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Growth and Nutrients Analysis in Marine Macroalgae

Pakawan Setthamongkol[1,2], Suriyan Tunkijjanukij[1], Kriengkrai Satapornvanit[3] and Jintana Salaenoi[1,2,*]

ABSTRACT

Macroalgae are one of the most important marine, living, renewable resources and are used for human consumption, animal feed and fertilizer in many countries. Nine species of macroalgae belonging to the Chlorophyta and Rhodophyta divisions—*Chaetomorpha crassa, Chaetomorpha linum, Ulva rigida, Caulerpa racemosa*, *Caulerpa brachypus, Caulerpa lentillifera, Caulerpa taxifolia, Gracilaria tenuistipitata* and *Gracilaria fisheri*—were cultured in a closed system with Guillard's f/2 medium for 3 wk. After rearing, *C. lentillifera* achieved the highest mean (± SD) growth rate, followed by *U. rigida* and *C. crassa* at 7.28 ± 0.69, 2.66 ± 0.83 and 2.64 ± 0.91 g.d^{-1}, respectively. In addition, *C. taxifolia* had the highest mean (± SD) protein 33.83 ± 0.21% and lipid 3.26 ± 0.44% contents, and the maximum mean (± SD) carbohydrate 67.84 ± 0.04%, fiber 34.29 ± 0.40% and ash 47.80 ± 0.87% contents were found in *U. rigida, C. crassa* and *C. lentillifera,* respectively. Knowledge of the essential compositions in the macroalgae could lead to the potential development of novel seaweed products in the future.

Keywords: macroalgae, growth, seaweed, culture, Guillard's f/2 medium

INTRODUCTION

Macroalgae, popularly known as seaweeds in marine ecosystems, are ecologically and biologically important for other living organisms by being primary producers in the ocean (Banerjee *et al*., 2009). They contain significant amounts of protein, lipid, minerals and vitamins for human nutrition (Manivannan *et al*., 2008). In Asian countries, macroalgae have been used as human food, animal feed, and fertilizer since ancient times (Satpati and Pal, 2011). At present, industrial utilization is largely confined to extraction for biochemicals and secondary metabolites to produce health-promoting food, drugs and medical materials (Samarakoon and Jeon, 2012). Nutrient contents of seaweeds vary according to species, geographical distribution, season and principal environmental factors such as the water temperature, salinity, light and nutrients and mineral availability (Pena-Rodriguez *et al*., 2011). Generally, brown algae contain less protein (3–15% dry weight) than green (9-26% of dry weight) and red seaweeds (maximum 47% dry weight) according to Fleurence (1999). *Acanthopora spicefera* contains 18.9% protein, followed by *Caulerpa racemosa* (18.3%) and *Padina gymnospora* (10.5%) according to Rameshkumar *et al*. (2012). The concentration of carbohydrate is also higher in most green algae, followed by brown and red algae (Kumar *et al*., 2010) In addition, green algae have shown

[1] Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
[2] Center for Advanced Studies in Tropical Natural Resources, NRU-KU, Kasetsart University, Bangkok 10900, Thailand.
[3] Deparment of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: ffisjid@ku.ac.th

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a higher lipid content than red and brown algae (Rohani-Ghadikolaei *et al*., 2012), with 2.1% in *Ulva* sp., followed by *Gracilaria* sp. (1.8%) and *Padina* sp. (1.7%) according to Dhargalkar and Pereira (2005).

Few studies have been done on cultivated specimens. Most descriptions are based on natural stocks collected from coastal areas and other submerged strata in the intertidal and shallow, subtidal zones (Marinho-Soriano *et al*., 2006). Studies on the biochemical properties of marine macroalgae in the same environment are insufficient. Although seaweeds in Thailand are extensive, they are relatively underutilized, with most of them used mainly as animal feeds and fertilizers in coastal villages (Ratana-arporn and Chirapart, 2006). In addition, knowledge about their nutritional composition is still limited (Rameshkumar *et al.*, 2012). Therefore, this study was conducted to compare the growth rates of nine marine macroalgae under culture conditions, as well as to determine their chemical compositions such as total protein, carbohydrate, fiber, lipid and ash. The biochemical information of macroalgae will be essential for the commercial food products and health supplements industries.

