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# Evaluation of amino acids in *Kappaphycus Sp*. to assess its applicability as nutrient

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## ABSTRACT

This paper presents the details of estimation of amino acids available in Kappaphycus sp. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Amino acids were estimated by HPLC method. Total 18 amino acids were found in the dried species. From the studies, it is observed that lysine is the major constituent and followed by asparagines, histidine, isoleucine, phenylalamine, tryptophan. The studies showed that *Kappaphycus sp* could be used as a food supplement.

**Keywords:** Seaweeds, red algae, *Kappaphycus*, amino acids

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## INTRODUCTION

Seaweed species are rich in beneficial nutrients, in countries such as China, Japan and Korea, they have been commonly utilized in human alimentation. Seaweeds have been consumed in Asia since ancient times. Further, marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical uses [1, 2]. This wide range in mineral content, not found in edible land plants, is related to factors such as seaweed phyllum, geographical origin and seasonal, environmental and physiological variations [2]. Ke Li et al. [3] determined various chemical constituents of the red alga *Grateloupia turuturu*. Protein aminoacids are usually considered to possess little, if any, taxonomic interest. Indeed, on account of their universal occurrence in living matter, variations of profiles are necessarily quantitative and plausibly affected not only by genetically controlled factors, but also by differences in physiological and environmental conditions. Consequently, most of the taxonomically oriented work has been devoted to the non-protein counterparts, supposedly of greater taxonomic potential. Nevertheless, evidence is being slowly accumulated that amino-acid profiles of protein extracts from different species show meaningful correlations. The distribution of unbound protein amino acids has been given still less attention, although early investigation had provided some evidence of possible taxonomic value. Christine Dawczynski et al. [4] analysed the nutritional compositions of 34 edible seaweed products of various species such as *laminaria, undaria pinnatifida, hizikia fusiforme, porphyra*.

In general, from the critical review of literature, it has been observed that the most studies on the nutrient contents of seaweeds have concerned fresh plants. Little is known of the effects of processing by drying or canning. The present investigation aims at on determination of amino acid contents in *Kappaphycus sp.*

## MATERIALS AND METHODS

Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Algae samples were cleaned at epiphytes and necrotic parts were removed. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the primary estimation of amino acids. This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing.

### Qualitative and quantitative estimation of amino acids

Free and protein amino acids were estimated by O-pthaldialdehyde method described by Rajendra [5]. Extractions of free amino acids and soluble proteins from the algal tissues are described elsewhere. Concentrated 80% ethanolic extract was directly used for qualitative and quantitative estimation of free amino acids. For protein amino acids, protein in the extract was

precipitated by adding equal volume of 10% TCA and dried in *vacuo*. To know quantities of dried protein (usually 75 mg), 2.0 ml of 6.0 N HCl was added and hydrolyzed at 110$^{o}$C for 18 hrs. After the hydrolysis, the hydrolysates were allowed to evaporate to dryness and the dried material was used for HPLC analysis.

**Reagents**

Borate buffer (0.4M)

Boric acid (2.47g) was dissolved in 100 ml of water and pH adjusted to 9.5 with 4.0N NaOH.

Methanol tetrahydrofuron

The reagent was prepared by the addition of 30 ml of tetrahydrofuron to 970 ml of methanol.

Ortho-pthaldialdehyde reagent

Anhydrous ortho-pthaldialdehyde (50 mg) was dissolved in a mixture of 2.0 ml of methanol, 8.0 ml of borate buffer and 5.0 ml of β-mercaptoethanol. The reagent was prepared fresh for every estimation.

**Procedure**

One milliliter of ortho-pthaldialdehyde reagent was added to 200 ml of the amino acid sample, mixed thoroughly and kept undisturbed for 2 minutes for derivatization. The sample was then filtered and 20 ml was injected in to the HPLC for analysis.

**Operational condition of HPLC**

| | | |
|---|---|---|
| The instrument | : | LACHROM L-700 and D-70000 HPLC |
| Column | : | C 18' 4.6 X 250 mm, 5 µm packing |
| Mobile phase A | : | 0.1 M acetate buffer (pH 7.2) |
| Mobile phase B | : | 3% tetrahydrofuron in methanol |
| Flow rate | : | 1.5 ml/min. |
| Gradient | : | 10 – 42% b for 15 min.,<br>42% B for 10 min.<br>42% - 50% B for 3 min.,<br>50% - 70% B for 7 min.<br>70% - 90% B for 4 min.,<br>90% - 100 % B for 1 min.<br>100% B for 2 min.<br>100% - 10% B for 1 min.<br>10% B for 2 min. |
| Detector | : | fluorescence, 9 µl flow cell F1-2 |
| Excitation filter | : | 305-395 nm |
| Emission filter | : | 430-470 nm |
| Sensitivity | : | 0.005 Abs |

