Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5233

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 1210-1216 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Amino Acid and Fatty Acid Composition of Seaweeds (*Ulva reticulata* and *Sargassum cinctum*): A Novel Natural Source of Nutrition

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Received: 17.07.2017 | Revised: 22.08.2017 | Accepted: 27.08.2017

ABSTRACT

Seaweed important renewable resource in the marine environment and have been a known natural source of nutritional compounds with health benefit. 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 and fatty acid profiling were carried out. Amino acid were analyzed using UHPLC (Ultimate High Performance Liquid Chromatography) unit and Fatty acids were identified and quantified as FAME (Fatty Acid Methyl Ester) using GC-MS (Gas chromatography Mass Spectrophotometer) unit. Results revealed that the numbers of amino acids were observed in U. reticulata (15) and S. cinctum (14) with more than 50% essential amino acids (EAA) to total amino acid (TAA) whereas PUFAs content were accounted 67.04%, 60.78% respectively with less than one dietary n-6/n-3 ratio. Therefore results indicated that both seaweeds can be considered as alternative source of amino acid and fatty acid for feed supplement and animal nutrition.

Key word: *Ulva reticulata, Sargassum cinctum, Amino acid, Fatty acid.*

INTRODUCTION

Seaweed refers to any large marine benthic algae that are multicellular, macrothallic, 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[1]. 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.

They are harvested and utilized as the sources of food, feed, phycocolloids, fertilizer, energy, medicines, cosmetics and nutraceuticals besides being used in biotechnological, bioremediation and aquaculture applications[2, 3, 4].

Cite this article: Ishakani, A.H., Vadher, K.H., Kadri, R.M. and Patel, M.R., Amino Acid and Fatty Acid Composition of Seaweeds (*Ulva reticulata* and *Sargassum cinctum*): A Novel Natural Source of Nutrition, *Int. J. Pure App. Biosci.* **5(5):** 1210-1216 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5233

In India, seaweeds are generally being used as raw materials for the production of agar, alginates and seaweed liquid fertilizer, in spite of their great potential as therapeutic health booster, beauty enhancer and the source of nutrition. Hence it becomes essential to popularize and to establish seaweeds as health food.

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[5], McDermid and Stuercke[6], Ortiz[7], Marsham[8], Chakraborty and Santra[9], Matanjun[10] 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[11]. Seaweed genus *Ulva* and *Sargassum* are almost extensively available during the all month of seaweed growing season at Veraval coast. Therefore, in present investigation the analysis amino acid and fatty acid profiling of *Ulva reticulata* and *Sargassum cinctum* will be beneficial for evaluating their nutritional value as human and animal nutrition.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh marine seaweeds *Ulva reticulata* and *Sargassum cinctum* were collected from the rocky shore regions of Veraval coast (21º 35' N, 69º 36' 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.

Amino Acid Profiling

(a) Hydrolysis: Sample biomass (3-10 mg dry weight and 10-20 mg 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^{o}C for 16 hrs with dry bath. After hydrolysis, 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: The 100µl of hydrolysates 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.

(c) 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 died 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.

Fatty Acid Profiling

(a) Lipid extraction: One Gram of each sample powder was extracted with chloroform-methanol mixture (2:1 v/v) using a glass pestle and mortar with a pinch of glass powder. The homogenate were filtrated (funnel with a folded defatted filter paper containing sodium sulphate) to recover the liquid phase and the filter residue re-homogenized with a second volume of chloroform- methanol. The filtrate were washed with 0.2 volumes (4 ml for 20 ml) of 0.85% NaCI solution and phases are

vigorously mixed. The mixture was poured into a separating funnel and allowed to decant. The lower chloroform phase containing lipids were collected and evaporated under vacuum in a rotary evaporator to bring down to a concentration of 2-3 ml. Further evaporation of chloroform was done under oven and residue was weighed to quantify the amount of lipid extracted. The lipid residue was re-dissolved in chloroform/methanol (2:1, v/v) and then stored in a 25 mL conical flask with glass stopper at -20°C until needed.

(b) Preparation of Fatty Acid Methyl Esters (FAME): Fatty acid methyl esters (FAME) was prepared from the isolated lipids thus obtained by heating at 45°C with adding 2ml of 0.5N Methanolic NaOH for 5 min, followed by with adding 2 ml of BF3 to esterification for 2 min then with adding 5ml of n-Heptan to to recover the methyl esters in organic phase for 8 min using Heating Mental. The mixture was washed with saturated NaCl solution and two phases was formed. The upper layer, an organic phase containing the fatty acid methyl ester (FAMEs) was pipette out and stored in 10 ml all glass vials with until further analysis.