MATERIALS AND METHODS

Algal samples and culture systems

Nine species of macroalgae consisting of seven species of green algae (Chlorophyta), namely, *Chaetomorpha crassa, C. linum, Ulva rigida, Caulerpa racemosa*, *C. brachypus, C. lentillifera* and *C. taxifolia*, and two species of red algae (Rhodophyta), namely, *Gracilaria tenuistipitata* and *G. fisheri*, were pre-cultivated for 1 wk in f/2 medium (Guillard and Ryther 1962; Guillard 1975) in a greenhouse with natural light. Before starting the experiment, each sample was blotted with a towel to remove excess water, trimmed, weighed to produce approximately 30 g and then placed in an aerated 10 L glass aquarium. The culture conditions were: 30 parts per trillion (ppt) filtered natural seawater with the f/2 medium consisting of 0.075 $g.L^{-1}$ $NaNO_3$ and 0.005 $g.L^{-1}$ $NaH_2PO_4.H_2O$ (Guillard and Ryther 1962; Guillard 1975), pH 8 with temperature between 28 and 32 °C. All culture systems were replicated three times for each species. Each medium was changed every week while the weights of the algae were recorded and growth rates were analyzed for 3 wk. The growth rates of macroalgae were determined as the average values from triplicate replications. The calculation of growth rate was based on Lobban *et al.* (1987). After 3 wk culture, all macroalgae were collected and washed in freshwater to remove salt, contaminants and epiphytes. The macroalgae were dried for 48 hours at 60 °C, ground and stored in a dry place for nutritional analysis.

Nutritional analysis

The composition of the macroalgae (protein, ash, fiber, moisture and lipid contents) was determined at the end of the third week according to standard methods (Association of Official Analytical Chemists, 1990). The carbohydrate contents were calculated using the formula: Carbohydrates = [100% - (%protein + %lipid + %ash + %water)], according to Ortiz *et al.* (2009). The results were expressed as a percentage of the component by dry weight. All data were analyzed statistically and derived from triplicate replications using analysis of variance. The significance test among mean values of treatments used Duncan's New Multiple Range Test (Duncan, 1955) with a 95% confidence interval. The results were expressed as the mean ± SD of the data.

RESULTS AND DISCUSSION

Growth rate of macroalgae

Nine species of macroalgae were cultured in the same medium and under the same environmental conditions. The results showed that after 3 wk, the highest growth rate was achieved by *Caulerpa lentillifera* (7.28 ± 0.69 $g.d^{-1}$) followed by *Ulva rigida* (2.66 ± 0.83 $g.d^{-1}$) and *C. crassa*

(2.64 ± 0.91 $g.d^{-1}$). The growth rates of the other species were in the range 0.12 ± 0.02 to 1.48 ± 0.17 $g.d^{-1}$ (Figure 1). Pugdeepun (2001) studied *C. lentillifera* cultured under laboratory conditions with Bold's basal medium and found that the highest growth (5.66% per day) was at salinity 30 ppt in the second week, after which, algal growth gradually declined and the algae died in the fifth week. Worasingh *et al.* (2007) tested seaweed culture with the f/2 medium over 60 d and found that *Acanthophora spicifera*, *C. lentillifera* and *Enteromorpha clathrata* had high growth rates at 1.5, 2.66 and 3.72 $g.d^{-1}$, respectively, in 15, 25 and 15 d, respectively. The medium used is important for the cultivation of macroalgae as it will affect the growth and biochemical accumulation in algae, so Grund medium, Provasoli's ES medium and the f/2 medium are generally used in seaweed culture (Harrison and Berrges, 2005; West, 2005). For macroalgal cultures, Grund medium is suitable for a number of Rhodophyta species and Provasoli's ES medium is similar to Grund medium but the former is more complete with trace metals and vitamins (Malachlan, 1979). The f/2 medium is satisfactory for marine microalgae and macroalgae and is suitable for commercial algae cultivation (West, 2005).

