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Short Communication

The Nutritional Value of Edible Freshwater Alga *Cladophora* sp. (Chlorophyta) Grown under Different Phosphorus Concentrations

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ABSTRACT

Culture of an edible freshwater alga *Cladophora* sp. was subjected to phosphorus (P) supply (1.07-14.78 mg L^{-1} of PO_4-P) in order to determine its proximate compositions, vitamins, minerals and carotenoid compositions. The alga was mass cultured by using 10% of canteen wastewater with the addition of di-potassium hydrogen orthophosphate at the concentrations of 0, 5, 10, 15 and 20 mg L^{-1}. The results showed that with increased P concentration, protein, vitamin A, P, β-carotene, lutein and zeaxanthin contents increased but carbohydrate content decreased indicating that P supply could enhance carotenoid production and some nutritional values of this alga. © 2011 Friends Science Publishers

Key Words: *Cladophora*; Edible algae; Nutritional value; Carotenoid composition

INTRODUCTION

Among the major freshwater edible algae of Thailand are *Cladophora, Microspora*, *Nostoc*, *Nostochopsis* and *Spirogyra*. In Thailand edible freshwater algae, *Cladophora* is known as Kai. It is abundant in the Nan and Mekong rivers in the Northern part of Thailand. The local people around these rivers collect it for domestic consumption and it is sold in the markets and is now cultured by using canteen wastewater for fish supplement feed (Traichaiyaporn *et al*., 2010). Many reports on nutritional value of *Cladophora* show that in contains significant amounts of protein and carotenoids, which are essential for human and fish nutrition (Khuantrairong & Traichaiyaporn, 2009; Traichaiyaporn *et al*., 2010). Studies on *Cladophora* have been reported especially on its taxonomy and nutritional values (Powtongsook, 2000; Peerapornpisal *et al*., 2006; Khuantrairong & Traichaiyaporn, 2009) but reported of relationship between its nutrition and environmental are few.

Algae are significant source of human food, especially in Asia and cultivated for nutrition and pigments for supplemental use as human food and animal feed (Promya *et al*., 2008). 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 (Whyte, 1987; Doroudi *et al*., 2003).

Studies on effect of phosphorus on nutritional values have been reported in algae *Chaetomorpha linum* (Menendez *et al*., 2004), *Skeletonema costatum*, *Chaetoceros muelleri* (Leonardos & Lucas, 2000) and the higher plants lentil (Zeidan, 2007; Togay *et al*., 2008) and forage maize (Eltelib *et al*., 2006). This study assessed the nutrition of a macroalga *Cladophora* sp. culture in different phosphorus concentration i.e., proximate compositions, vitamins, minerals and carotenoid compositions contents.

MATERIALS AND METHODS

Canteen wastewater preparation: Canteen wastewater (1000 L) was collected from canteen wastewater clarifier of Maejo University and allowed to settle in an open-air cement pond for 3 weeks for the breakdown of solid organic wastes by microorganisms. The water was then filtered through an 80 μm plankton net filter (Promya *et al*., 2008). The filtrate was analyzed for pH (8.64), total hardness (145.50 mg L^{-1}), orthophosphate phosphorus (4.84 mg L^{-1}), ammonia nitrogen (3.05 mg L^{-1}) and nitrate nitrogen (6.44 mg L^{-1}). Then the filtrate was diluted to 10% for algal cultivation.

Culture conditions: *Cladophora* sp. was obtained from Algae and Water Quality Research Unit, Chiang Mai University, Thailand. The alga was bath cultured by attachment on plastic nets (60 g m^{-2}) in cement raceway ponds (size 1.2×2.3×0.5 m) using 10% canteen wastewater, with continuous flow of 0.15 m s^{-1} for 12 weeks (Khuantrairong & Traichaiyaporn, 2009; Traichaiyaporn *et al*., 2010). A complete randomize design (CRD) was carried

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out using 4 treatments with 3 replications by addition of 4 concentrations of di-potassium hydrogen orthophosphate (K_2HPO_4) at 5, 10, 15 and 20 mg L^{-1} (treatment 1, 2, 3 & 4, respectively) to the cultured media and the control group was 10% canteen wastewater without adding K_2HPO_4.

Analysis method: Algal samples at 12 weeks of culturing were harvested, washed, air dried and freeze-dried. The samples were analyzed for the purposes:

Proximate composition, vitamin and mineral analysis: The protein, fat, ash, moisture and fiber were determined by standard AOAC (2000) methods. Carbohydrate was determined according to the method of Crampton and Harris (1969). The vitamin A content was calculated on the basis of β-carotene content according to the method of Tee and Lim (1991) as vitamin A (retinol equivalent, RE) = (μg β-carotene)/6. The level of four vitamins (B_1, B_2, C & E) and six minerals (Ca, Fe, K, Mg, P & Zn) were determined by in house method base on AOAC (2000).

