



## Amino acids in the seaweeds as an alternate source of protein for animal feed

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### Abstract

The nutritional value of six tropical seaweeds (*Sargassum wightii*, *Ulva lactuca*, *Kappaphycus alvarezii*, *Hypnea musciformis*, *Acanthophora spicifera* and *Gracilaria corticata*) as complementary source of dietary proteins for human and animal nutrition based on amino acid profile was evaluated. All these species showed similar non-essential amino acid patterns in which aspartic and glutamic acids constituted together a large part of the amino acid fraction (25.2% to 29.5%). Among these, *Hypnea musciformis* possessed higher amino acid content and better amino acid profile and all of them were generally rich in phenylalanine, tyrosine, threonine and tryptophan and deficient in methionine, cysteine, leucine and lysine. Except *U. lactuca* all others showed a balanced amino acid profile comparable to FAO reference pattern. Seaweeds being rich in minerals, vitamins, polyunsaturated fatty acids as well as phycocolloids, partial substitution of costly protein sources in animal feeds with seaweed protein may improve feed quality while reducing the cost.

**Keywords:** Seaweeds, amino acid profile, animal nutrition, protein source

### Introduction

Seaweeds are macroscopic marine algae, a renewable natural resource, found growing in large quantities along the coasts of India. The major seaweed growing areas include Gulf of Mannar, Gulf of Kutch, Palk Bay, Lakshadweep and Andaman and Nicobar islands. Though they have wide industrial, medicinal and agricultural applications, the country presently harvests only about 22,000 tonnes of macro-algae annually. This forms a mere 2.5% of the potential harvest of 870,000 tonnes (Anon, 2003). The principal uses of seaweeds are as sources of phycocolloids, as fodder, as fertilizer and for direct use in human nutrition (Abbott, 1996). Seaweeds cannot be considered as a main source of energy but they have nutritional value regarding vitamin, protein and mineral contents (Norziah and Ching, 2000). According to Chapman and Chapman (1980), 100g seaweed provide more than the daily requirement of Vit. A, B<sub>1</sub> and B<sub>12</sub> and two thirds of Vit.C. They are also natural sources of hydrosoluble and liposoluble vitamins, such as thiamine and riboflavin, *b*-carotene and tocopherols, as well as of long-chain polyunsaturated essential fatty acids from the *omega*-3 family such as eicosapentaenoic acid (Khotimchenko *et al.*, 2002), which may reduce the risk of heart disease, thrombosis and atherosclerosis (Mishra *et al.*, 1993). There is, therefore, interest in the use of seaweeds in the development of low-cost, highly nutritive diets for human and animal nutrition, especially animal nutrition since sea vegetables are able to accelerate the

growth of oysters, tilapia, salmon, trout, etc., all of great commercial interest (Fleming *et al.*, 1996; Horn, 1989).

Marine macroalgae could be a potential low-cost source of protein for fish. Various studies on the relationship between the nutritive value of feeds and culture organisms have demonstrated that the content of essential amino acids and fatty acids is the principal factor in their dietary value (Metailler *et al.*, 1981; Watanabe *et al.*, 1983; Tibaldi and Lanari, 1991). For good growth and survival of fish, the same 10 essential amino acids as well as the unsaturated fatty acids of the *omega* - 3 series are essential dietary nutrients for fish (Bell *et al.*, 1986). This is because many marine animals have a limited ability to synthesize these compounds from precursor molecules (Watanabe *et al.*, 1983; Kanazawa *et al.*, 1979). The primary objective of the present study is to examine the amino acid composition of the six common species of macroalgae available in India; *Sargassum wightii*, *Ulva lactuca*, *Kappaphycus alvarezii*, *Hypnea musciformis*, *Acanthophora spicifera* and *Gracilaria corticata* in order to determine if these algae are potential source of dietary protein for fish, poultry, cattle, as well as human beings.

### Materials and methods

Seaweed samples were collected from Kerala coast; *G. corticata*, *H. musciformis*, *A. spicifera* and *S. wightii* from Thikkodi and *U. lactuca* and *K. alvarezii* from Thangasseri, Quilon. Since marine algae are known to

differ in their nutritional quality with time, sampling was carried out during one growing season, in July. The samples were washed well in distilled water to remove excess salt and dried in an oven at 60°C for 12 hours. For amino acid analysis, triplicate sub-samples were hydrolysed with 6 N hydrochloric acid in evacuated sealed tubes for 24 h at 110°C. The hydrolysed sub-samples were then analyzed in Waters Pico-Tag HPLC, Amino acid Analysis System (Column: Pico-Tag amino acid analysis column, 3.9(150 mm); detector: Waters 2489 Dual  $\lambda$  ( absorbance Detector). For tryptophan analysis, samples were alkali hydrolyzed since the amino acid will undergo denaturation under low pH.