(c) GC-MS Analysis: Fatty acid composition was analysed using GC-MS after derivatization of fatty acid to methyl ester (FAMEs). Analysis was carried out with TRACE 1310 GC fitted with TSQ 8000 EVO MS system and Triplus auto analyzer (Thermo, US). The system parameter for analysis were, the oven temperature 1 min initial hold at 50^0C, temperature raised from 50-150^0C at the rate of 20^0 C per min followed by a hold of 15 min. at 150^0C, temperature raised from 150-240^0C at the rate of 20^0C per min and a final hold of 2 min at 240^0C. Helium was used as a carrier gas with column flow rate 1ml. The MS conditions were ionization voltage 70 ev, MS transfer line temperature 250^0C, ion source temperature 230^0C, foreline pressure 70 m torr, mass range of 40-500 and the scan time equal to GC run time.

RESULTS AND DISCUSSIONS

Amino Acid Profiling

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 both seaweeds were contained 15 (*U. reticulata*) and 14 (*S. cinctum*) numbers of amino acids which were exhibited in almost similar patterns. The quantity of total amino acid was accounted 10.30 (*U. reticulata*) and 9.79 (*S. cinctum*) mg/100 mg (DW). The both seaweed *U. reticulata* and *S. cinctum* were contained relatively higher percentage of glutamic acids (11.53, 11.02 %) and aspartic acid (10.45, 10.16 %) respectively which are responsible for the special flavour and taste of seaweeds. Similar results were also obtained in previous studies by Wong[12], Kumar [13] and Gressler [14]. The quantities of the individual essential amino acids were ranged from 0.48 to 0.93 (*U. reticulata*) and 0.29 to 0.97 (*S. cinctum*) mg /100 mg (DW). As essential amino acid arginine in *U. reticulata* and phenylalanine in *S. cinctum* were accounted higher while all other were present in significant amount in both seaweeds. The distribution of more than 50% essential amino acids among both the seaweed species was corroborate the previous study by Kumar[13] and Ratana-arporn[15] as well as reflecting as alternative nutrient sources of amino acids for human and animal consumption.

Fatty Acid Profiling

Fatty acid composition and the Chromatograms of fatty acid profile of both seaweeds were shown in Table-2 and Fig.-2 respectively. In present investigation the quantity of total fatty acid (TFAs) accounted 445.97 (*U. reticulata*) and 80.67 (*S. cinctum*) µg/g of FAME. The quantity of unsaturated fatty acid in *U. reticulata* (70.34%) and *S. cinctum* (68.74%) was higher than saturated fatty acids which are in agreement with previous reports[16, 17]. Dominance of palmitic acid of both seaweeds is in agreement compare to previous reports for same genus *spp.* by Rohani-Ghadikolaei[11,] Debbarma[18] reported that Oleic acid was the most dominant monounsaturated fatty acid (MUFA) in the seaweeds whereas in present study Eladic Acid (2.34 and 4.66 %) was dominated as MUFAs. The contribution of polyunsaturated fatty acids PUFA to total amino acid was relatively

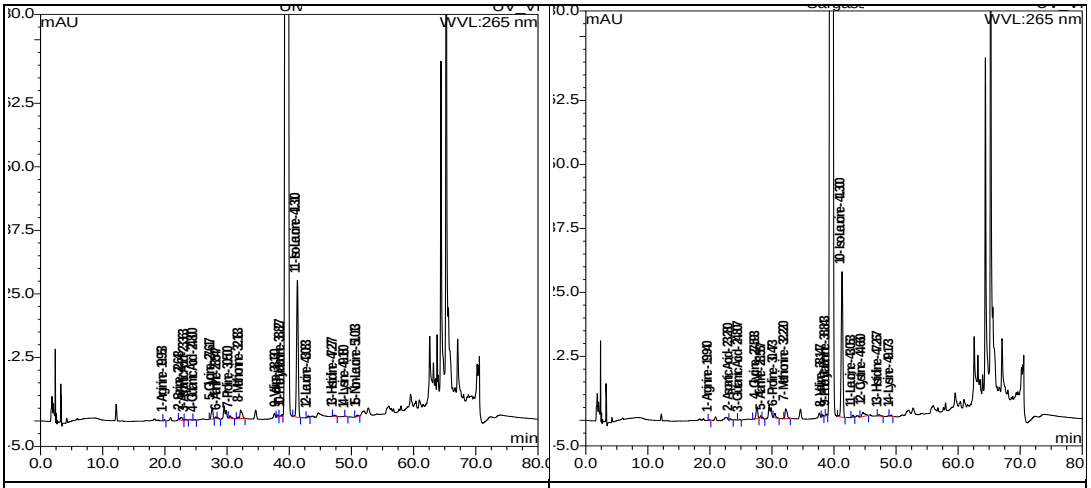
higher in *U. reticulata* (67.04%) and *S. cinctum* (60.78%) which is in agreement with previous that marine algae are rich source of PUFAs[19, 20]. In present investigation the accounted concentration of the health-beneficial PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of both seaweeds was relatively higher; AA was higher in *U. reticulata* and lower in *S. cinctum*; essential PUFAs such linolenic acid was lower in both seaweed whereas linoleic acid was lower in *U. reticulata* and higher in *S. cinctum* than those previously reported by Matanjun[10]; Chakraborty[8]; Rohani-Ghadikolaei[11] and Debbarma[24]; Ortiz[7] for the same genus species. Consumption of long-chain omega-3 PUFA are known to be beneficial to human health[21] and together with a low dietary n-6/n 3 ratio, are fundamental for a diet with cardio-protective benefits. In the present study both seaweed contained the highest total n-3 PUFA content with the less than 1 n-6/n-3 ratio which may be considered as potential source of fatty acid for dietary nutrition.