Nutritional composition of macroalgae

Macroalgae are substantially different from terrestrial plants in terms of their chemical composition, as well as physiological and morphological features (Jung *et al.*, 2013). They are a valuable sources of protein, fiber, vitamins, polyunsaturated fatty acids, macro and trace elements, as well as important bioactive compounds (Ortiz, *et al.*, 2006). In addition, the biochemical composition of seaweeds varies and is affected by the species, nutrition and environment (Kumar *et al.*, 2010). The protein, lipid, carbohydrate, fiber, ash and moisture contents of the nine cultured macroalgae in the f/2 medium at the end of the third week were determined. Protein has crucial functions in all algal biological processes; their activities can be described by enzymatic catalysis, transport and storage, and mechanical sustentative control (Rameshkumar *et al.*, 2012). High protein accumulation was found in the green algae, *C. taxifolia* (33.83 ± 0.21%) and *C. linum* (30.70 ± 0.70%) as shown in Figure 2a, which was higher than in *C. taxifolia* (10.90–11.70%) and *Chaetomorpha media* (15.93–19.40%) according to Shanmugam *et al.* (2001) and *C. taxifolia* (23.78%) collected from Seeniappa Dharga in India (Murugaiyan *et al.*, 2012). These results

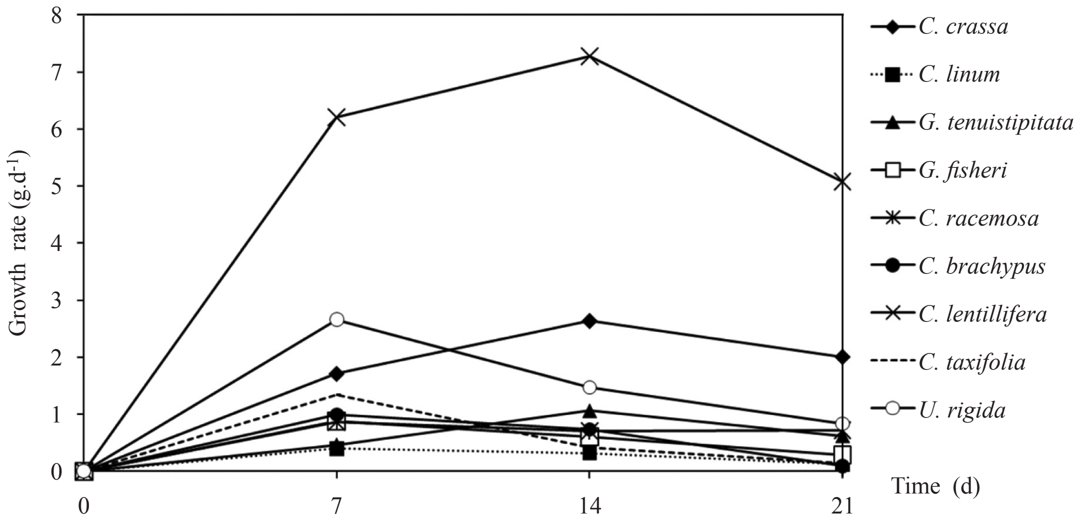

Figure 1 Growth rates of nine macroalgae cultured in f/2 medium for 3 wk.

show that the same species growing in different environments can have different amounts of nutrition. In general, green and red seaweeds contain higher protein contents (10–30%) than brown seaweeds (5–15%) according to Benjama and Masniyom (2011). Most of the nine species sampled in the current study had protein contents ranging from 23.41 ± 0.10 to 26.71 ± 0.40%, which was higher than in *C. racemosa* (18.3%) collected from Palk Bay (Rameshkumar *et al*., 2012) and *C. racemosa* (24.55%) collected from Seeniappa Dharga, India (Murugaiyan *et al*, 2012).

