Proximal analysis of seagrass species from Laguna de Términos, Mexico

Análisis proximal de los pastos marinos de la Laguna de Términos, México

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ABSTRACT

This paper examines chemical nutritional aspects of three seagrass species (*Thalassia testudinum* König, *Halodule wrightii* Ascherson, and *Syringodium filiforme* Kützing) found at Laguna de Términos, Campeche, Mexico during the rainy season of 2004, following analysis methods described by the Association of Official Analytical Chemists. High protein (8.47-10.43%), high crude fiber (15.70-19.43%), high ash (23.43-38.77%) high nitrogen-free extract contents (37.27-45.37%), and low lipid levels (0.83-2.13%) were common features of the three species analyzed. Given its chemical contents and the World Health Organization reference standards, particularly the high protein (10.43%), high ash (23.43%), high fiber (19.43%), high nitrogen-free extract (45.37%) and low lipids (2.13%), *S. filiforme* appears to be a noteworthy potential dietary supplement and a nutrient source for human consumption. Another use of this high-protein seagrass could be in producing food for aquaculture fish.

Key words: *Halodule wrightii,* Laguna de Términos, proximate analysis, *Syringodium filiforme, Thalassia testudinum*.

RESUMEN

El presente trabajo consiste en la caracterización química nutrimental de tres especies de pastos marinos (*Thalassia testudinum* König*, Halodule wrightii* Ascherson y *Syringodium filiforme* Kützing) de la Laguna de Términos, Campeche, México, durante la temporada de lluvias de 2004 siguiendo los métodos de análisis descritos por la Asociación Oficial de Químicos Analíticos. Altos niveles de proteína (8.47-10.43%), de fibra cruda (15.70-19.43%), de ceniza (23.43-38.77%) de extracto libre de Nitrógeno (37.27-45.37%) así como bajos niveles de lípidos (0.83-2.13%) fueron una característica común de las tres especies analizadas. Considerando la composición química y los estándares de referencia de la Organización Mundial de la Salud, en particular el alto contenido de proteína (10.43%), de ceniza (23.43%), alto contenido de fibra (19.43%), de extracto libre de Nitrógeno (45.37%) y bajo nivel de lípidos (2.13%) medido para *S. filiforme*, sugiere su aplicación como un buen suplemento alimenticio y fuente de nutrimentos para el humano. El uso de pastos marinos con alto contenido de proteínas para la producción de alimentos para peces podría ser otra aplicación de este recurso marino.

Palabras clave: Análisis químico proximal, *Halodule wrightii,* Laguna de Términos, *Syringodium filiforme, Thalassia testudinum*.

INTRODUCTION

Seagrass meadows are prominent components of the littoral zone of tropical and temperate seas, provide habitat and food for organisms, and modulate sedimentary and biogeochemical processes (Duarte & Chiscano, 1999). In general, seagrasses are assigned to two families, Potamogetonaceae and Hydrocharitaceae, encompassing 12 genera of angiosperms containing about 50 species (Hemminga & Duarte, 2000). In addition to their local importance, seagrasses are significant contributors to the primary production of the global ocean (Duarte & Chiscano, 1999), which suggests that they could be analyzed further as a potential human-food source (Montaño *et al.*, 1999). Seagrasses have been used as human food especially by coastal populations (Hemminga & Duarte, 2000), and for a variety of remedial purposes in folk medicine (e.g. treatment of fever and skin diseases) (De la Torre-Castro & Ronnback, 2004). In some countries, seagrasses are used as medicine, food, fertilizer, and livestock feed (Rengasamy *et al.*, 2013).

The nutrient concentration of aquatic resources has been relatively well documented (Duarte, 1992). There are a numerous reports regarding the nutritional content of seaweeds (Rhodophyta, Phaeophyta, and Chlorophyta) as a potential food source (Plaza *et al*., 2008; Gupta & Abu-Ghannam, 2011; Mohamed *et al*., 2012). Yet, there is still a dearth of studies on the nutritional composition of seagrasses and their possible utilization as a source of human food, and information regarding the Gulf of Mexico in particular is limited to the reports by Dawes (1986, 1990) that showed high levels of ash and protein in three seagrass species.

