditionally in different regions across the world (Chandini et al. 2008). Macroalgal species are commercially utilized worldwide and consumed as human food. Generally, products from macroalgae were considered as low calorie foods with high contents of minerals, vitamins, proteins and carbohydrates (Kumari et al. 2009). Freshwater macroalgae are a rich source of structurally novel and biologically active metabolites. Primary or secondary metabolites produced by these organisms may be potential bioactive compounds of interest in pharmaceutical industry (Kamenarska et al. 2000). To date, many chemically unique compounds with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals.

Fresh water macro-algae *Spirogyra* is a genus of filamentous green algae of the order Zygnematales. *Spirogyra* is commonly known as pond silk, water silk, or mermaid's tree because of their bright green silky look; and natural growth in freshwater habitats such as clean eutrophic water, small stagnant water bodies, streams, shallow water, ponds and rivers. There are more than 400

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species of *Spirogyra* existing in the world (John et al. 2002). In Thailand, an edible freshwater alga, *Spirogyra* (known locally as Tao), has been considered a source of food for the north and northeast of Thailand, since *Spirogyra* consists of proteins, lipids, carbohydrates, dietary fibers, multivitamins, and mineral substances. Factor such as cell size and shape, rate of ingestion, digestibility and biochemical composition determine the nutritive quality of algae and their utility as food. *Spirogyra varians* is common in northern Thailand. Consequently, this study was to examine the *Spirogyra varians* characteristic identification, pigments, protein, carbohydrate, lipid, Phenol content and minerals.

Methodology

Plant materials for identification

The freshwater macroalgae samples collected from slow running fresh water stream at Pangyang, MaeTeang district, Chiang Mai Province, Thailand, were transferred to 2 L labelled transparent plastic bottles. Same sampling location was used for entire experiment. Samples collected by traditional method using bamboo stick (Figure 1), were transported to the plant physiology and technology laboratory, program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai-50290, Thailand, for species identification in March 2015. The algal species was washed with distilled water to remove macro/microscopic contaminations. The algal samples were observed under light microscope and were then visualized with a Nikon Eclipse 80i microscope and photographs were taken with attached digital camera. Macroalgae were identified by comparing the morphology with taxonomic keys and literature (Prescott 1951; Randhawa 1959, Vidyavati 1995, Kargupta and Jha 2004, Taft 2009). According to its morphology and macro/microscopic observations, it was identified as *Spirogyra varians* (Figure 2 and Figure 3).

Harvest and sample preparation

A total of 10 kg of wet weight of *Spirogyra varians* was harvested (Figure 2), in March 2015. The algae were exhaustively rinsed immediately with freshwater to remove sand and debris. Upon arrival at the laboratory, the algal samples were washed again with distilled water and manually sorted to remove epiphytes. The total and homogeneous samples were dried in continuous air flow (35 °C, 72 h). The dried alga was then milled in a mechanical grinder for 5 min to obtain a fine and homogeneous powder. The powder was stored in hermetic bags in a dry and dark area at room temperature (25 °C) until use.

Figure 1. Traditional harvesting method

Figure 2. Harvested Macroalgae sample

Pigments extraction processes and analysis

Spectrophotometric method was performed for pigment determination of macroalgae extract. Weighed samples, having been put separately in 96% methanol (50 ml for each gram), were homogenized with homogenizer at 1000 rpm for one minute. The homogenate was filtered and was centrifuged at 2500 rpm for ten minutes. The supernatant was separated and the absorbance were

read at 400-700 nm on Schimadzu spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific). It was recorded that chlorophyll-a showed maximum absorbance at 666 nm, chlorophyll-b at 653 nm and total carotene at 470 nm. After extraction, pigments concentration was determined spectrophotometrically and chlorophyll (Chl) content (Chl-a, Chl-b, Total Chl-(a+b)) and total carotene was calculated according to the formulas of Lichtenthaler and Wellburn (1985). Following formulas were used:

Chl-a = $(15.65 \times A_{666} - 7.340 \times A_{653})$

Chl-b = $(27.05 \times A_{653} - 11.21 \times A_{666})$

Total Chl-(a+b) = chlorophyll-a + chlorophyll-b

Total carotene = $(1000\ A_{470} - 2.860$ Chl-a $- 129.2$ Chl-b/245)

Protein measurement

Nitrogen content was determined by Kjeldahl method and analytical procedure was adopted from the standard method (APHA-AWWA and WPCF, 2005). The findings were expressed in percent of dry weight. Protein content was measured by multiplying the nitrogen content of dried algae samples by a conversion factor 6.25 (Ryan et al., 2010).

Carbohydrate estimation

The total carbohydrate was estimated by phenol-sulphuric acid method according to Dubois et al. (1956) with glucose as standard.

Lipid estimation

According to Bligh and Dyer (1959), the lipids were extracted and total lipid content was determined gravimetrically.

Total phenolic content determination

The concentration of total phenols in the extracts was determined by UV spectrophotometer using using Folin-Ciocalteu method as described previous investigators (Gao et al. 2000; Kumar et al. 2015).

