

Seaweeds (*Ulva reticulata* and *Sargassum cinctum*) as Alternative Source of Amino Acid for Mud Crab Feed

Sumara M. Ayaz K.* and K. H. Vadher

Department of Aquaculture

College of Fisheries Science, Junagadh Agricultural University, Veraval 362265

*Corresponding Author E-mail: sumaraayaz@gmail.com

Received: 2.05.2020 | Revised: 15.06.2020 | Accepted: 23.06.2020

ABSTRACT

Seaweeds form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. Seaweeds and other dietary feed components are rich in proteins, vitamins, carbohydrates, fibre, lipids, and minerals and are easy to obtain. Some species of algae may contain more protein, carbohydrates, and fat than the ingredients traditionally used in trash fish diets in crab. Nutritional study of seaweeds compounds has been scarce, and is estimated mainly from their biochemical and chemical composition. For that purpose to evaluate the nutrition quality of seaweed *Ulva reticulata* and *Sargassum cinctum* based on amino acid profiling was carried out.

Results revealed that the numbers of amino acids were observed in *U. reticulata* (15) and *S. cinctum* (13) with more than 55% essential amino acids (EAA) to total amino acid (TAA). The quantities of the individual essential amino acids were ranged from 0.07 to 1.82 (*U. reticulata*) and 0.29 to 1.97 (*S. cinctum*) mg /100 mg (DW). Therefore results indicated that both seaweeds can be considered as alternative source of amino acid for feed supplement and animal nutrition.

Keyword: Amino acid, Mud crab, *Sargassum cinctum*, *Ulva reticulata*

INTRODUCTION

Interest in fish and shellfish nutrition has been increasing markedly over the past two decades, largely due to the global increase in aquaculture production and the awareness of the farming community on the requirement of balanced supplementary feeding for sustainability in the production. In aquaculture nutrition it becomes absolutely essential to

supply feed to meet the nutritional requirements for enhanced growth and productivity (De-Silva, 1993).

Mud crab (*Scylla serrata*) is an important economic fishery commodity. The demand of the global mud crab market is increasing every year. The demand for mud crab increases because of its high nutritional content so that it is demanded by many people.

Cite this article: Sumara, M.A.K., & Vadher, K. H. (2020). Seaweeds (*Ulva reticulata* and *Sargassum cinctum*) as Alternative Source of Amino Acid for Mud Crab Feed, *Ind. J. Pure App. Biosci.* 8(3), 561-566. doi: <http://dx.doi.org/10.18782/2582-2845.8187>

The high market demand encourages farmers to increase the production of mud crab (*Scylla serrata*). Mud crabs (*Scylla serrata*) have the potential to be cultivated because of their advantages, including rapid growth, larger size than other species, disease resistance, and high selling prices.

In several research reported the digestibility of some vegetable raw materials is high (above 85%) in feed to crabs and they have a digest potential to be ingredients in mangrove crab (Catacutan et al., 2003; Pavasovic et al., 2004; Nguyen et al., 2014; Azra & Ikhwanuddin, 2016).

Seaweeds form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. Reports on the uses of seaweeds have been cited as early as 2500 years ago in Chinese literature (Tseng, 2004). The long history of seaweed utilization for a variety of purposes has led to the gradual realization that some of their constituents are more superior and valuable in comparison to their counterparts on land. There are in the nature different types of antimicrobial compounds present that play an important role in the natural defense of all kinds of living organisms (Ilhami et al., 2003).

Seaweed refers to any large marine benthic algae that are multicellular, macrothalic, and differentiated from most algae that are of microscopic size. These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. Reports on the uses of seaweeds have been cited as early as 2500 years ago in Chinese literature. The long history of seaweed utilization for a variety of purposes has led to the gradual realization that some of their constituents are more superior and valuable in comparison to their counterparts on land

In order to fully exploit the nutritional value of seaweeds, several studies on the biochemical and nutritional composition of various seaweeds collected from different parts of the world have been conducted by Rupérez, McDermid and Stuercke, Ortiz,

Marsham, Chakraborty and Santra, Matanjun etc. Unfortunately, this type of study for seaweeds compounds has been scarce, and is estimated mainly from their biochemical and chemical composition.

The biochemical composition of marine seaweeds is generally known to be highly influenced by geographical location and local environmental conditions. Seaweed genus *Ulva* and *Sargassum* are almost extensively available during the all month of seaweed growing season at Okha coast. Therefore, in present investigation the analysis amino acid profiling of *Ulva reticulata* and *Sargassum cinctum* will be beneficial for evaluating their nutritional value as supplements ingredient in mud crab feed and animal nutrition.