Amino acids recovered are expressed in percentage with reference to dry weight of seaweeds. The level of amino acids (mg/g N) in different seaweeds is compared with the FAO reference pattern (FAO/WHO, 1981) and that of egg and milk protein (Orr and Watt, 1968) in order to evaluate their protein quality. The essential amino acid (EAA) score is calculated by the method of FAO/WHO (1981) as shown below:

Essential amino acid score =

$$\left[ \frac{\text{mg of EAA in 1 g of test protein}}{\text{mg of EAA in reference pattern}} \right] \times 100$$

and the limiting amino acid in each seaweed was found out.

## Results and discussion

The amino acid profiles of six species of seaweeds are presented in the Table 1. The total amino acid content ranged from 5 % in *K. alvarezii* to 20% in *H. musciformis*. They contained all the essential amino acids in different proportions, which accounted for 45 to 49 % of the total amino acid content which is comparable to that of the other seaweeds reported in earlier works; 37±38 % in *Porphyra tenera*, *Grateloupia turuturu*, *Ulva pertusa* and *Codium fragile* (Fujiwara-Arasaki *et al.*, 1984); 37-42 % in *U. lactuca* and *Gelidium amansii* (Ochiai *et al.*, 1987) and 36.5± 38.6% in *Ulva rigida* and *U. rotundata* (Fleurence *et al.*, 1995). All of the essential amino acids *i.e.* methionine, leucine, lysine, cysteine, phenylalanine, arginine, iso-leucine, threonine, tryptophan and valine and seven non-essential amino acids were found to be present in all the six species.

The total amino acid content in *H. musciformis* was 20% in which 10% was made up of essential amino acids. The ratio of essential amino acids to total amino acids was 0.49 *i.e.*, almost half of the amino acid. The result also indicated that the ratio of essential amino acids to non-essential amino acids was 0.95. This seaweed was rich in

Table 1. Amino acid profile of seaweeds (in % dry weight of seaweed)

Amino acid	Sa	Ul	Ka	Hy	Gr	Ac
Asp	1.80	1.59	0.66	2.46	1.99	1.42
Glu	1.15	1.40	0.73	2.67	1.85	1.21
Ser	0.54	0.94	0.39	1.45	1.12	0.40
Gly	0.45	0.71	0.28	0.95	0.85	0.33
His	0.45	0.31	0.08	0.40	0.33	0.38
Arg	0.60	0.89	0.46	1.04	0.89	0.51
Thr	0.71	0.99	0.39	1.78	1.23	0.50
Ala	0.44	0.85	0.25	0.97	0.81	0.34
Pro	0.38	0.41	0.17	0.82	0.58	0.31
Tyr	0.49	0.39	0.13	0.46	0.44	0.62
Val	0.50	0.66	0.31	1.07	0.90	0.44
Met	0.37	0.19	0.19	0.43	0.15	0.32
Cys	0.25	0.10	0.06	0.21	0.12	0.13
Ile	0.55	0.38	0.21	0.90	0.63	0.37
Leu	0.55	0.72	0.33	1.33	1.06	0.49
Phe	0.63	0.60	0.24	0.91	0.76	0.54
Trp	0.21	0.13	0.13	0.61	0.41	0.24
Lys	0.50	0.46	0.20	1.13	0.68	0.43
Total	10.58	11.73	5.21	19.59	14.80	8.99

Sa: *S.wightii*, Ul: *U.lactuca*, Ka: *K.alvarezii*,

Hy: *H.musciformis*, Gr: *G.corticata*, Ac: *A.spicifera*

aspartic acid, glutamic acid, serine, threonine, leucine and lysine and poor in cysteine, methionine, histidine and tyrosine. Compared to other seaweeds it contained higher quantity of threonine, tryptophan and lysine. The limiting amino acids were methionine and cysteine and the amino acid score obtained was 0.9. *G. corticata* contained 15% amino acids out of which 7% consisted of essential amino acids. The ratio of essential amino acid to total amino acid was 0.47 and that of essential amino acid to non-essential amino acid was 0.88. It was found to be rich in aspartic acid, glutamic acid, serine, glycine, arginine, threonine, alanine, valine and leucine while deficient in methionine and cysteine. Among the six seaweeds, this species contained higher amounts of valine and leucine. The limiting amino acids were methionine and cysteine with an amino acid score of 0.50 (Table 1).