Minerals analysis

Samples for mineral analysis were dissolved in hydrochloric acid and analyzed through Atomic Absorption Spectrophotometer (AAS) following the procedures described by (AOAC, 2000).

Statistical Analysis

All the values or readings are the result of mean of three replicates. Data is reported as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel.

Results and Discussion

Morphological identification of alga

Spirogyra is an unbranched filamentous alga. It is commonly occurring in freshwater habitats of Thailand. *Spirogyra* species are characterized by spirally coiled chloroplasts and sexual reproduction by means of conjugation. Vegetative growth of *Spirogyra* can be recognized by three characteristics: (1) type of cross walls (plane, replicate, semi-replicate or colligate), (2) cell length and width and (3) chloroplast numbers (Prescott 1951; Randhawa 1959, Vidyavati 1995, Kargupta and Jha 2004, Taft 2009). The morphology of algal specimen was studied carefully and the specimen was identified as *Spirogyra varians* (Hassall) Kützing. Figure 3 shows the morphology of *S. varians* observed under a light microscope.

Systematic classification

Kingdom - Plantae
Division -Algae
Order-Conjugales/Zygnematales
Family- Zygnemataceae
Species -Karnalae
Sub Kingdom -Thallophyta
Class- Chlorophyceae
Sub-order- Zygnemideae
Genus- Spirogyra
Species - *Spirogyra varians* (Hassall) Kützing

The taxonomic description and identification characters were as follows: vegetative cells 30-70 μm long, 30-38 μm wide; one or several chloroplasts band-shaped, spirally arranged within the cell, pyrenoids present; septum with or without folded structure; conjugation scalariform; female gametangium swelled toward male gametangium; zygospores ellipsoidal or broad ellipsoidal or spherical in shape (45-65 μm long, 30-35 μm wide).

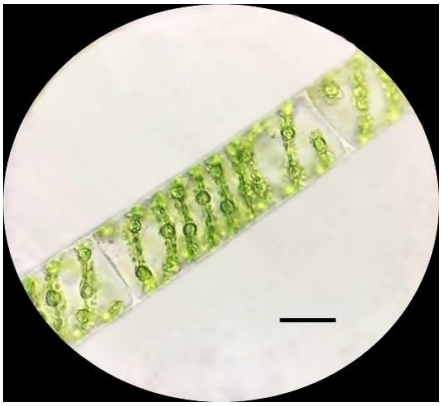

Figure 3 Microscopic pictures of macroalgae *Spirogyra varians*

Pigments of S. varians

In general, *Spirogyra sp*. contains chlorophyll-a and chlorophyll-b which are responsible for its green color. However, in some culture/stress conditions, the macroalga appears yellow or orange due to the presence of secondary pigments (carotenoids). Chlorophyll is essential for photosynthesis, since it absorbs light energy, which is subsequently converted into chemical energy bound in biomass. Conventionally biologists, ecologists and industrialists consider biomass measurement. Amount of algal Chl/carotenoids and its efficiency varies depending on the algal species, taxonomic composition and physicochemical and biological factors (Ramaraj et al. 2013). To measure the chlorophylls and carotenoids contents, the absorption of 96% methanol extracts from *S. varians* was detected with a spectrophotometer. Results are presented in Table 1. Chl-a, Chl-b, total chlorophyll and total carotenoids results were average as 6.0 ± 0.22 μgmL^{-1}, 3.0 ± 0.03 μgmL^{-1}, 9.0 ± 0.19 μgmL^{-1} and 1.8 ± 0.17 μgmL^{-1}, respectively.

Chlorophyll is an essential and valuable bioactive compound that can be extracted from algal biomass. It is used not only as an additive in pharmaceutical and cosmetic products but also as a natural food coloring agent. Additionally, it has antimutagenic properties (Hosikian et al. 2010). Chlorophyll and its derivatives also have profound antioxidant properties (Lanfer-Marquez et al. 2005). Chlorophyll derivatives such as pheophorbide and pheophytin b have always been known as strong antioxidants (Hosikian et al. 2010). Therefore, chlorophyll has a wide range of applications due to its coloring effect, antioxidant and antimutagenic properties. Macroalgae, *S. varians* seems to be a promising alternative source for chlorophyll.

Table 1: Chlorophylls and total carotenoids contents in *Spirogyra varians*

S. No.	Parameter	Mean (µg/ml)	Range (µg/ml)
1	Chlorophyll-a	6.0	5.7 - 6.2
2	Chlorophyll-b	3.0	3.0 - 3.1
3	Total chlorophylls	9.0	8.8 - 9.2
4	Total carotenoids	1.8	1.7 - 2.0

As freshwater macroalgae were grown under various physicochemical conditions (e.g., high light, rapid temperature changes, and drought), and various carotenoids are produced to adapt to the environmental conditions, in this study, we focused on total carotenoids estimations. Carotenoids are compounds with pharmaceutical, high industrial and economical value, for example, the astaxanthin is recognized as a potential anti-oxidant, and could prevent cancer and cardio-vascular problems (Giordano et al. 2012). Accordingly, one of the main advantages of the use of macroalgae as a carrier of carotenoids is their positive impact on human health.