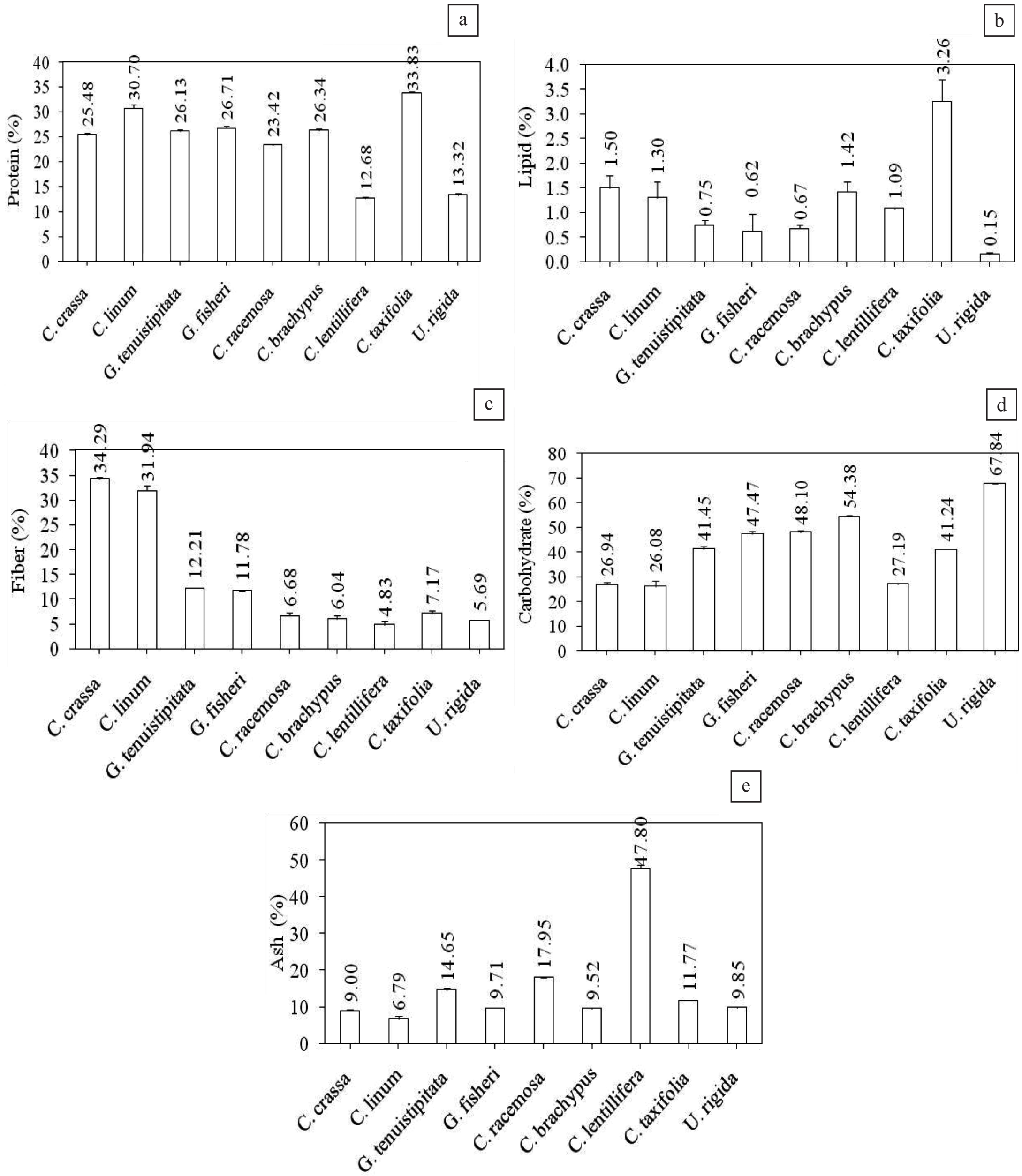

Figure 2 Nutritional analysis of nine macroalgae cultured in f/2 medium: (a) Protein content; (b) Lipid content; (c) Fiber content; (d) Carbohydrate content; and (e) Ash content. (Error bars show ± SD.)

The current research results were compared to Fleurence (1999) who reported a range of 10–26% in the protein content in *Ulva* spp. while *U. fasciata* had 12.3% in collections from the Hawai'ian islands (McDermid and Stuercke, 2003). However, most marine macroalgae have a greater protein content than terrestrial plants and animal products, such as raw broccoli (4.4%), fresh whole milk (3.3%) and raw carrot (0.7%) according to U.S. Department of Agriculture (2001).