The aim of this paper involved examining the chemical nutritional content in three seagrass species from Laguna de Términos, Campeche, Mexico: *Thalassia testudinum* König*, Halodule wrightii* Ascherson, and *Syringodium filiforme* Küitzing during the rainy season of 2004.

Knowledge of the chemical composition of seagrass is important both for the assessment of the nutritional value of marine invertebrate or vertebrate herbivores and for the evaluation of potential sources of protein, carbohydrates, and lipids for commercial use or for possible human consumption. We note that, like most flora, the nutrient content of seagrass is affected by external factors such as geographic location, environmental conditions, seasons, and sampling conditions (Renaud & Luong-Van, 2006).

MATERIALS AND METHODS

Laguna de Términos (Fig. 1) is the largest coastal lagoon in Mexico (Carvalho *et al*., 2009). Situated at the southern extreme of the Gulf of Mexico, between latitudes N18° 27’ 37” and N 18° 47’ 36” and longitudes W 91° 14’ 44” and W 91° 53’ 55”, the lagoon has a total surface area is 2,500 km^2 and a mean depth of 3.5 m. It is connected to the Gulf of Mexico by two openings. Three rivers provide most of the freshwater input to the lagoon: Palizada, Chumpán, and Candelaria. The climate is determined by three seasons: dry, rainy, and north winds. The dry season commonly lasts from March to May and rains are heaviest from June to October. The north-winds season lasts from November to February (David & Kjerfve, 1998). In this system, seagrass are important components of coastal ecosystems and include three species: *Thalassia testudinum, Halodule wrightii*, and *Syringodium filiforme. T. testudinum,* commonly known as turtle grass, is a major component of the seagrass community, providing food for urchins, sea turtles, and fishes, and habitat for a diverse population of epiphytes (Pirog, 2011). *H. wrightii,* also known as shoal grass, is found in the intertidal zones of shallow waters with sandy or muddy substrates at depths of 0 to 2 m. It is well established from the southeastern United States to South America; it occupies the shallowest waters in the Gulf of Mexico and is often exposed during low tides (McGovern & Blankenhorn, 2007). *S. filiforme* commonly known as Manatee grass, is an important component of seagrass beds in shallow warm waters. It typically grows at depths ranging from 1 to 3 m and is found in the sublittoral zone of marine waters with sandy or muddy substrate (Duarte *et al*., 2007).

Using a hand shovel, we collected triplicate samples of each seagrass species during the 2004 rainy season (October) in the north region of the lagoon (Fig. 1). They were placed into plastic bags, stored on ice, and transported to the laboratory, where they were washed with distilled water to remove sand and surface debris, and holdfasts and epiphytes were removed. Rhizomes, thoroughly cleaned, were weighed (Sartorius Analytical Balance with 0.01 mg resolution) and then dried at 115° C for 5 h until a constant dry wet was obtained.

Proximate analysis was carried out on samples of the three species following the methods of analysis described by the Association of Official Analytical Chemists (AOAC, 2000). Analysis included the determination of crude protein, crude lipids, crude fiber, dry matter, nitrogen-free extract, and ash.