MATERIALS AND METHODS

2.1 Site of experiment

Fresh Seaweeds *Ulva reticulata* and *Sargassum cinctum* were collected from the rocky shore regions of Okha coast (22° 28'0" N, 69° 4'0" E), Saurashtra region of Gujarat. Amino acid profiling was carried out at the CSIR- Central Salt & Marine chemicals Research Institute, Bhavnagar.

2.2 Sample Collection and Preparation

Fresh marine seaweeds *Ulva reticulata* and *Sargassum cinctum* were collected from the rocky shore regions of Okha coast (22° 28'0" N, 69° 4'0" E), Saurashtra region of Gujarat. The collected seaweeds were washed thoroughly with tap water in order to remove epiphytes and other marine organisms and then fixed on herbarium sheet for preparation of voucher specimen and then identified using the standard literature. The seaweeds species was then dried under shade at room temperature and dried seaweeds samples were ground well by using mixer grinder and sieved using a nylon sieve in order to remove seaweed fiber.

2.3 Amino acid profiling

2.3.1 Reagents

- a) **100mM or 0.1N HCL (As Diluents):**
8.212ml of HCL was taken with
991.788ml distilled water

- b) **Buffer:** 1.115gm of Tetra-methyl ammonium chloride and 2.035gm of Sodium acetate trihydrate were dissolved in 1 L distilled water. The 3.5 pH was adjusted with glacial acetic acid and filtered through 0.45µ nylon membrane.
- c) **Organic phase:** 1960 ml of acetonitrile was mixed with 40 ml methanol
- d) **Mobile Phase A:** 900 ml of Buffer was mixed with 100 ml of organic phase
- e) **Mobile Phase B:** 100 ml of Buffer was mixed with 900 ml of organic phase
- f) **50% NaOH:** 5mg of NaOH pellets dissolved in 100 ml distilled water
- g) **Borate Buffer:** 6.18gm of boric acid powder was dissolved in 100ml distilled water and the 6.2 pH was adjusted with 50% NaOH.
- h) **FMOC Reagent:** 100mg of FMOC reagents was diluted in 25 ml of dried acetone.
- i) **n-Hexane**

2.3.2 Sample preparation

(a) Hydrolysis

Sample biomass (3-10 mg of dry weight or 10-20 mg of wet weight) was taken in a heat stable test tube; added 100µl of 0.1N HCL, 800µl of 6N HCL, 100 µl of Nor-Leucine st (1000 ppm) and 10µl of phenol and sealed the tube. The hydrolysis was carried out at 110°C for 16 hrs with dry bath. After hydrolysis, the test tube was opened and the contents were transferred to 10 ml standard flask. Added 0.5 ml of 50% NaOH in standard flask and made volume up to 10 ml with diluents (0.1N HCL).

(b) Derivatization

After hydrolysis, the 100µl of hydrolysates or standards or diluent for blank sample were taken into 10 ml falcon tube; added 900 µl of borate buffer, 1ml of FMOC and mixed thoroughly; added 4ml of n-Hexane and vortexed for 45 second. Two layers were formed, the upper layer was discarded and the lower layer was collected into the UHPLC injection tube or vial and seal. Then vials were

loaded onto the auto sampler tray ready for analysis.

2.3.3 UHPLC Analysis

UHPLC vial with collected sample was loaded into the tray of auto sampler. Then 25 µl of sample was injected to an amino acid analyzer equipped with column (C 18' 4.6 X 25 mm, 5 µm packing) and diode array detector (265nm Wavelength). The Column was run with mobile phase A and B at flow rate of 1.5 ml/min. The column gradient was maintained as 10 - 50% B for 45 min., 50% B for 5 min., 90% B for 10 min., 100% B for 2 min., 100% B for 5 min., 10 % B for 2 min., 10% B for 6 min. Standard amino acid mixture (25µl) was also run separately and then the chromatograms of standard and sample were compared and quantified.

