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 Ulva 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, and B12, 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 nutritious 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
Department 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 λ (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:

\[
\text{Essential amino acid score} = \left( \frac{\text{mg of EAA in 1 g of test protein}}{\text{mg of EAA in reference pattern}} \right) \times 100
\]

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 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).

\[
\text{Total} = 10.58 \quad 11.73 \quad 5.21 \quad 19.59 \quad 14.80 \quad 8.99
\]

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Sa</th>
<th>Ul</th>
<th>Ka</th>
<th>Hy</th>
<th>Gr</th>
<th>Ac</th>
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<td>Asp</td>
<td>1.80</td>
<td>1.59</td>
<td>0.66</td>
<td>2.46</td>
<td>1.99</td>
<td>1.42</td>
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<td>Glu</td>
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<td>0.73</td>
<td>2.67</td>
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</tr>
<tr>
<td>Ser</td>
<td>0.54</td>
<td>0.94</td>
<td>0.39</td>
<td>1.45</td>
<td>1.12</td>
<td>0.40</td>
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<tr>
<td>Gly</td>
<td>0.45</td>
<td>0.71</td>
<td>0.28</td>
<td>0.95</td>
<td>0.85</td>
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</tr>
<tr>
<td>His</td>
<td>0.45</td>
<td>0.31</td>
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<td>Arg</td>
<td>0.60</td>
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<td>Thr</td>
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<tr>
<td>Ala</td>
<td>0.44</td>
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<td>0.97</td>
<td>0.81</td>
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<td>Pro</td>
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<td>0.41</td>
<td>0.17</td>
<td>0.82</td>
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<td>0.31</td>
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<tr>
<td>Tyr</td>
<td>0.49</td>
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<td>0.13</td>
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<tr>
<td>Val</td>
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<td>1.07</td>
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<tr>
<td>Met</td>
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<td>0.19</td>
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<tr>
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<td>0.25</td>
<td>0.10</td>
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<td>0.13</td>
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<tr>
<td>Ile</td>
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<td>0.21</td>
<td>0.90</td>
<td>0.63</td>
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<tr>
<td>Leu</td>
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<td>Phe</td>
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<td>0.13</td>
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</tr>
<tr>
<td>Lys</td>
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<td>0.20</td>
<td>1.13</td>
<td>0.68</td>
<td>0.43</td>
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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. alvarezi 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), 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. alvarezi. Similarly H. musciformis contained maximum amount of essential amino acids (49%) and K. alvarezi 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

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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,

![Graphs showing amino acid profile comparison](chart.png)

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. alvarezi*; 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. alvarezi 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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