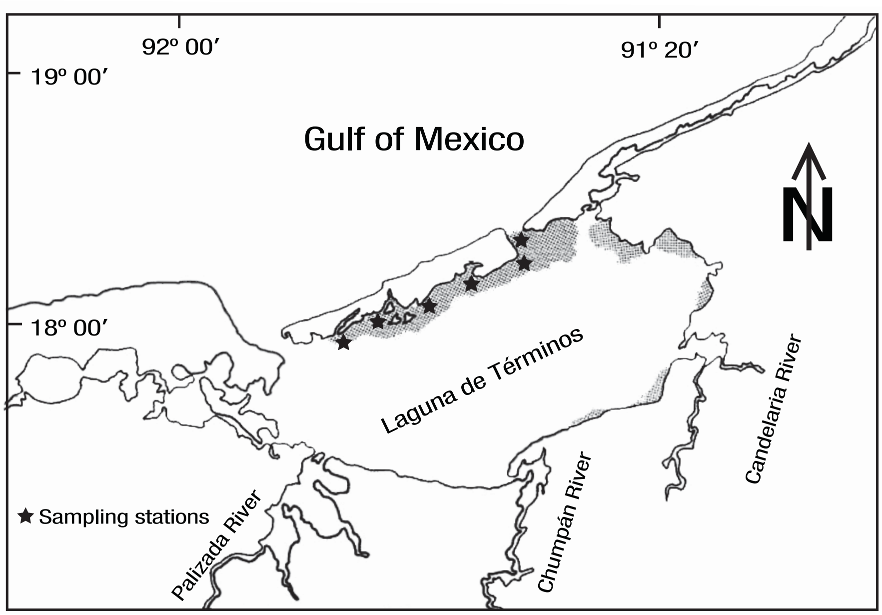

Figure 1. Study area and sampling stations. Laguna de Terminos, Campeche, Mexico.

The Kjeldahl method was employed to determine the nitrogen-free extract and the crude protein (x 6.25). Crude lipids were extracted by continuous heat extraction of all soluble substance in petroleum ether. The organic matter and ash contents were determined based on methods outlined in AOAC by combustion at 550° C during 5 hours; the final ash was considered to be the mineral portion of the sample. The replicates of each sample were used for statistical analysis and the values were reported as mean ±1 standard error (SE).

RESULTS

Table 1 shows the results of the proximate analysis (mean ±SE) of the three species of seagrass.

The crude protein value (Fig. 2a) was higher for *S. filiforme* at 10.43%, followed by *T. testudinum* with 8.47%, and *H. wrightii* with 8.10%. In contrast, the crude-lipid content was significantly different (p <0.001), with low content in all samples, i.e., 2.13, 2.33 and 0.83% for *S. filiforme, H. wrightii* and *T. testudinum,* respectively (Fig. 2b). The crude fiber content (Fig. 3a) was higher for *S. filiforme* with 19.43%, followed by *H. wrightii* with 19.03%, and *T. testudinum* with 15.70%. The dry matter content (Fig. 3b) was higher for *T. testudinum* with 12.27%, followed by *S. filiforme* with 11.50%, and *H. wrightii* at 9.47%. The difference observed for these two groups was p=0.008.

The nitrogen-free extract content (Fig. 4a) was higher for *S. filiforme* with 45.37%, followed by *H. wrightii* with 42.87%, and *T. testudinum* with 37.27%; ash content (Fig. 4b), an indicator of total mineral or inorganic content of the sample, was high for the three species: 23.43% for *S. filiforme,* 27.23% for *H. wrightii* and 38.77% for *T. testudinum*. The difference for these two groups was p=0.081.

Table 1. Proximate analysis values (means ±1 standard error (SE)) of three seagrass species from Laguna de Términos, Campeche, Mexico, during the rainy season of 2004.

	Crude Protein (%)	Crude Fiber (%)	Crude Lipids (%)	Ash (%)	Nitrogen-free extract (%)	Dry matter (%)
Syringodium filiforme	10.43±0.26	19.43±0.23	2.13±0.20	23.43±0.30	45.37±0.27	11.50±0.26
Halodule wrightii	8.10±0.38	19.03±0.14	2.33±0.24	27.23±0.30	42.87±0.26	9.47±0.27
Thalassia testudinum	8.47±0.32	15.70±0.21	0.83±0.22	38.77±0.15	37.27±0.12	12.27±0.33

