WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 10, 1428-1438. Research Article ISSN 2277– 7105

AMINO ACIDS COMPOSITION OF TWO *ULVA* SPECIES FROM SOUTHEAST COAST OF TAMIL NADU, INDIA

[1]Anbalahan N., [1]Nandakumaran T., [2]Manivannan M. and [2]*G. Subramanian

[1]PG and Research Department of Botany, Kandaswami Kandar's College, Velur, Namakkal - 638 182.

[2]PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal – 637 002.

Article Received on
07 July 2019,

Revised on 28 July 2019,
Accepted on 18 August 2019,

DOI: 10.20959/wjpr201910-15733

***Corresponding Author**
G. Subramanian
PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal – 637 002.

ABSTRACT

The aim of the present study concentrated on amino acids content of two green algal seaweeds namely *Ulva fasciata*, and *Ulva intestinalis* collected from southeast coast of Tamil Nadu, India. Amino acids of the seaweeds were evaluated by using HPLC. Depending on the species, the quantity of each amino acid varied. Of the total amino acids, essential amino acids were higher than non-essential amino acids in two seaweeds investigated.

KEYWORDS: Amino Acids, Essential Amino acids, *Ulva fasciata*, *Ulva intestinalis*, South East Coast of Tamil Nadu.

INTRODUCTION

The first commercial production of the amino acid, glutamic acids, was started in Japan in as early as 1908. It was Ikeda (of Japan) who first identified that glutamic acid (in the form of monosodium glutamate) possesses taste enhancing properties. In the early days, monosodium glutamate (MSG) was extracted from the vegetable proteins (wheat and soy). It was only in 1957, the large scale industrial production of MSG by using microorganisms commenced. Today, commercial production of amino acids is one of the biggest industries worlds over with an annual increase in the demand by about 10%. Glutamic acid continues to be the largest producer among the amino acids, followed by lysine, methionine, threonine and aspartic acid. Amino acids have a wide range of applications; because of they are 65% in food industries, 30% in feed additives and 5% in pharmaceutical industries.[1,2]

Protein and amino acids 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.[3] This present study is to find out the profile of amino acid of an individual species of selected marine green algal seaweeds

Studies in free amino acids of some Indian marine algae[4] indicate that these algae possess all the essential amino acids needed in the human diet. The greatest importance of the amino acid in the seaweed is mainly due to the occurrence of amino acids like methionine and tryptophan which are not available in other vegetable food materials. The amino acid contents of some marine algae were reported by Lewis and Gonzalves.[5-7,8] The maximum amino acid was observed in the red algae *Acanthophora spicefera* (27.12%) when compared with brown and green seaweeds. The minimum amino acid was observed in the red algae (15.32%) when compared to brown and green seaweeds. In general, there were not many seasonal variations in these biochemical constituents in all the algae. The protein content amino acid to and lipid varies from species to species. It was observed that green algae *Ulva lactuca* has the maximum lipid content (2.10) followed by *Enteromorpha flexusa* (1.75%), *E.intestinals* (1.32%), *Caulerpa scalpelliformis* (1.85%), *Chaetomorpha luna* (1.33%.[9,10]

In most analyses of amino acid composition in marine algae, glutamic acid, and aspartic acid represent the highest proportions of amino acids.[11-13] Generally, the amino acids are as protein constituents, they are either as free amino acids or their salts. For humans, glutamate is the major component of the savory, the fifth basic taste called umami from its characterization in kelp.[14,15] Glutamic acid content may decrease after several successive harvests of *Pyropia yezoensis.*[16] Other amino acids (alanine and glycine) also contribute to distinctive flavors of some marine algae.[13] The non-proteinaceous amino acid taurine is especially abundant in marine red algae.[16] Although taurine is not an EAA for adults, it is a component of bile acids that complex and lower cholesterol in the bloodstream.[17]

During periods of nutrient limitation such as during the summer stratification of coastal waters, however, macroalgal protein content decreases, and the relative proportions of amino acids change.[18-20]

MATERIALS AND METHODS

The selected green algal seaweeds namely *Ulva fasciata,* and *Ulva intestinalis*, from the southeast coast of Tamil Nadu, India was taken up for this present research work. They are occurring on the rocky substratum and shallow muddy waters along the inter-tidal and sub-tidal regions of coastal waters.