Carotenoid compositions analysis: β-carotene, lutein and zeaxanthin were extracted following the method described by Yoshii *et al*. (2004) with some modifications. The samples (0.5 g) were extracted on ice in the dark with 25 mL of 100% acetone, stored in the dark at $-20^{o}C$ for 18 h and the supernatant was filtered. Investigation and quantification of these pigments were performed by reverse phase high liquid chromatography (HPLC) with method modified from Mostaert *et al.* (1998) and Yoshii *et al*. (2004), using a C_{30} carotenoid column (5 μm; 250 × 4.6 mm). The flow rates were 1.0 mL min^{-1} at 0-8 min and 2.0 mL min^{-1} at 8-18 min. The mobile phase was methanol: chloroform (4:1). Peak responses were determined by a UV-Vis at the absorbance of 456 nm. The carotenoids peaks were identified with compared by pure β-carotene, lutein and zeaxanthin standards from Sigma Chemical Company.

Statistical analysis: For all analyses, the mean and standard deviation were calculated and reported. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were adopted for statistical analyses and comparative studies of proximate compositions, vitamins, minerals and carotenoids of *Cladophora* sp. growth under different phosphorus concentrations.

RESULTS

Proximate composition: The proximate composition of *Cladophora* sp. indicated that the alga contained: protein (10.71-17.69%); lipid (2.04-2.56%); ash (14.71-16.86%); moisture (9.87-11.32%); fiber (20.67-26.10%) and carbohydrate (52.54-60.98%). Protein contents increased under high phosphorus (P) concentration, while carbohydrate contents decreased. Statistical analysis showed that protein and carbohydrate contents were significantly different among the treatments (Table I).

The vitamins contents (in mg 100 g^{-1}) of *Cladophora* sp. culture at different P concentrations were observed as follows: vitamin A (0.33-1.61); vitamin B_1 (0.05-0.06); vitamin B_2 (0.05-0.06); vitamin C (1.89-1.94) and vitamin E (5.96-6.00). Statistical analysis indicated that increasing the concentration of phosphorus increased of vitamin A content in *Cladophora* sp., but had no effect on vitamin B_1, B_2, C and E contents (Table I).

The levels of Ca, Fe, K, Mg, P and Zn contents (in mg 100 g^{-1}) were similar among the treatments and ranged from 6585-6863, 29.40-30.87, 2512-2658, 241.60-255.30 and 1.87-1.95, respectively. The P contents ranged from 69.17-111.79 mg 100 g^{-1}. There was significant difference among treatments and the highest value was observed in treatment 4 which addition of 20 mg L^{-1} K_2HPO_4 (Table I).

Carotenoid composition: Fig. 1 shows the HPLC chromatogram of carotenoid compositions in the extracted sample of *Cladophora* sp. β-carotene, lutein and zeaxanthin contents (in μg g^{-1}) of this species culture at different phosphorus concentrations were observed as 20.01-96.59, 172.80-437.28 and 24.62-171.75, respectively (Table I). The highest values of these pigments were observed in treatment 4, which addition of 20 mg L^{-1} K_2HPO_4, whereas the lowest values were observed in the control group. Statistical analysis showed that the levels of β-carotene, lutein and zeaxanthin were significantly different among all the treatments (Table I) suggesting that phosphorus effected the increase in carotenoid compositions production of *Cladophora* sp.

DISCUSSION

The biochemical composition of algae varies between species and according to culture conditions (Leonardos & Lucas, 2000; Martínez-Fernández *et al*., 2006). It is influenced by growth phase and culture medium composition as well as environment factors (Brown *et al*., 1993; Valenzuela-Espinosa *et al*., 2002). In the present study, proximate composition, vitamin and mineral contents of *Cladophora* sp. i.e., protein, vitamin A and P increased with increased phosphorus levels in culture media, while carbohydrate decreased. The previous studies have been shown the environmental conditions influence to algal nutritional values. Khuantrairong and Traichaiyaporn (2009) suggested that P content of *Cladophora* sp. was positively correlated with phosphorus levels in culture medium. Leonardos and Lucas (2000) reported that phosphorus limitation affected to decrease protein content of a microalga *Chaetoceros muelleri*.

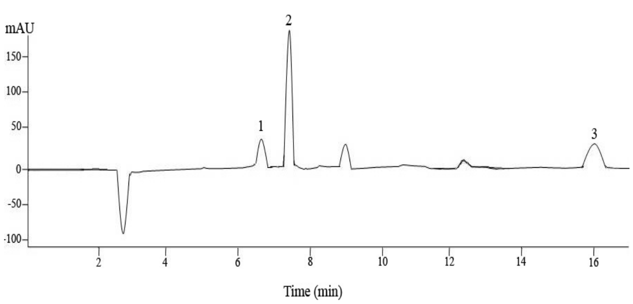
Elenkov *et al*. (1996) reported that high salinity affected increasing lipid content of *Cladophora vagubunda*. In addition, *C. siwaschensis* living in high salt water was considerably increased calcium and phosphorus content (Alfimov & Proshkina-Lavrenko, 1961). Auer and Canale (1982) observed that high dissolved P in water induced an increase in tissue P content of *Cladophora*. Moreover, enrichment of P in culture media increased tissue P of a green macroalga *C. linum* (Menendez *et al*., 2004). In the higher plants, Zeidan (2007) and Togay *et al*. (2008) stated