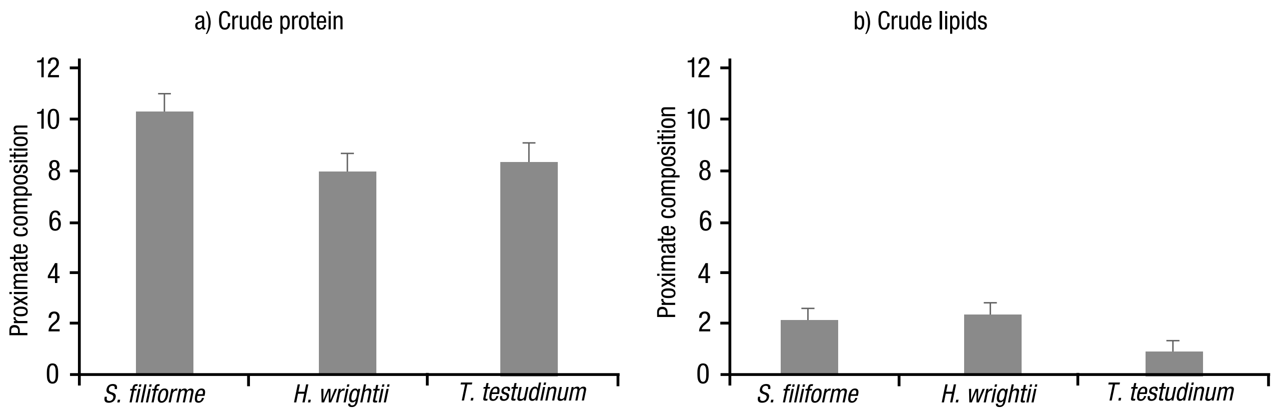

Figure 2a-b. Proximate composition of three selected seagrass species from Laguna de Términos, Campeche, Mexico, during the rainy season of 2004: a) Crude protein, and b) Crude lipids. Values represent means ±1 standard error (SE).

DISCUSSION

Seagrass productivity can surpass that of wheat, corn, and sugar beets (Rollon & Fortes, 1990). The high productivity of seagrass suggests that it should be further explored as a potential food source for humans (Montaño *et al.*, 1999).

In this study, protein content ranged from 8.47 to 10.43%, and *S. filiforme* was found to have the most. These concentrations were similar to other published data for the seagrass *Enhalus acoroides* Linnaeus f. royle, which is traditionally consumed in some countries (Montaño *et al.,* 1999). The values observed in this study were comparable to those of Dawes (1986, 1990), who reported crude protein values for *S. filiforme* (~10%) on the West Coast of Florida (Table 2). Recently, similar concentrations of protein (~14%) were observed for the seagrass *Cymodocea rotundata* Ehrenberg (Table 2) in the Gulf of Mannar, India (Rengasamy *et al.,* 2013). As suggested by Athiperumalsami *et al.* (2008), high protein levels in seagrasses point to an interesting possibility for exploiting natural marine resources in processed form to alleviate problems of protein deficiency in developing countries. In comparison with most industrially-exploited seaweeds (Rhodophyta, Phaeophyta, and Chlorophyta), the protein fraction of brown seaweed

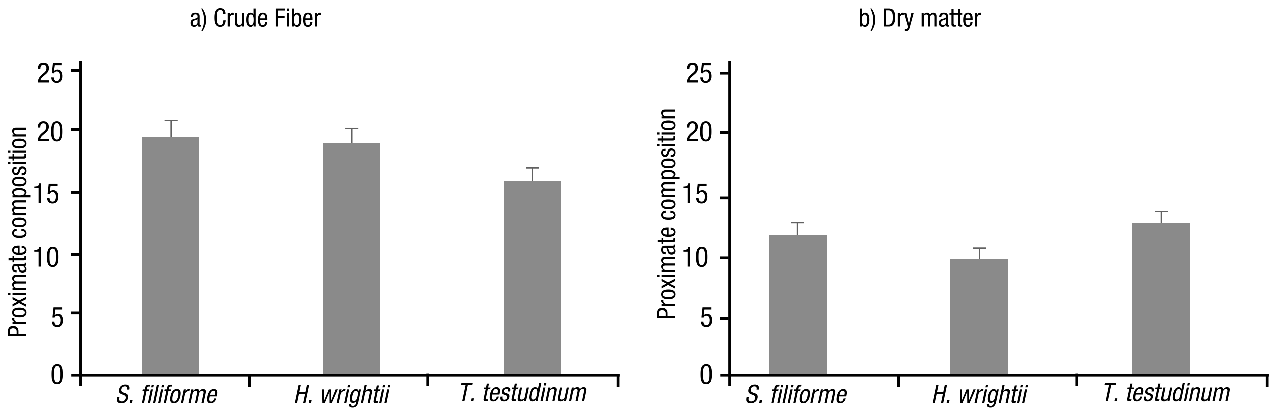

Figure 3a-b. Proximate composition of the three selected seagrass species from Laguna de Términos, Campeche, Mexico, during the rainy season of 2004: a) Crude fiber, and b) Dry matter. Values represent means ±1 standard error (SE).