Amino Acid Analysis on HPLC

Extraction of crude amino acids.[21]

The collected marine algal sample of seaweeds weighing approximately 1 kg fresh weight and air-dried under shade in dark condition for 3 days. The shade dried samples were milled using a maxi grinder machine as a fine powder. Then the powdered samples of each specimen weighing 100 grams were transferred into 250 ml of Erlenmeyer conical flasks containing 150 ml of 75% ethanol for extraction for 5 days under the dark condition in room temperature. The extraction was repeated at least thrice until the extracts were colourless. The extracts were combined and kept each extraction type separately were centrifuged at 12000 rpm for 10 minutes. The supernatants were concentrated using flash evaporator at 45^0C under reduced pressure. The extracts were stored at 0^0C to further study.

Reduction of disulfide compounds and deproteinization

In a test tube, 1 mg crude amino acid extracts prepared from seaweeds and 1 ml of β-mercaptoethanol was added and mixed thoroughly, allowed to stand for 5 minutes at room temperature. Then 0.5 ml of ice-cold methanol was added and mixed thoroughly using vortex mixture for 5 minutes and allowed the tubes to stand for 15 minutes in an ice bucket. Then the mixture was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected and immediately processed by HPLC analysis.[22]

Peak identification and quantification

Amino acids were detected based on the retention time established for the individual amino acid under defined experimental conditions. The linearity of the peak areas for different concentrations, ranging from 20 - 200 picomoles, of individual amino acids, was determined. The calculation was based on the area under peak established for a given amino acid of known concentration.

Free and protein amino acids were estimated by O-pthaldialdehyde method described by Rajendra.[23] 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 an 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 tetrahydrofuran: The reagent was prepared by the addition of 30 ml of tetrahydrofuran to 970 ml of methanol. 50 mg of Ortho-pthaldialdehyde was dissolved in a mixture of 2.0 ml of methanol, 8.0 ml of borate buffer and 5.0 ml of β-mercaptoethanol is known as Ortho-pthaldialdehyde reagent which is prepared always freshly.

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 2 µl was injected into the HPLC for analysis.

The 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% tetrahydrofuran in methanol
Flow rate : 1.5 ml/min.
Gradient : About 10 to 42% of B for 15 min., 42% of B for 10 min. 42% - 50% of B for 3 min., 50% - 70% of B for 7 min. 70% - 90% of B for 4 min., 90% - 100 % of B for 1min.100% of B for 2 min.100% - 10% of B for 1 min.10% of 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

Column Conditions

Column : DENALI C18 5MICROMM- 4.6 mm x 150 mm

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 : About 0 to 75% of B over 15 min.

Temperature : Ambient 23° C

Detection : 254 nm

Sample : 5 microlitre of aminoacids standards mixture.

RESULTS AND DISCUSSION

In the present investigation, biochemical characteristics such as amino acids, from the extract of two green seaweeds selected from the southeast coast of Tamil Nadu.

Results on amino acids profiles of *Ulva fasciata*, and *Ulva intestinalis* were obtained through HPLC and presented in tables 1 and 2. Twenty amino acids were identified and estimated. The analysis revealed the presence of nine essential amino acids and eleven non-essential amino acids. Quality and quantity of amino acids vary according to algal species.

In the present investigation, two green seaweeds were evaluated for various amino acids using HPLC. Depending on the species, the quantity of each amino acid varied. Of the total amino acids, essential amino acids were higher than non-essential amino acids in two seaweeds investigated. However, the number of essential amino acids was nine whereas non-essential amino acids were eleven. Essential amino acids(EAA) recorded were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine and the non-essential amino acids (NEAA) alanine arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, glutamic acid, proline, serine, and tyrosin were recorded in the selected two seaweeds.