*U. lactuca* contained 12% amino acids in which essential amino acids constituted 5 %. The ratio of essential amino acids to total amino acids was 0.45 and that of essential amino acid to non-essential amino acids was 0.8. Amino acids in this species were constituted mainly of aspartic acid, glutamic acid, serine, glycine, arginine, threonine, alanine and leucine and were poor in cysteine, tryptophan and methionine. The serine, glycine and ala-

nine contents were higher than those in all other seaweeds. The amino acid score of the species was 0.7, the limiting amino acid being lysine. Total amino acid content in *S. wightii* constituted 12% of its dry weight and the major amino acids contributed were aspartic acid, glutamic acid, arginine, threonine, isoleucine and phenylalanine. Compared to other seaweeds, this species was rich in aspartic acid, histidine, isoleucine and cystine, but poor in tryptophan. The amino acid score was 0.75 and the limiting amino acid was leucine.

*A. spicifera* contained 9% amino acids/100g out of which essential amino acids contributed 4%. The essential amino acid to total amino acid ratio was 0.45 and that of essential amino acid to non-essential amino acid was 0.82. The major amino acids were aspartic acid, glutamic acid, tyrosine, arginine, threonine, phenylalanine and leucine, and were poor in cysteine. Phenylalanine, methionine and tyrosine content was higher in this species. The amino acid score was 0.77 and the limiting amino acid was leucine. The total amino acid content in *K. alvarezii* was 5% in which 2% was made up of essential amino acids. The ratio of essential amino acids to total amino acids was 0.45 and that of essential to non-essential amino acids was 0.82. It was poor in cystine and histidine. Glutamic acid and arginine content in this species was higher compared to other seaweeds. The limiting amino acid was lysine with an amino acid score of 0.69. Thus the major limiting amino acids among the six seaweed species were methionine and cysteine, lysine and leucine. According to Wong and Cheung (2000), limiting amino acids of *H. musciformis* and *U. lactuca* protein concentrates were the sulphur containing amino acids (methionine and cysteine) and lysine. Arasaki and Mino (1973) and Fujiwara-Arasaki *et al.* (1984), also have reported similar observations from *Ulva pertusa*, *Codium fragile*, *Porphyra tenera* and *Grateloupia turuturu*.

All seaweed samples exhibited similar non-essential amino acid patterns in which aspartic and glutamic acids constituted together a large part of the amino acid fraction (25% to 30%). In the brown seaweed, *Fucus* sp. these two amino acids could represent between 22% and 44% of the total amino acids (Munda, 1977). In the green seaweeds, the level of these two amino acids represent up to 26% and 32% of the total amino acids as in *Ulva rigida* and *U. rotundata*, respectively (Fleurence *et al.*, 1995). However, the glutamic and aspartic acid levels seem to be lower (Fujiwara-Arasaki *et al.*, 1984; Indegaard and Minsaas, 1991) in red seaweed species such as *Palmaria palmata* and *Porphyra tenera* (14 and 19% of the total amino acids, respectively). According to Mabeau *et al.* (1992), the high levels of aspartic and glutamic acids