Biochemical composition of S. varians

The protein, carbohydrate and lipid content of *S. varians* are shown in Table 2. Many algae have the ability to produce protein in reasonable amount of their dry weight, making them good protein sources for organic fertilizer, animal feed and human nutrition supplement (Dawczynski et al. 2007). Protein is the most important component contributing to the nutritional value of food. Measurements of nitrogen by Kjeldahl or total nitrogen by elemental analysis are less susceptible to interferences, and nitrogen-to-protein conversion factor quantification was reliable measurement for algal protein (González López et al. 2010). Accordingly, *S. varians* protein content was estimated by Kjeldahl method (APHA-AWWA and WPCF, 2005; Ryan et al. 2010), quantitative

analysis of protein content ranged between 9.47% and 14.68%. Analyses of total protein in algae are often done in order to search new sources of protein supplements.

Chemically, carbohydrates are molecules that are composed of carbon, hydrogen and oxygen, made up of sugars, starches, cellulose and lignin. Carbohydrate is an essential component for metabolism and metabolic processes. Wijesekara et al. (2011) stated that algal carbohydrates can provide human health benefits in the form of anticoagulants, antivirals, dietary fibers and antioxidants. The types and abundance of carbohydrates vary strongly between algae species (Chennubhotla 1996; Dawczynski et al. 2007). Carbohydrate content of *S. varians* ranged from 10.63% and 28.58% along with the maximum carbohydrate content was observed.

Table 2: Total protein, carbohydrate and lipids contents in *Spirogyra varians*

S. No.	Parameter	Mean ± SD (%)	Range (%)
1	Total protein	16.7 ± 1.5	12.0-24.4
2	Total carbohydrates	55.7 ± 2.4	42.8-62.0
3	Total lipids	18.1 ± 0.7	14.8-21.0

In general, macroalgae showed considerable lipid contents, and the total lipid content in *S. varians* was between 14.8% and 21.0%; their polyunsaturated fatty acid (PUFA) contents are superior to those of the terrestrial vegetables. They are rich in C18 and C20 PUFAs with nutritional implications and are thus, studied extensively for biotechnological, food, feed, cosmetic and pharmaceutical applications (Darcy-Vrillon 1993; Chandini et al. 2008). Long-chain n-3 PUFAs, such as EPA and DHA, have various beneficial clinical and nutraceutical applications (Kumari et al. 2009).

Total Phenolic and mineral content of S. varians

Phenolic compounds are considered as one of the most important classes of natural antioxidants. Chemically, polyphenols can be divided into several classes, including phenolic acids, flavonoids, isoflavonoids, stilbenes, lignans, and phenolic polymers (ex. tannins). These compounds such as flavonoids, phenolic acids, and tannins are considered to be main contributors to the antioxidant activities (Ferguson et al. 2004; Manach et al. 2004; Cornish and Garbary 2010). Furthermore, these antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities (Machu et al. 2015). Phenolic compounds play an important role in algal cell defense against abiotic and biotic stress. The total phenolic content of *S. varians* was evaluated as gallic acid equivalents (GAE) in miligram per gram (mgg^{-1}). Total phenolic content of *S. varians* was analyzed using the most appropriate extraction method considering the strenuousness of the laboratory procedure and cost-effectiveness. In this study, the total phenolic content was shown 44.52 ± 1.08 mg g^{-1} in methanolic extracts.

Mineral elements play an important role in regulating many vital physiological processes in the human body, such as regulation of enzyme activity (cofactor or metalloenzyme), skeletal structures (e.g., calcium and phosphorus), neuromuscular irritability and clotting of blood (calcium). Generally, the trace minerals are found in macroalgae (Matanjun et al. 2009). Commonly, environmental features of each region such as the salinity, temperature and pH of the water, sampling seasonality and age of the fronds influence the uptake and accumulation of the mineral and trace elements. In this study, mineral content of macroalgae *S. varians* was demonstrated as calcium 445.9±0.1 mg/100g, magnesium 366.7±0.7 mg/100g, iron 141.3±0.2 mg/100g, zinc 5.2±0.4 mg/100g and copper 2.1±0.6 mg/100g. The amounts of calcium and magnesium contents were higher in *S. varians*. Based on the results obtained from the present study, it was verified that important minerals contents are available in *S. varians*. Our finding reveals that *S. varians* may act as an important source of minerals, which are essential for human nutrition.

Conclusions

The freshwater algae used in this study were identified as *Spirogyra varians*, filamentous green algae. The edible macroalgae, *S. varians*, green algae commonly available in northern Thailand, was analyzed for its biochemical and mineral composition. The biochemical and nutritional composition of *S. varians* reveals that this macroalgae has an appreciable amount of pigments, dietary protein, carbohydrate and minerals content.

Nutraceutical components of *S. varians* were much higher. Thus, results of the present study conclude that *S. varians* is a potential health food in human diets and may be of use to the food industry as a source of ingredients with high nutritional value. *S. varians* can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of freshwater macroalgae based products.

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