**AMINO ACIDS – METHOD OF ANALYSIS**

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Column: DENALI C18 5MICROMM
4.6 mm x 150 mm ( cat no. DEN-5C18-15046)
Flow : 1ml/minute
Mobile Phase : A= 20 : 80 ACETONITRILE : 25 Mm potassium Phosphate, Ph 3.3 B = 80: 20
CAN : 25 Mm potassium Phosphate , Ph 3.3
Gradient : O TO 75% B over 15 min
Temperature : AMBIENT 23° C
Detection : 254 nm
Sample : 5 microlitre of aminoacids standards mixture

## RESULTS AND DISCUSSIONS

The standard graph for Amino acid is shown in Figure 1. The various components available in the standard amino acid are given in Table 1. Figure 1 also shows the various components of amino acids available in *Kappaphycus* sp. Table 1 also presents the quantity and qualitative information on the various components available in amino acid. From the Table 1, it can be observed that the standard deviation (SD) for all the components is less than 0.05.

**Table 1: Components of standard amino acid & *Kappaphycus sp***

| **Amino acid standard** | | | **Amino acids in red algae *(Kappaphycus*)** | | |
|---|---|---|---|---|---|
| **Component name** | **R.T.** | **AREA %** | **R.T.** | **AREA %** | **Various components /100gm** |
| Aspartic acid | 4.03 | 6.343 | 4.05 | 1.490 | 0.565±0.01 |
| Glutamic acid | 6.47 | 2.546 | 6.48 | 0.657 | 1.12±0.012 |
| Asparagine | 9.23 | 1.365 | 9.24 | 0.434 | 1.045±0.021 |
| Serine | 10.22 | 3.067 | 10.21 | 0.897 | 0.1121±0.02 |
| Gultamine | 11.67 | 1.275 | --- | ----- | ------ |
| Glycine | 13.45 | 11.152 | 13.45 | 7.566 | 0.1012±0.015 |
| Threonine | 14.89 | 3.565 | 14.89 | 3.909 | 0.5067±0.01 |
| Arginine | 18.08 | 13.565 | 18.08 | 4.454 | 0.52012±0.02 |
| Alanine | 19.53 | 6.921 | 19.53 | 3.787 | 0.706±0.001 |
| Cystine | 21.67 | 6.787 | 21.67 | 0.399 | 0.2012±0.01 |
| Tyrosine | 24.76 | 4.556 | 24.76 | 4.375 | 0.3415±0.012 |
| Histidine | 27.25 | 0.245 | 27.25 | 0.699 | 1.15±0.02 |
| Valine | 29.90 | 8.343 | 29.90 | 3.787 | 0.314±0.001 |
| Methionine | 31.41 | 0.756 | ---- | ----- | ---------- |
| Iso-leucine | 36.62 | 7.554 | 36.62 | 3.967 | 1.126±0.01 |
| Phenyl alanine | 37.93 | 4.620 | 37.93 | 9.898 | 1.454±0.02 |
| Leucine | 39.34 | 14.41 | 39.34 | 4.677 | 0.9067±0.002 |
| Taurine | 41.11 | 0.545 | --- | ---- | ---------- |
| Lysine | 43.39 | 1.105 | 43.39 | 38.060 | 2.075±0.01 |
| Proline | 45.11 | 0.616 | 45.11 | 6.979 | 0.3235±0.012 |
| Tryptophan | 46.12 | 0.656 | 46.12 | 0.853 | 1.11±0.01 |
| | | 100.000 | | 100.000 | |