Table 1: Amino acids composition of seaweeds

Amino acids mg/100mg DW	***Ulva reticulata***	***Sargassum cinctum***
Arginine	0.93	0.57
Threonine	0.00	0.00
Methionine	0.48	0.56
Valine	0.57	0.69
Phenylalanine	0.82	0.97
Iso-Leucine	0.51	0.57
Leucine	0.89	0.60
Cystine	0.00	0.51
Histidine	0.50	0.62
Lysine	0.52	0.29
Total EAA	**5.22**	**5.39**
Serine	0.50	0.00
Aspartic Acid	1.08	0.99
Glutamic Acid	1.12	1.08
Glycine	1.03	0.88
Alanine	0.50	0.66
Proline	0.58	0.78
Nor-Leucine	0.27	0.00
Total None-EAA	**5.08**	**4.40**
Total AA	**10.30**	**9.79**

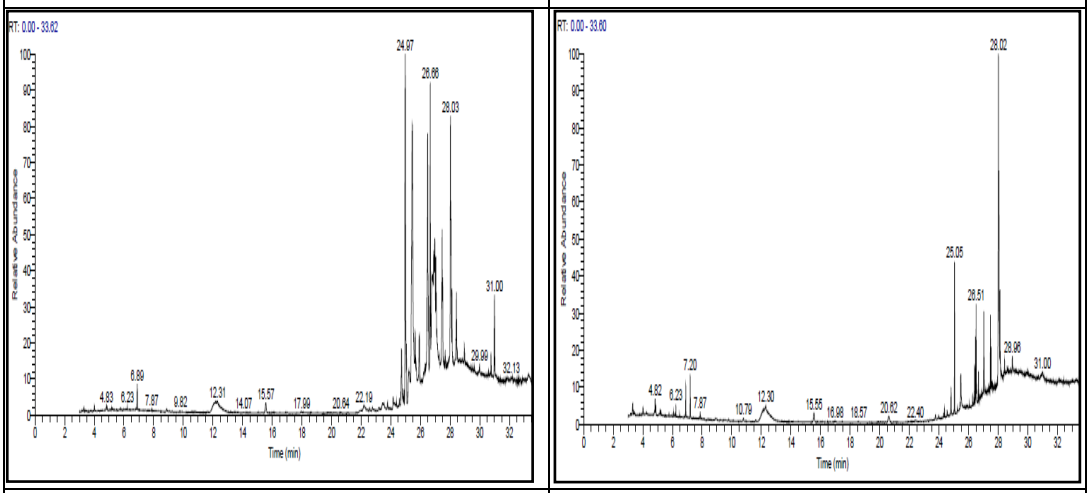
Table 2: Fatty acids composition of seaweeds

Fatty acid (µg/g FAME)	***U. reticulata***	***S. cinctum***
Hexanoic acid	0.27	1.09
Octanoic_acid	0.24	0.43
Deconoic_acid	0.23	0.34
Undeconoic_acid	0.06	0.07
Dodeconoic	0.58	0.72
Trideconoic_acid	0.08	0.14
Pentadecanoic acid	0.25	0.63
Palmitic_acid	110.04	21.35
Heptadeconoic_acid	20.22	0.1
Docosanoic_acid	0.3	0.35
∑ **Saturated FAs**	**132.27**	**25.22**
Oleic acid	1.97	0.28
Eladic Acid	10.42	3.76
Eicosenoic acid	1	2.22
Erucic acid	0.7	0.01
Nervonic acid	0.62	0.15
∑ **MUFAs**	14.71	6.42
Linolenic_acid	0.29	0.41
Eicosapentaenoic acid (EPA)	126.1	28.32
Eicosatrienoic acid (ETE)	5.14	0.37
Docosahexaenoic acid (DHA)	37.56	7.36
Linoleic acid	0.28	9.66
Arachidonic acid (AA)	126.22	0.33
Ecosadienoic_acid	3.4	2.58
∑ **PUFAs**	298.99	49.03
∑ **TFAs**	**445.97**	**80.67**



Ulva reticulata | ***Sargassum cinctum***

Fig. 1: Chromatograms of amino acid profiling of seaweeds



Ulva reticulata | ***Sargassum cinctum***

Fig. 2: Chromatograms of fatty acid profiling of seaweeds

CONCLUSION

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.

Acknowledgement

The first author gratefully acknowledges the ever willing and sincere help offered by the authorities of College of Fisheries Science, Junagadh Agricultural University,Veraval for granting the permission and providing all the necessary facilities right from the beginning of the work to pursue Ph. D. studies. Also thankful to Central Institute of Fisheries Technology, ICAR- Regional center, Veraval for providing working facilities during the tenure of research work.

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