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Nutritional Composition of the Colonial Ascidian *Eudistoma viride* and *Didemnum psammathodes*

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In the present investigation the colonial ascidians *E. viride* and *D. psammathodes* were collected from the rocks of near Hare Island, Tuticorin coast. The collected ascidian samples were tested for biochemical assays (carbohydrate, protein, lipid, amino acids and fatty acids). All these assays were followed by standard methods. In bio chemical analysis protein content in *E. viride* and *D. psammathodes* tissues showed 3.78 and 3.62 μgmL^{-1}, total carbohydrate content 2.15 and 2.2 μgmL^{-1}, crude fiber content 9.2 and 7.9 μgmL^{-1}and total free amino acid content 3.2 and 3.9 μgmL^{-1}. From the amino acid analysis leucine, lysine, isoleucine, threonine, methionine, histidine and arginine shows high amount in *E. viride* and *D. psammathodes* tissues. The total lipid content in ascidians *E. viride* and *D. psammathodes* tissues showed 0.23 and 0.32 μgmL^{-1} respectively. In fatty acids estimation, *E. viride* and *D. psammathodes* shows 31.71 % and 32.96 % total saturated fatty acids (Σ of SFAs), 24.55 % and 25.52 % total mono unsaturated fatty acids (Σ of MUFAs) and 29.07 % and 33.59 % total poly unsaturated fatty acids (Σ of PUFAs). The results from this study may help in developing appropriate diets for human nutrition.

Keywords: ascidian, nutrient composition, food, feed.

Marine invertebrates are being widely used as food and feed supplement around the world. The spaial and temporal patchiness of food in nature, periods of food deprivation are common in the lives of many animals (Mehner and Wieser, 1994). Ascidians are conspicuous and important members of shallow benthic communities (Monteiro *et al.*, 2002). Ascidians are a well-known prochordates naturally prey for many animals including flat worms, molluscs, rock crabs, starfishes, sea birds and sea otters. It constitutes one of the important marine living renewable resources with high protein, glycogen and minerals as compared with other animal foods (Ali, 2004; Tamilselvi Tamilselvi, 2008). It is considered as highly nutritious seafood. Therefore, information on their growth, production and biochemical and energetic composition (Peterson *et al.*, 1995) is important in modeling the flow of material and energy within marine benthos.

Ascidians are also as food in the form of various preparations in many parts of the world including Chile (Probecho), France (Figueodemer, violet), Korea (Meongge), Italy (limone di mare, uova di mare), Japan (hoya, maboya) etc. Recently interest has been developed in the class Ascidiacea, paricularly the family Pyuridae. The tunic of the pyurid ascidian *Microcosmus hartmeyeri* and mantle bodies of *Halocynthia roretzi* and *H.*

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auranlum are farmed and eaten in Japan (Nanri *et al.*, 1992). In Europe, *Microcosmus sabatieri* and *M. vulgaris* are consumed whereas; *Pyura chilensis*is an important food item in Chile (Davis, 1995). Though a lot of literature is available on the food products prepared from a number of marine organisms like prawn, molluscs, fishes etc. Ascidians are also farmed and fished in many parts of the world for food. Consumption just after harvest is favored for the best flavour as a metallic taste may become evident as the product becomes less fresh. Food quality has fundamental implications for the ecology of all species. Though ascidians are one of the commercially important sources of sea food in various parts of the world, their utilization is not popular like other sea food due to lack of perception combined with the conservative food habits of the people in India. Since the biochemical parameters are important for reflecting the food value. In the present study this investigation deal with a few biochemical assays like estimation of carbohydrate, protein, lipid, amino acids and fatty acids have been planned to evaluate the nutritional significance of ascidian *E. viride* and *D. psammathodes.*

MATERIALS AND METHODS

Specimen Collection and Identification

Ascidians were collected as common and persistent biofoulants from the rocks of Hare Island Tuticorin Coast (Lat. 8° 46' 20. 72" N and Long. 78° 11' 57. 91" E), India (Fig.1.) by SCUBA diving at the depth ranging from 1 to 3 m between September, 2010. Fig. 2 shows the colonies of *E. viride* and *D. psammathodes*. The samples were thoroughly washed with treated sea water and removed sand, mutt and overgrowing organisms at the site collection and transported to laboratory and specimens were identified by the standard literature of Monniot and Monniot, (2001); Cole and Lambert, (2009); Kott, P. (2001). A Voucher specimen No: AS 2234 and AS 2233 has been deposited in the Museum (National Collections of Ascidians) of the Department of Zoology, A.P.C. Mahalaxmi College for women, Tuticorin - 628002.

Sample preparation

Ascidians tissues (10 g) were shade dried for few days. The dried tissues were made into powder for biochemical analysis.

Protein estimation

Protein concentration was estimated by the method of Bradford *et al.* (1976). Bovine serum albumin (2 $mgmL^{-1}$) was used as reference standard concentrations 20, 40, 60, 80 and 100 µg/100 µL. The assay relies on the binding of the dye Coomassie Blue G250 to the protein molecule measured calorimetrically at 595 nm.

Carbohydrate estimation

The total carbohydrate was estimated by following the Phenol-sulphuric acid method of Dubois *et al.*, (1956). Glucose (1 μgmL^{-1}) was used as a as reference standard concentrations 20, 40, 60, 80 and 100 $\mu g\mu L^{-1}$. The absorbance was measured at 490 nm in a spectrophotometer (HITACHI-220S UV).