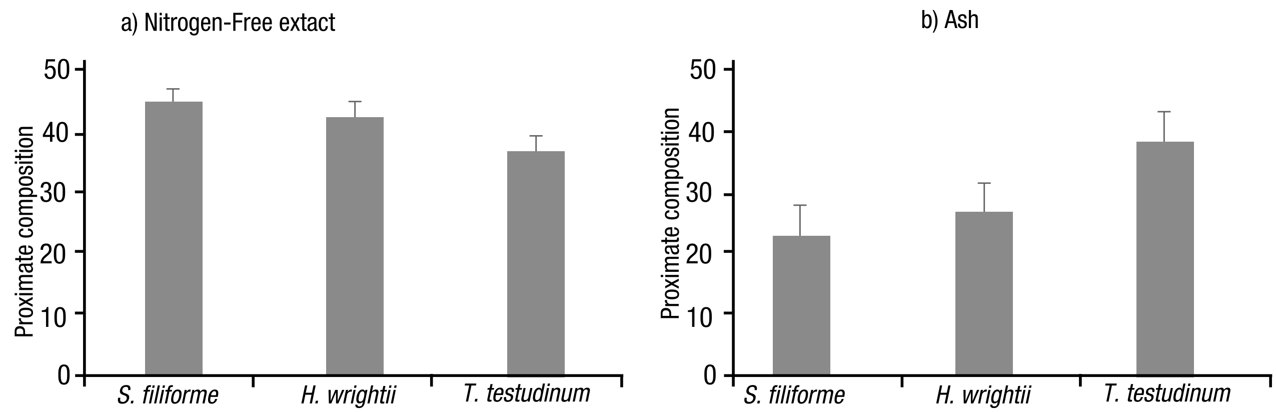

Figure 4a-b. Proximate composition of three selected seagrass species from Laguna de Términos, Campeche, Mexico, during the rainy season of 2004: a) Nitrogen-free extract, and b) Ash. Values represent means ±1 standard error (SE).

is low (~15%) compared with that of green or red seaweed (~47%) (Arasaki & Arasaki, 1983). Except for *Undaria pinnatifida* Harvey, which has a protein level between 11 and 24%, most industrially-exploited brown seaweed (*Laminaria digitata* Hudson*, Ascophyllum nodosum* Linnaeus*, Fucus vesiculosus* Linnaeus, and *Himanthalia elongate* Linnaeus), have a protein content ~15%, similar to that of the seagrass *S. filiforme* studied here. Recently, the crude protein level for the African Yam Bean (*Sphenostylis stenocarpa* Hochst. ex A. Rich) has been reported at ~19% (Table 2) (Ndidi *et al.*, 2014), which is similar to *S. filiforme.*

Significant variations in the lipid content of seagrasses species have been attributed to age, stage of growth, and ecological variations. The crude-lipid-content value presented in this study for *S. filiforme* (2.13%) was consistent with those reported for *E. acoroides* (1.40%) by Rengasamy *et al.* (2013). By contrast, the lipid content reported by Pradheeba *et al.* (2011) for *E. acoroides* (3.2%) was slightly higher than the values found by this study. In comparison, the lipid content in seaweeds is generally low, ranging from 0.12% for *Jania rubens* Linnaeus to 6.73% for *Laurencia papillosa* C.Agardh (Polat & Ozogul, 2009). Low lipid levels are also reported for *Codium fragile* Suringar, *Gracilaria chilensis* C. J. Bird*, Macrocystis pyrifera* Linnaeus (0.7-1.5%), *Eucheuma cottonii* Weber-van Bosse*, Caulerpa lentillifera* J. Agardh and *Sargassum polycystum* C. Agardh (0.29-1.11%) (Matanjun *et al.,* 2009; Ortiz *et al.,* 2009), of the same order, particularly for *S. filiforme* (2.13%), as the values found herein.

In this study, the fiber content was higher for *S. filiforme* (19.43%). The high proportion of fiber in this seagrass is in line with that reported for the seagrass *C. rotundata* (Rengasamy *et al.*, 2013). Many components of the dietetic fiber show antioxidant and immunological activity (Suzuki *et al.,* 2004). The World Health Organization (WHO) recommends a fiber intake of 22-23 g for each 1000 kcal of food (Kanwar *et al.*, 1997). Dietary fiber is necessary for digestion, elimination of wastes, and contraction of the muscle walls of the digestive tract (Rengasamy *et al.*, 2013). Recently, a dietary pattern containing low lipids and high fiber products (as observed for *S. filiforme* in this study) was associated with a lower risk of breast cancer (Kushi *et al.*, 2012).