Total numbers of nine essential amino acids were detected in the selected two algal seaweeds such as *U. intestinalis,* and *U. fasciata*. All the nine EAA were detected in two algal seaweeds such as *U. fasciata,* and *U. intestinalis*.

EAA 10.66 mg/g of dry algae was in *U. fasciata.* In *U. fasciata* the significantly high amount of EAA was lysine (11.58%), and the less amount of EAA was valine (0.60%). The

remaining EAAs leucine, histidine, isoleucine, tryptophan, phenylalanine, and methionine were 7.88%, 7.07%, 6.41%, 7.41%, 6.55% and 4.69% of the total EAA of *U. fasciata* respectively.(Tables -1, 2)

EAA 10.52 mg/g of the dry weight of alga was in *U. intestinalis.* In *U. intestinalis* the significantly high amount of EAA was lysine (13.71%), and the less amount of EAA was threonine (1.43%). The remaining EAAs phenylalanine, tryptophan, histidine, isoleucine, leucine, methionine and valine were 6.39%, 6.55%, 6.79%, 8.78%, 9.23%, 2.28% and 1.74% of total EAA of *U. intestinalis* respectively.

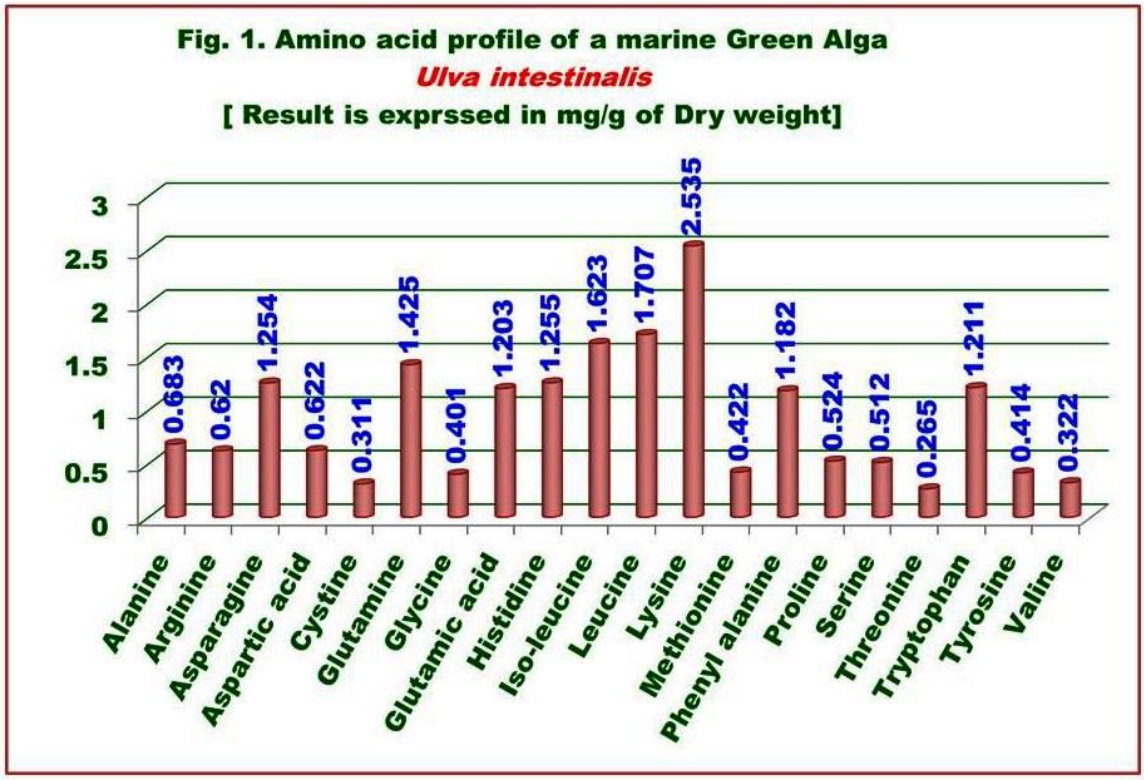
Table. 1: Amino Acid Profile of Two Marine Green Algae (Result is expressed in mg g^{-1} of dry wt.).