Lipids provide much greater efficacy in oxidation processes than other biological compounds and they constitute a convenient storage material for living organisms (Miller, 1962) In macroalgae, the lipids are widely distributed, especially in several resistance stages (Rameshkumar *et al.*, 2012). Most macroalgae have a low lipid content and it only provides a very low amount of energy and is low in calories (Ratana-arporn and Chirapart, 2006). In the present study, the lipid content ranged from the low values of 0.62 ± 0.35 to 1.5 ± 0.25% in all species except for *C. taxifolia* (3.26 ± 0.44%) as shown in Figure 2b. The lowest lipid content was found in *U. rigida* (0.15 ± 0.03%). This result was consistent with Ortiz, *et al*. (2006) who reported 0.3% lipid accumulation in *U. lactuca*.

Polysaccharides of macroalgae cannot be digested by humans and they are regarded as a new source of dietary fiber and food ingredient (Ratana-arporn and Chirapart, 2006). The fiber content was significantly higher in *C. crassa* (34.29 ± 0.40%) and *C. linum* (31.94 ± 0.94%), whereas the other species had 4.83 ± 0.72 to 12.21 ± 0.02% (Figure 2c). The results of this experiment showed higher contents than those in terrestrial plants such as raw broccoli (2.6%), raw carrot (2.4%) and oranges (1.7%) according to U.S. Department of Agriculture (2001).

Carbohydrate is one of the most important components for metabolism and it supplies the energy needed for respiration and other important processes (Rameshkumar *et al.*, 2012). The concentration of carbohydrate was high in all species in this study. The carbohydrate content in *U. rigida* was 67.84 ± 0.04%, followed by *C. prolifera* (54.38 ± 0.60%) as shown in Figure 2d. These results were similar to those reported by Rameshkumar, *et al*. (2012). The carbohydrate contents of *G. fisheri* and G. tenuistipitata were 47.47 ± 0.83 and 41.45 ± 1.05 %, respectively, which were higher than that for *G. folifera* (22.32%) collected on the Mandapam coast in India (Manivannan *et al*., 2008).

The ash content of *C. lentillifera* (47.80 ± 0.87%) was significantly higher than that of the other species. The ash content ranged from 6.79 ± 0.66 to 17.95 ± 0.04% as shown in Figure 2e. These figures were consistent with the results of Benjama and Masniyom (2011) who reported an ash content of seaweeds between 8 and 40%. Most seaweed has a greater ash content than terrestrial plants and animal products; in addition, some of the trace elements found in seaweeds are rare or absent in terrestrial plants (Rameshkumar *et al*., 2012). The moisture content of the nine seaweeds ranged from 2.30 to 6.42%.

Finally, the growth and chemical composition of marine macroalgae are significantly affected by their environmental conditions and are particularly dependent on taxonomical classes and species (Jung *et al.,* 2013). For example, Jayasankar (1993) revealed that the seasonal variation in the carbohydrate content of *Sargussum wightii* varied from 6.65% in March to 15.18% in January. In addition, different species of seaweed also have different qualitative and quantitative nutrient requirements. Thus, the different species have different growth rates and rates of biochemical accumulation.

CONCLUSION

Macroalgae can be considered as promising plants for the future. They are one of the most important marine living resources with high nutritional value. As plants with a unique structure and biochemical composition, macroalgae could be exploited for their various properties, such as for food, energy, medicine, cosmetics and for biotechnology. Nine macroalgae, namely, *Chaetomorpha crassa, C. linum, Ulva rigida, Caulerpa racemosa*, *C. brachypus, C. lentillifera, C. taxifolia, Gracilaria tenuistipitata* and *G. fisheri* were cultivated to determine their original and nutritional characteristics of interest, and the results showed the following ranges: 33.83–12.68% protein, 3.26–0.15% lipid, 34.29–5.69% fiber, 67.84–26.08% carbohydrate and 47.80–6.7% ash. The surrounding environment, nutrients and species of macroalgae are factors affecting the different biochemical substances accumulated in the plants. Further studies are required on the effect of the environment on biochemical production in marine microalgae. The information gained will be essential to the development of commercial products based on cultivated marine macroalgae.

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