Nitrogen-free extract (NFE) consists of carbohydrates, sugars, starches, and a major portion of materials classified as hemicellulose in feeds (AOAC, 2000). Castro-González *et al.* (1991) indicated that the seaweed *Macrocystis pyrifera* contains ~46% of nitrogen-free extract;

Table 2. Comparative proximate composition values for different species of the [1]present study; [2]Aketa & Kawamura, 2001; [3]Dawes & Guiry, 1992; [4]Shams *et al.*, 2013; [5]Rengasamy *et al.*, 2013; [6]Dawes, 1986; [7]Dawes, 1990; [8]Ndidi *et al.*, 2014.

Seagrasses	Crude Protein (%)	Crude Fiber (%)	Crude Lipids (%)	Ash (%)	Nitrogen-free extract (%)	Dry Matter (%)
Syringodium filiforme[1] Kütz	10.43	19.43	2.13	23.43	45.37	11.50
Halodule wrightii[1] *Asch.*	8.10	19.03	2.33	27.23	42.87	9.47
Thalassia testudinum[1] Banks *et* Sol. ex K.D.Koenig	8.47	15.70	0.83	38.77	37.27	12.27
Cymodocea serrrulata[2] (R.Br.) Asch. *et* Magnus	7.5	*	*	*	*	16.8
Halophila ovalis[2] (R.Brown) J.D.Hooker	6.2	*	*	*	*	14.3
Zostera capricorni[2] Irmisch ex Asch.	5.00	*	*	*	*	17.30
Zostera marina[3] L.	10.4	*	2.5	*	*	24
Cymodocea nodosa[4] (Ucria) Ascherson	51.04	*	10.08	*	*	*
Posidonia oceanica[4] (L.) Delile	60.75	*	4.05	*	*	*
Enhalus acoroides[5] (L.f.) Royle	13.8	20.3	1.4	26.63	2	*-
Thalassia hemprichii[5] (Ehrenb. ex Solms) Asch.	5.61	16.76	2.3	27.36	2.3	-*
Halodule pinifolia[5] (Miki) Hartog	7.45	16.5	9.56	24.16	2.61	-*
Syringodium isoetifolium[5] (Asch.) Dandy	9.16	21.4	5.27	22.16	1.03	-*
Cymodocea serrulata[5] (R. Br.) Asch. *et* Magnus	6.63	23.93	2.41	21.23	1.98	-*
Cymodocea rotundata[5] Ascherson *et* Schweinfurth	14.4	25.2	2.81	18.4	1.76	-*
Thalassia testudinum[6] Banks ex König	10	*-	1.4	41	*	10
Syringodium filiforme[6] Küetz	10	*	1.5	34	*	10
Halodule wrightii[6] Asch.	10	*	1.2	36	*	10
Thalassia testudinum[7] Banks ex König	13	20.5	3.8	41-	*	*
Syringodium filiforme[7] Küetz	10.7	18.7	*	*	*	*
Halodule wrightii[7] Asch.	17.5	25.5	*	*	*	*
Aquatic Macrophytes						
Elodea densa[2] (Planch.) Casp.	20.5	*	3.3	*	*	9.8
Alternanthera philo[2] Kuntze	15.6	*	2.7	*	*	14.5
Eleocharis acicularis[2] (L.) Roem. *et* Schult	22.5	*	3.6	*	*	11.1
Terrestrial Plants						
Alfalfa[2]	19.5	*	0.7	*	*	18.3
African Yam Bean[8]	19.00	9.29	2.84	2.64	54.22	*

*Not available data

results presented here concur, but were considerably higher than the 23% reported by El-Deek and Brikaa (2009) for seaweed used in poultry diets.