were responsible for the special flavour and taste of the seaweeds. Moreover, the seaweeds were generally rich in glycine and alanine but poor in histidine, which was also consistent with the results of seaweed proteins such as in *U. pertusa*, *C. fragile*, *P. tenera*, *G. turuturu* (Arasaki and Mino, 1973), *U. rigida*, *U. rotundata*, *U. lactuca* and *Gelidium amansii* (Ochiai *et al.*, 1987). It is reported that there are some pronounced differences between the amino acid profiles of Rhodophyceae (red seaweeds) and Chlorophyceae (green seaweeds), especially the sulfur-containing amino acids in the former were higher than in the latter (Qasim, 1991). According to Wong and Cheung (2000), the sulfur-containing amino acids of *Hypnea* species were about 5% and that of *U. lactuca* 3% of the total amino acids. Results from the present study also indicated variations in the distribution of different amino acid groups among the three classes of seaweeds. Except in the case of *G. corticata*, sulphur containing amino acids, cysteine and methionine were higher in all other red seaweeds than that of green seaweed, *U. lactuca*. The values ranged from 3% for *H. musciformis* to 5% for *A. spicifera*, while *U. lactuca* contained 3% only. The brown seaweed, *Sargassum wightii* had the highest value, 6%. Similarly aromatic amino acids (tyrosine, phenylalanine and tryptophan) and acidic amino acids (aspartic acid and glutamic acid) were also lower in green seaweed (10% and 26% respectively). Values obtained for red seaweeds were 10% to 16% in the case of aromatic amino acids and 26% to 29% for acidic amino acids. *S. wightii* contained 13% aromatic amino acids and 28% acidic amino acids. But *U. lactuca* contained higher aliphatic amino acids compared to green and brown seaweeds. Neutral and basic amino acid content in green seaweed was also comparatively higher than red and brown seaweeds. The higher alanine content obtained for *U. lactuca* (7%) with respect to red and brown seaweeds was similar to the values obtained in previous studies; *U. lactuca* 10% (Wong and Cheung, 2000); *Ulva* sp. 7-8% (Fleurence *et al.*, 1995). If the species wise difference is considered, it can be seen that maximum aromatic and acidic amino acids were present in *A. spicifera*, aliphatic amino acids in *G. corticata*, sulphur containing amino acids in *S. wightii*, neutral amino acids in *Ulva*, and basic amino acids in *K. alvarezii*. Similarly *H. musciformis* contained maximum amount of essential amino acids (49%) and *K. alvarezii* the minimum (45%).

The comparison of the amino acid composition of seaweeds with FAO reference pattern (FAO/WHO, 1981) and those of other food proteins (Orr and Watt, 1968) allows us to obtain a first estimate of the nutritional value of seaweed proteins. It is evident that most of the seaweeds seemed to be able to contribute adequate levels of total

essential amino acids according to the FAO requirement pattern (Fig.1). Leucine, lysine and valine were the only amino acids deficient in *S. wightii* and the levels of all others were higher with respect to the reference pattern.

Methionine + cysteine, phenylalanine + tyrosine, threonine and tryptophan content in *S. wightii* were even higher than that in egg, human and cow milk protein. *U. lactuca* was found to be deficient in four amino acids (Isoleucine,