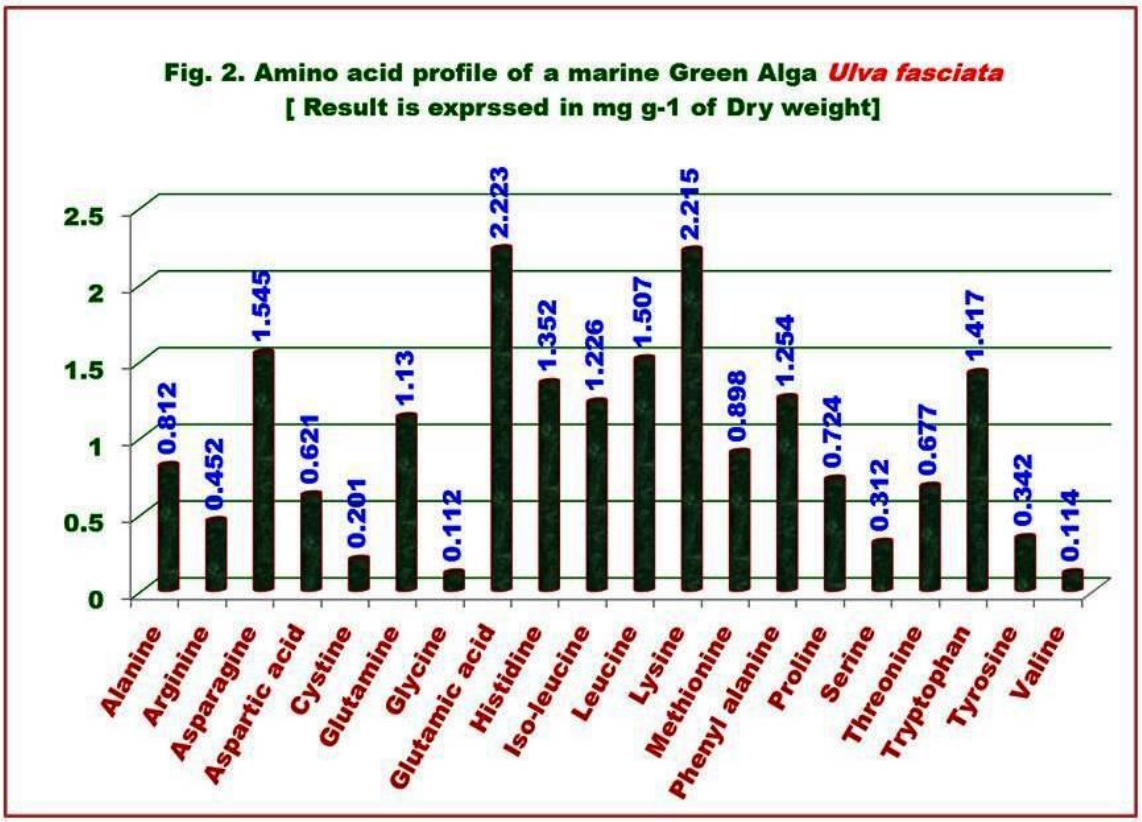
S.No.	Amino Acids	*Ulva intestinalis*	*Ulva fasciata*
Essential Amino Acids [EAA]			
01	Histidine	1.255 ± 0.02	1.352 ± 0.03
02	Iso-leucine	1.623 ± 0.12	1.226 ± 0.01
03	Leucine	1.707 ± 0.03	1.507 ± 0.04
04	Lysine	2.535 ± 0.12	2.215 ± 0.21
05	Methionine	0.422 ± 1.03	0.898 ± 0.23
06	Phenylalanine	1.182 ± 0.02	1.254 ± 0.22
07	Threonine	0.265 ± 0.14	0.677 ± 0.04
08	Tryptophan	1.211 ± 0.11	1.417 ± 0.05
09	Valine	0.322 ± 0.15	0.114 ± 0.11
Non-Essential Amino Acid s [NEAA]			
10	Alanine	0.683 ± 0.02	0.812 ± 0.11
11	*Arginine*	0.621 ± 0.11	0.452 ± 0.06
12	Asparagine	1.254 ± 0.15	1.545 ± 0.07
13	Aspartic acid	0.622 ± 0.01	0.621 ± 0.02
14	Cystine	0.311 ± 0.03	0.201 ± 0.01
15	Glutamine	1.425 ± 0.04	1.131 ± 0.01
16	Glycine	0.401 ± 0.11	0.112 ± 0.03
17	Glutamic acid	1.203 ± 0.36	2.223 ± 0.05
18	Proline	0.524 ± 0.41	0.724 ± 0.16
19	Serine	0.512 ± 0.01	0.312 ± 0.22
20	Tyrosine	0.414 ± 0.02	0.342 ± 0.44