Seagrass mineral content is higher than that of land plants and animal products (Ortega-Calvo *et al.*, 1993). Sweet corn has a lower content (2.6%), while spinach has an exceptionally high mineral content (20.4%) for a land plant (Rupérez, 2002). The ash content in this study was higher for *T. testudinum* (38.77%) and lowest for *S. filiforme* (23.43%). Similar ash content was reported for *E. acoroides* (24.6%) and *T. hemprichii* Ehrenb. ex Solms (22.6%) by Yamamuro & Chirapart (2005). Highest ash content (~50%) has been reported in *Halophila ovalis* R. Brown in Australia (Yamamuro *et al.*, 2001). The ash values obtained in the three species analyzed herein fell well within the wide ranges reported (8 to 40%) for some seaweeds (Rupérez, 2002).The proximate composition values in this study, in particular the high protein, high ash and low lipid content for *S. filiforme*, were comparable to those reported in terrestrial plants. Table 2 shows values reported for some seagrasses as well as macrophytes and terrestrial plants. Except for the seagrass *Posidonia oceanica* Linnaeus, the crude protein values reported herein agree with other species (both aquatic and terrestrial). For example, reported African Yam Bean values (Table 2) are of the same order as the seagrasses analyzed in this study, suggesting that the latter can be an important food supplement to help meet the recommended daily adult intake of some minerals and trace elements. The proximate composition of seagrasses has been correlated to seasonal periods, with low values in the winter months and high values in the spring and summer months. This cyclic response corresponds to a winter dieback or slowdown of growth and a summer growth peak due to a continued buildup of photosynthesis (Dawes, 1986). Thus, we expect a temporal variation in seagrasses from Laguna de Términos, given the wide climate variation in the area.

In summary, the chemical contents reported in this study, in particular the high protein, high ash and low lipid content would appear to make *S. filiforme* a good dietary supplement and source of nutrients for human consumption. Given these high nutritional levels, this seagrass could also be used as food for farmed fish. For example, protein requirements for the optimal growth of Nile tilapia (*Oreochromis niloticus)* depend on the source of the protein, the size of the fish, its age, and the energy content of its diet, reported to vary from as high as 45-50% for first feeding larvae, 35-40% for fry and fingerlings, and 25-35% percent for juveniles. The best protein digestibility occurs at 25 °C, and the optimum dietary protein to energy ratio was estimated around 110 to 120 mg per kcal digestible energy for fry and fingerling, respectively (Stickney, 2006). Tilapia require about 40-45% protein for optimum reproduction, spawning efficiency, and larval growth and survival.

The lipid nutrition of farmed tilapia has been studied by Ng and Chong (2004). The minimum requirement of dietary lipids in tilapia diets is 5%, but improved growth and protein utilization efficiency has been reported for diets with 10-15% of lipids.

The daily protein requirement of common carp is about 1 g/kg body weight for maintenance and 12 g/kg body weight for maximum protein retention. The efficiency of nitrogen utilization for growth is highest with a protein intake of 7 to 8 g/kg body weight/day. Crude protein levels ranging from 20% to 38% appear to satisfy the fish optimally. This level has been determined by using semi-purified diets containing a single high-quality protein source. When the diet contains sufficient digestible energy, the optimal protein level can be effectively kept at 15-30% (Watanabe, 1982). As an omnivorous fish, common carp (*Cyprinus carpio)* can effectively utilize both lipids and carbohydrates as dietary energy sources. The enrichment of the digestible energy content from 13 to 15 MJ/kg diet by addition of lipid at levels of 5-15% to diets did not result in higher growth rates or improved net protein utilization (Takeuchi *et al*., 1979). Increasing dietary lipid seems to increase its body deposition.

In Mexico, as in other countries, new possibilities for using seagrasses could exist in developing functional foods for human nutrition, particularly the protein-rich species. In addition, high ash and nitrogen-free extract content were a common feature in the three species studied. Based on our results, these seagrasses, used as a food supplement, could help fulfill the recommended daily adult requirements of some macro-minerals and trace elements. Lipid content of all seagrass was generally low. Yet, like any other ingredient, seagrasses have to meet the industrial specifications and official consumer safety regulations dealing with microbiological quality and heavy metal contents. More research is needed to evaluate the nutritional value of seagrass, especially in the fields of biochemical analysis. The use of seagrass with high protein levels as a pellet binder in the production of foods for aquaculture fish could be another application of this marine plant resource.

Proteins are the primary components of living things. The presence of high protein levels in seagrasses indicates that they may have increased value as a food or that a protein base bioactive compound may be isolated in the future.

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