RESULTS AND DISCUSSIONS

3.1 Amino Acid Profiling

The nutritive evaluation of seaweeds *Ulva reticulata* and *Sargassum cinctum* on the basis of amino acid composition was carried out. In all 17 amino acids involving 10 essential amino acids and 7 non-essential amino acids have been detected using high performance liquid chromatography. Data and Chromatograms of essential and non essential amino acids of investigated seaweeds are shown respectively in Table-1 and Fig. 1. In present studied *Ulva reticulata* all essential except threonine and all non- essential amino acid were present. While in *Sargassum cinctum* except threonine all essential and except aspartic acid and tyrosine all non-essential amino acids have been detected. In studied both seaweeds were contained 15 (*U. reticulata*) and 13 (*S. cinctum*) numbers of amino acids which were exhibited in almost similar patterns. The quantity of total amino acid was accounted 10.21 (*U. reticulata*) and 10.05 (*S. cinctum*) mg/100 mg (DW). The both seaweed *U. reticulata* and *S. cinctum* were contained relatively higher percentage of methionine acids (17.82%, 19.60 %) and serine acid (10.96 %, 10.74 %) respectively which are responsible for the special role in the synthesis, structure and function of

proteins. Similar results were also obtained in previous studies by Y. R. Alissianto *et al.*, 2018; Kumar and Ratana-arporn. This present study has demonstrated that amino acid methionine can potentially be used in addition on feed toward the growth and retention on mud crab (*Scylla serrata*). The quantities of the individual essential amino acids were ranged from 0.07 to 1.82 (*U. reticulata*) and 0.29 to 1.97 (*S. cinctum*) mg /100 mg (DW). As essential amino acid methionine in *U. reticulata* and *S. cinctum* were accounted higher while all other were present in significant amount in both seaweeds. The distribution of total amino acids of *Ulva reticulata* and *Sargassum cinctum*, 5.63 mg and 5.66 mg amino acid/100 mg (DW) sample corresponding to 55.14 and 56.31 % respectively were made up of essential amino acids among both the seaweed. Presence of 55-59% of essential amino acid in *K. alvarezii* and *H. musciformis* and more than 50% in *C.*

lentillifera and *U. reticulata* (Ratana-arporn and Chirapart, 2006; Kumar and Kaladharan, 2007) occurred in various regions of the sea, corroborate with the present study. species was corroborate the previous study by Kumar and Ratana-arporn as well as reflecting as alternative nutrient sources of amino acids for human and animal consumption. Studies showed the percent level of amino acid found in seaweeds (*U. reticulata* and *S. cinctum*) that might be used as ingredients in a formulated crab diet. The availability and quality of these ingredients are good and they are suitable for the aqua-feed industry. These are also widely used ingredients in aquaculture diets as they have good nutrient profiles, are readily digested by crustacean and are cost effective compared other ingredients. Therefore results indicated that seaweeds (*U. reticulata* and *S. cinctum*) can be considered as alternative source for feed supplement and animal nutrition.

Table 1: Amino acids composition of seaweeds

Amino acids mg/100mg DW	<i>Ulva reticulata</i>	<i>Sargassum cinctum</i>
Histidine	0.93	0.57
Arginine	0.50	0.61
Threonine	0.00	0.00
Valine	0.57	0.69
Methionine	1.82	1.97
Cystine	0.07	0.00
Iso-Leucine	0.49	0.60
Leucine	0.23	0.31
Phenylalanine	0.50	0.62
Lysine	0.52	0.29
Total EAA	5.63	5.66
Aspartic Acid	0.00	0.00
Glutamic Acid	1.08	0.99
Serine	1.12	1.08
Glycine	1.03	0.88
Alanine	0.50	0.66
Proline	0.58	0.78
Tyrosine	0.27	0.00
Total None-EAA	4.58	4.39
Total AA	10.21	10.05