Table. 2. Amino Acid Profile of Two *Ulva* species (Result is expressed in % of Dry Wt.)			
S.No.	**Amino Acids**	***Ulva intestinalis***	***Ulva fasciata***
Essential Amino Acids [EAA]			
1	**Histidine**	6.79	7.07
2	**Iso-leucine**	8.78	6.41
3	**Leucine**	9.23	7.88
4	**Lysine**	13.71	11.58
5	**Methionine**	2.28	4.69
6	**Phenylalanine**	6.39	6.55
7	**Threonine**	1.43	3.54
8	**Tryptophan**	6.55	7.41
9	**Valine**	1.74	0.60
Non-Essential Amino Acids [NEAA]			
10	**Alanine**	3.69	4.24
11	**Arginine**	3.35	2.36
12	**Asparagine**	6.78	8.07
13	**Aspartic acid**	3.36	3.25
14	**Cystine**	1.68	1.05
15	**Glutamine**	7.71	5.91
16	**Glycine**	2.17	0.59
17	**Glutamic acid**	6.51	11.62
18	**Proline**	2.83	0.724
19	**Serine**	2.77	0.312
20	**Tyrosine**	2.24	0.342

Total numbers of eleven non-essential amino acids (NEAA) were detected in the selected two marine algae such as green algae (Tables – 1, 2). All the eleven non-essential amino acids (NEAA) were detected in the two marine green algal seaweeds. (Tables – 1, 2).

Among the two seaweeds, a maximum of total and non-essential amino acids was recorded in *U. fasciata* followed by *U. fintestinalis* respectively. Of the 11non-essential amino acids glutamic acid and glutamine were recorded the highest of 11.62%, and 7.71% in *U. fasciata* and *U. intestinalis* respectively. (Tables – 1, 2).





The amino acid composition in free or bound form observed a significant difference between protein and amino acid contents of red, brown and green algae.[24, 25] In the present study, 9 essential amino acids and 11 non-essential amino acids were recorded from the crude amino

acids of two seaweeds. Between the two seaweeds, a maximum of total and essential amino acids was recorded in *U. fasciata* followed by *U. intestinalis*. The total non-essential amino acid is significantly high in *U. fasciata* followed by *U. intestinalis.* The distribution pattern of these amino acids reveals some significant differences between the two species investigated.

CONCLUSION

In the present investigation is the essential and non-essential amino acids characteristics potential of seaweeds occurring along the coast of regions of Tamil Nadu, India. With the above extraction of seaweeds, essential and non-essential amino acids were analyzed. Results of amino acids profiles were obtained through HPLC analysis. Twenty amino acids were identified and estimated. The analysis revealed the presence of nine essential amino acids and eleven non-essential amino acids. Quality and quantity of amino acids vary according to algal species. Essential amino acids(EAA) recorded were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine and the non-essential amino acids (NEAA) alanine arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, glutamic acid, proline, serine, and tyrosin were recorded in the selected two seaweeds. The distribution pattern of these amino acids reveals some significant differences in the two species investigated. This present research work highlight that this is a rapidly advancing area of algal science with a particular focus on the key research required to assess better the health benefits of algae or algal products. There are rich opportunities for phycologists in this emerging algal field.

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