Table I: Nutritional values of *Cladophora* sp. cultured at different phosphorus concentrations

Nutritional values	Amount of nutritional values				
	Addition of K_2HPO_4 (mg L^{-1})				
	0	5	10	15	20
Proximate (%)					
Protein	10.71±1.14[a]	11.94±0.55[a]	15.00±0.88[b]	15.46±0.47[b]	17.69±1.75[c]
Lipid	2.04±0.78[a]	2.56±0.45[a]	2.29±0.93[a]	2.09±0.30[a]	2.46±1.19[a]
Ash	15.29±1.11[a]	15.56±0.67[a]	15.14±1.52[a]	14.71±0.64[a]	16.86±0.84[a]
Moisture	10.98±1.91[a]	11.11±1.19[a]	9.87±0.75[a]	11.32±0.86[a]	10.45±1.24[a]
Fiber	23.05±2.63[a]	26.10±2.72[a]	25.4±2.93[a]	20.67±1.28[a]	22.65±3.78[a]
Carbohydrate	60.98±1.57[c]	58.83±1.63[b]	57.70±1.21[b]	56.43±0.47[b]	52.54±2.26[a]
Vitamin (mg $100g^{-1}$)					
Vitamin A	0.33±0.23[a]	1.02±0.15[b]	1.11±0.21[b]	1.59±0.40[c]	1.61±0.08[c]
Vitamin B_1	0.05±0.01[a]	0.06±0.02[a]	0.06±0.02[a]	0.05±0.01[a]	0.06±0.01[a]
Vitamin B_2	0.05±0.00[a]	0.06±0.01[a]	0.06±0.02[a]	0.06±0.01[a]	0.05±0.01[a]
Vitamin C	1.89±0.10[a]	1.91±0.04[a]	1.91±0.13[a]	1.93±0.11[a]	1.94±0.05[a]
Vitamin E	5.97±0.13[a]	6.01±0.01[a]	5.98±0.20[a]	5.96±0.08[a]	6.00±0.21[a]
Minerals (mg $100g^{-1}$)					
Calcium (Ca)	6,631±209.03[a]	6,657±14.14[a]	6,585±279.31[a]	6,863±211.42[a]	6,740±169.00[a]
Iron (Fe)	29.91±1.93[a]	29.40±0.88[a]	29.55±0.64[a]	30.87±1.93[a]	29.57±1.08[a]
Potassium (K)	2,658±200.11[a]	2,652±137.18[a]	2,512±149.20[a]	2,652±136.47[a]	2,633±43.13[a]
Magnesium (Mg)	241.60±18.67[a]	245.45±6.43[a]	251.55±17.18[a]	254.00±5.66[a]	255.30±8.20[a]
Phosphorus (P)	69.17±1.17[a]	71.03±1.40[a]	87.82±3.24[b]	94.55±1.42[b]	111.79±13.07[c]
Zinc (Zn)	1.91±0.04[a]	1.94±0.09[a]	1.91±0.01[a]	1.95±0.16[a]	1.87±0.09[a]
Carotenoid (μg g^{-1})					
β-carotene	20.01±13.99[a]	61.03±9.26[b]	66.38±12.83[b]	95.56±24.11[c]	96.59±5.10[c]
Lutein	172.80±48.11[a]	296.70±16.97[b]	395.39±18.17[c]	370.80±1.32[c]	437.28±26.38[d]
Zeaxanthin	24.62±12.49[a]	72.17±3.00[b]	79.40±2.27[b]	92.47±7.17[b]	171.75±46.69[c]

*Data in the same row with different superscripts are significantly different ($p<0.05$)

Fig. 1: HPLC chromatogram of carotenoid compositions extracted from *Cladophora* sp. Peaks are lutein (1), zeaxanthin (2) and β-carotene (3)



that P increased protein content in lentil. In addition, Ciereszko *et al*. (2005) suggested that P deficiency decreased protein content in *Arabidopsis*. On the other hand, result of experiment by Eltelib *et al.* (2006) showed that P had no effect on protein content in forage maize.

In the present study it was indicated that P increased carotenoid compositions contents of *Cladophora* sp., which agreed with study of Khuantrairong and Traichaiyaporn (2009). In addition, Celekli *et al*. (2009) reported that phosphate supply increased carotenoid production of a blue-green alga *Spirulina platensis*. On the other hand, Latasa and Berdalet (1993) suggested that the synthesis of pigments in a dinoflagellate *Heterocapsa* sp. stopped under P limitation. In contrast, enhancement of astaxanthin in a green alga *Haematococcus pluvialis* (Brinda *et al*., 2004) as well as β-carotene, zeaxanthin and violaxanthin in marine microalga *Nannochloropsis gaditana* (Forján *et al*., 2007) were observed under P limitation. Leonardos and Geider (2005) stated that P and nitrogen ratio was related to carotenoid production in cryptophyte *Rhinomonas reticulata*. These reports strongly suggested that phosphorus affected carotenoids production in *Cladophora* sp.

In conclusion, P supply had effect on the increase carotenoids production and some nutritional values of *Cladophora* sp. Further study should be investigated effect of other factors such on nutritional values of this alga.

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