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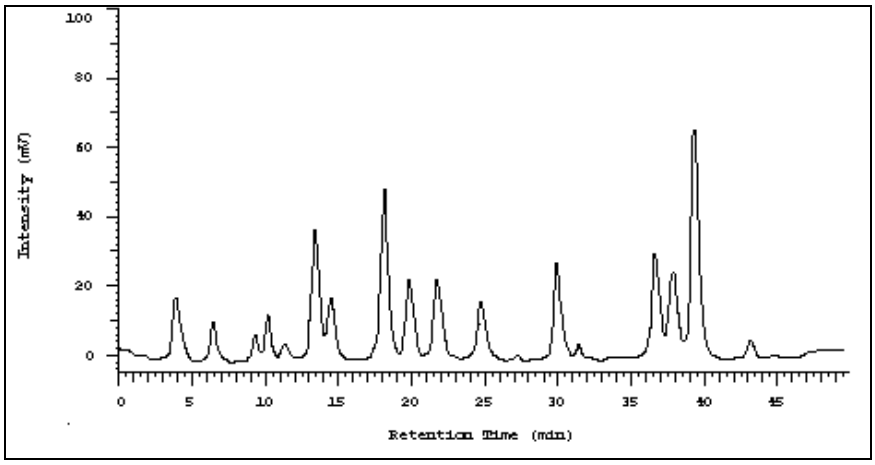
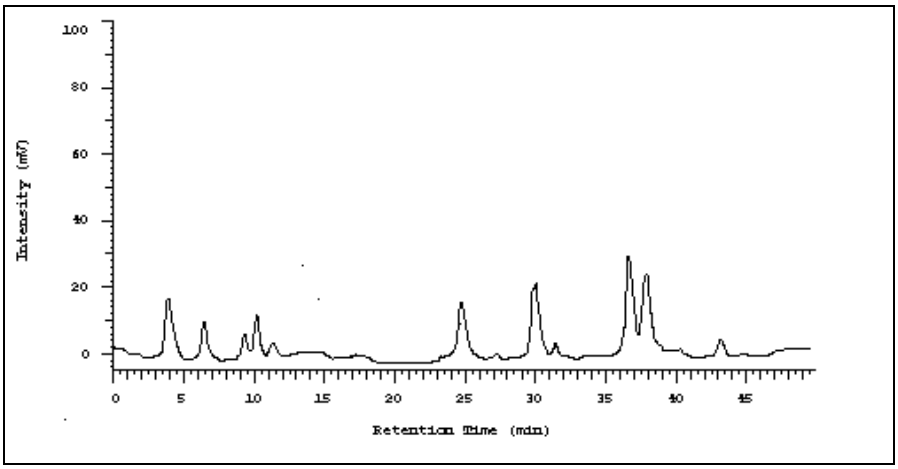
The dried sample of kappa.alvarezi was found to contain 18 amino acids, namely, asparagine, aspartic acid, glutamic acid, alanine, valine, glycine, arginine, serine, cystine, methonine, theronine, phenylalanine, tyrosine, isoleucine, leucine, histideine, lysine, tryptophan and praline. It can be observed from Table 1 that lysine content is the major component and the quantity being 2.075gms/100mgs. The other components, namely, phenylalanine (1.454gms), glutamic acids (1.12gms), isoleucine (1.126gms), histidine (1.15gms), tryptophan (1.11gms), methonine (1.078gm), asparagine (1.045gms) are followed by lysine. Further, it can be noted that glycine is much less (0.1012gms) quantity compared to all other components available in *Kappaphycus sp*.




**(a) Standard graph**




**(b) *Kappaphycus* sp.**

**Figure 1: Profile of Amino acids**

Giuseppe Impellizzeri et al. [6] quantitatively determined free protein amino acids in 30 red algae. In most of the species, aspartic acid (asparagine), glutamic acid (glutamine), alanine, glycine and serine dominate, while massive accumulation of proline (up to 80.5%) was observed in six species, all belonging to the family of Rhodomelaceae. Giuseppe Impellizzeri [7] analysed 18 red algae sp for amino acids and low-MW carbohydrates using different methods. It was

observed that all species of red algae examined had a similar composition of protein amino acids. All species contain low concentrations of basic amino acids and show a general prevalence of the same compounds (aspartic and glutamic acids, which often make up 50% or more of the total, alanine, glycine and serine). Christine Dawczynski et al. [4] examined different seaweed products for analysis of amino acids (aas), protein and dietary fibre. All essential aas were detected in the seaweed species tested and redalgae species featured uniquely high concentrations of taurine when compared to brown algae variteis. Dhamotharan [8] mentioned that the dried samples of brown algae (*padina tetrastromatica and stocchosperumum marginatum*) were found to contain 18 aminoacids, namely, aspartic acids, glutamic acid, asparagines, serine, glutamine, glycine, threonine, arginine, alanine, cystine, tyrosine, histidine, valine, methionine, isoleucine, phenyalanine, leucine and lysine). Analysis of amino acids by GC showed the presence of 18 amino acids in the tissues of the brown algae. In the present study showed that lysine is the major content of fatty acid. Interestingly, lysine was the major constituent in padina and accounted for 13% of the total amino acid content. Lysine is an essential aminoacid and has nutraceutical value. As compared to padina, the total amino acid content was 32% less with histidine as the major constituent in stoechospermum marginatum. Further lysine levels were relatively low in these tissues. The tissues of both algae showed high levels of asparagines in addition to having arginine and cystine indicating that these algae might be using asparagines as their transport form of N while arginine and cystine as the storage form. The aas fingerprint is distinct for the two algae. Dave and Chawhan [9] have reported high levels of lysine in the tissue of *caulerpa sp*. The observed levels of lysine in padina is more than that reported for the freshwater alga spirulina and less than that reported for another fresh water alga *dithophorooedogonia*. Rajeshwar et al. [10] separated the ultraviolet (UV) absorbing mycosporine – like amino acids (MAAs) from a marine red alga *Gracilaria* cornea using HPLC. The isolated MAAs were identified as porphyra-334 and/or shinorine by comparing them with various standards. Their results indicated a highly stable nature of MAAs against the environmental stress factors like UV-B and heat. In general, the amino acid profile is important for evaluating the nutritional value of algae proteins, but the digestibility of algae protein was not analysed.

## SUMMARY & CONCLUDING REMARKS

Amino acids were estimated on the *Kappaphycus sp*. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Amino acids were estimated by using HPLC method where as fatty acids by gas chromatography. Total 18 amino acids were found in the dried powder of species. Among all the amino acids, lysine is the major constituent and followed by asparagines, histidine, isoleucine, phenylalamine, tryptophan. The studies showed that red seaweeds could be used as a food supplement to meet the recommended daily intake of some essential minerals.

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