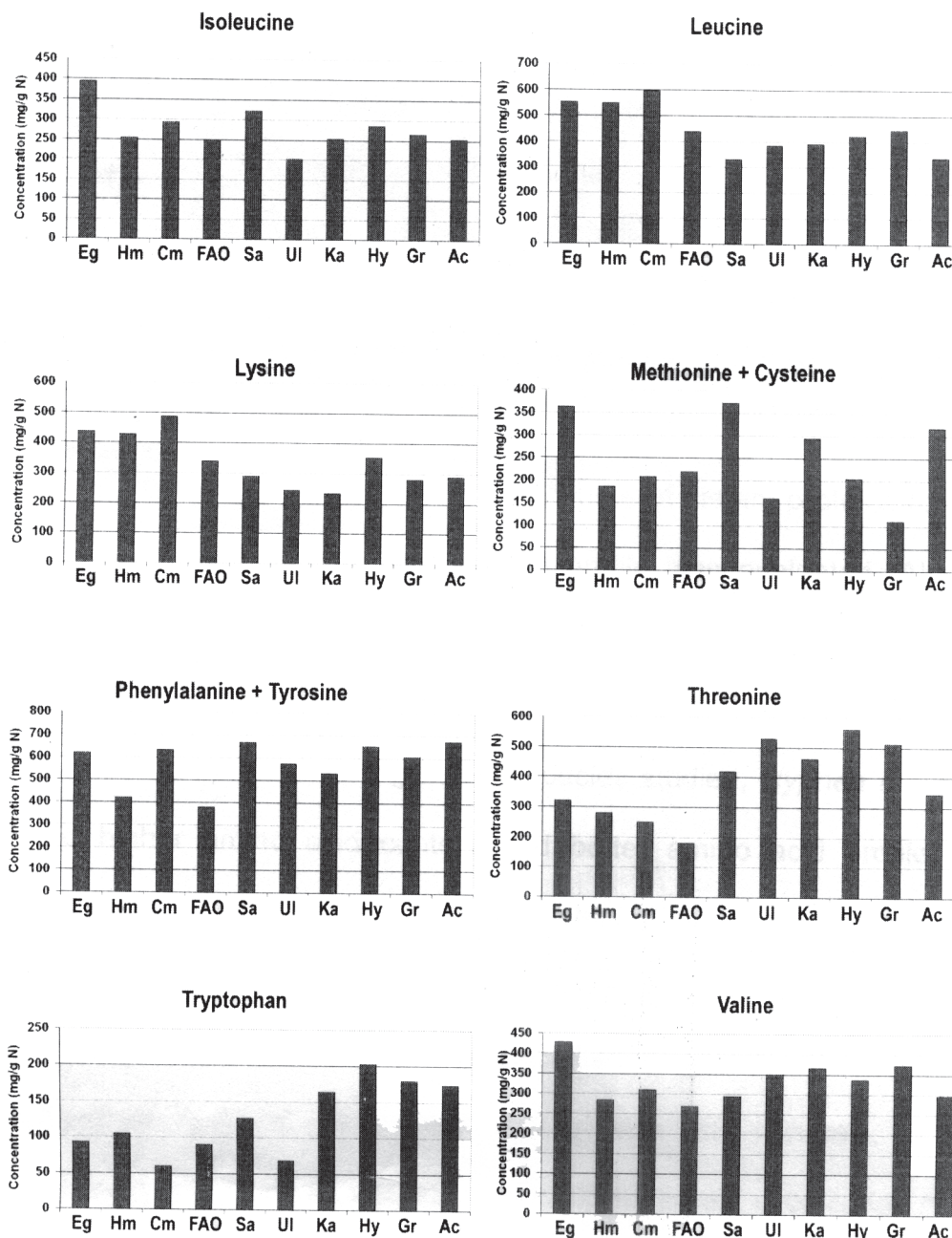


Fig. 1. Comparison of amino acid profile of seaweeds with that of FAO reference pattern, egg and milk proteins. Abbreviations: Eg: Egg; Hm: Human milk; Cm: Cow milk; FAO: FAO reference pattern; Sa: *S. wightii*; Ul: *U. lactuca*; Ka: *K. alvarezii*; Hy: *H. musciformis*; Gr: *G. corticata*; Ac: *A. spicifera*



leucine, lysine and methionine + cysteine) but had higher threonine content than that in egg protein. *K. alvarezii* was also deficient in leucine and lysine but had higher threonine and tryptophan content. *H. musciformis* was found to possess better amino acid profile than other seaweeds and methionine was the only deficient amino acid present. Phenylalanine + tyrosine, threonine and tryptophan levels were much higher than those in egg and milk proteins. Lysine and methionine levels in *G. corticata* were lower than those in FAO reference pattern. The phenylalanine + tyrosine, threonine and tryptophan levels in this species were equal to or even higher than that in egg, human and cow milk proteins. The deficient amino acids in *A. spicifera* were leucine and lysine but the levels of phenylalanine + tyrosine, threonine and tryptophan were higher than those in egg protein.

It can be concluded that all the six species of seaweeds are generally rich in aromatic amino acids, threonine and tryptophan and deficient in sulphur containing amino acids, leucine and lysine. Except *U. lactuca* all the five seaweeds showed a balanced amino acid profile comparable to that of FAO. It was found that seaweeds could be a complementary source of food proteins for human and animal nutrition. Besides as source of proteins, they can be used as excellent binders in formulated feeds as majority of them contain phycocolloids such as agar, algin and carrageenan. However, their nutritional values obtained here were based on chemical analysis only. Biological evaluation using human and animal feeding studies would be required to establish the nutritional value of these seaweeds, particularly the *in vivo* protein digestibility and bioavailability of the essential amino acids. Steps like biotechnological treatment by enzymatic degradation of algal fibres could be attempted to improve protein digestibility and to increase the nutritional value of these proteins. Also the use of seaweed proteins with good amino acid profile in feed for fish and cattle seems to be a promising way for the utilization of this marine resource that remains under-exploited in our coasts. This would also satisfy industrial needs, which require a partial substitution of animal meal by plant meal in fish feed. Results obtained from this study may help to blend different seaweed species in various proportions to achieve required level of amino acids in the forage/ formulated feed for the candidate species proposed to be farmed.

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