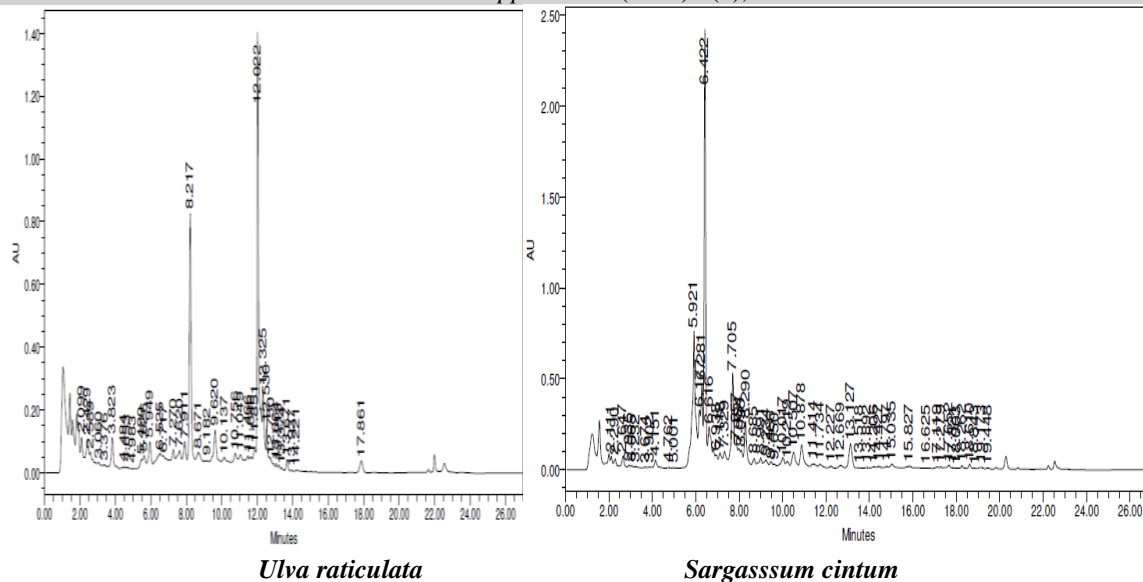


Fig. 1: Chromatograms of amino acid profiling of major diet ingredients

CONCLUSIONS

Therefore based on the results obtained from the present study it is concluded that both seaweeds have beneficial nutritional composition is elevated their value as human and animal nutrition as well as may add to their efficacy as a dietary supplement or as part of a balanced diet. The results of the present study demonstrated that mud crabs will accept formulated feeds using targeting ingredients in laboratory process or commercial extrusion. Hence, the development of selected ingredients diets for crabs is technically possible.

Acknowledgements

The first author gratefully acknowledges the ever willing and sincere help offered by the authorities of College of Fisheries Science, Junagadh Agricultural University, Veraval (Gujarat) for granting the permission to pursue postgraduate studies for providing all the necessary facilities right from the beginning of the work.

REFERENCES

- Allisianto, Y. R., Sandriani, Z. A., Rahardja, B. S., Agustono, & Rozi., (2017). The effect of amino acid lysine and methionine addition on feed toward the growth and retention on mud crab (*Scylla serrata*). *Earth and Environmental Science* 137, 1755-1315.
- Azra, M. N., & Ikhwanuddin, M., (2016). A review of maturation diets for mud crab genus *Scylla* broodstock: Present research, problems and future perspective. *Saudi Journal of Biological Sciences.*, 23(2), 257-267.
- Catacutan, M. R., Eusebio, P. S., & Teshima, S., (2003). Apparent digestibility of selected feed stuffs by mud crab, *Scylla serrata*. *Aquaculture.*, 216, 253–261.
- Chakraborty, S., & Santra, S. C. (2008). Biochemical composition of eight benthic algae collected from Sunderban, *Indian J. Mar. Sci.*, 37, 329–332.
- De Silva, S. S. (1993). Supplementary feeding in semi-intensive aquaculture systems. In: M.B. New, A. G. J. Tacon and I. Csavas (Editors), *Farm-Made Aquafeeds*. Proceedings of the Fao/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992, *FAO-RAPA/AADCP*, Bangkok, Thailand, pp. 24-60.
- Ilhami, G., Metin, T. U., & Munir, O. (2003). Evaluation of antioxidant and antimicrobial activities of Clary Sage. *Turk. J. Agric.*, 28, 25-33.

- Kumar, V. V., & Kaladharan, P., (2007). Amino acids in the seaweeds as an alternate source of protein for animal feed, *J. Mar. Biol. Ass. India.*, 49(1), 35-40.
- Marsham, S., Scott, G. W., & Tobin, M. L. (2007). Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem.*, 100, 1331–1336.
- Matanjun, P., Mohamed, S., Mustapha, N. M., & Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*, *J. Appl. Phycol.*, 21, 75–80.
- McDermid, K. J., & Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds, *J. Appl. Phycol.*, 15, 513–524.
- Nguyen, N. T. B., Chim, L., Lemaire, P., & Wantiez, L. (2014). Feed intake, molt frequency, tissue growth, feed efficiency and energy budget during a molt cycle of mud crab juveniles, *Scylla serrata* (Forsk., 1775), fed on different practical diets with graded levels of soy protein concentrate as main source of protein. *Aquaculture*. 434, 499–509.
- Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A., & Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea Antarctica*, *Food Chem.*, 99, 98-104.
- Pavasovic, M. (2004). Digestive profile and capacity of the mud crab (*Scylla serrata*) (Doctoral dissertation, Queensland University of Technology).
- Ratana-arporn, P., & Chirapart, A. (2006). Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata* Kasetsart, *J. Nat. Sci.*, 40, 75–83.
- Rupérez, P. (2002). Mineral content of edible marine seaweeds, *Food Chem.*, 79, 23-26.
- Tseng, C. K. (2004). The past, present and future of phycology in China. *Hydrobiologia.*, 512, 11- 20.