Crude fiber estimation

The crude fiber was recorded with the method described by American Association of Cereal Chemist, (1962). 1 g of dry sample and 50 mL of 1.25% H_2SO_4 was boiled for 30 min in 100 mL flask, then cooled and filtered. The residues were again boiled for 30 min in 1.25% of 50 mL NaOH and filtered.

Total free amino-acids estimation

Total free amino–acids were determined by the method described by Hamilton and Van Slyke, (1943). 10% pyridine (1 mL) was used as a reference standard. The absorbance was measured at 570 nm in a spectrophotometer (HITACHI-220S UV).

Amino acid estimation

The samples for free amino acids were prepared by the method of Huesgen *et al.* (1998). The amino acids were determined by an amino acid analyzer (Shimatzn-high performance Liquid chromatography, company-HITACHI, Detector-SPD 10 AVP, LP pump LC-10AT VP).

Lipid estimation

The amount of lipid was determined by the method described by Folch *et al.* (1957). 1gm dry samples were dried to constant weight in a drying oven (60°C, 24 hrs). Dried samples were homogenized with chloroform: methanol mixture (2:1 V/V), mixed in a vortex mix in 2800 rpm and filtered. The extract was shaken and equilibrated with 0.25 of its volume of a saline solution. The extracted lipids were concentrated by a rotary evaporator. Extracted lipids were weighed in vials using a micro electronic balance (±0.001 mg) in

order to calculate the total lipid content.

Fatty acid estimation

In fatty acid analyses following preprocess were followed saponification, methylation, extraction, base wash and FAMEs were separated by GC finally fatty acids were analyzed by gas phase chromatography according to method of Lepage, (1986). The fatty acid samples were analysed by gas phase chromatography (Network Gas Chromatograph model 6890N, Agilent Technologies, USA). Samples were injected by Split injector, split ratio 100:1, used column was Ultra -2 capillary column. Used software Sherlock version 4.5 with EUKARY data base (MIS Ver. No. 3.8, Microbial ID. Inc., Newark, Delaware).

RESULTS

In the present study the result clearly indicates the high amount of protein, carbohydrate, crude fiber present in their tissues. The ascidian *E. viride* and *D. psammathodes* tissues showed the presence of protein content in 3.78 and 3.62 μgmL^{-1}, total carbohydrate content 2.15 and 2.2 μgmL^{-1}, crude fiber content 9.2 and 7.9 $\mu gmL^{-1}$and total free amino acid content 3.2 and 3.9 μgmL^{-1}. The table 1. represents the presence of 15 essential and non-essential amino acids in the ascidian tissues. From the amino acid analysis leucine, lysine, isoleucine, threonine, methionine, histidine and arginine shows high amount in *E. viride* and *D. psammathodes* tissues. serine and glycine showed very trace amount in these ascidians. This estimation proves that amino acids are rich in ascidians *E. viride* and *D. psammathodes*. The total lipid content in *E. viride* and *D. psammathodes* tissues showed 0.23 and 0.32 μgmL^{-1} respectively. In fatty acids estimation, *E. viride* and *D. psammathodes* shows 31.71 % and 32.96 % total saturated fatty acids (Σ of SFAs), 24.55 % and 25.52 % total mono unsaturated fatty acids (Σ of MUFAs) and 29.07 % and 33.59 % total poly unsaturated fatty acids (Σ of PUFAs). In saturated fatty acids (SFAs), Palmitic acid (C16:0) shows high amount 10.72 and 11.63 mg/g, In mono unsaturated fatty acids (MUFAs), Cis-6-Palmitoleic acid (C16:1ω-6) show high amount of 6.13b% and 7.12 %, In poly unsaturated fatty acids (Σ of PUFAs), Eicosapentaenoic (C20:5ω -3) show high amount of 11.10 % and 10.34 % in both ascidian *E. viride* and *D. psammathodes* (Table. 2.).

DISCUSSION

As far as we know, human consumption of ascidians in Japan and Chile. Some ascidians (e.g. *Halocynthia roretzi*) is widely enjoyed as food in Japan, particularly in the Hokkaido and Johoku districts because of the high amount of protein, carbohydrate and other essential micronutrients (Nanri *et al.,* 1992). The people in the East and

Table 1. Amino acid contents in *E. viride* and *D. psammathodes*

S. No	Amino acids	*E. viride* (mg/g)	*D. psammathodes* (mg/g)
1.	Aspartate	56.8	71.2
2.	Glutamate	21.8	24.6
3.	Serine	T	T
4.	Histidine	168.4	177.4
5.	Glycine	T	21.3
6.	Threonine	295.6	312.5
7.	Alanine	89.5	72.1
8.	Arginine	365.4	401.2
9.	Tyrosine	124.3	156.7
10.	Valine	140.5	124.6
11.	Methionine	195.7	175.6
12.	Phenylalanine	105.2	97.8
13.	Isoleucine	231.2	254.1
14.	Leucine	582.3	540.9
15.	Lysine	344.5	385.4

*T (Trace) = < 0.1

Table 2. Fatty acids contents in ascidian *E. viride* and *D. psammathodes*

Carbonchain	Fatty acids	*E. viride*(%)	*D. psammathodes* (%)
C10:0	Capric acid	-	-
C11:0	Undecyclic acid	-	-
C12:0	Lauric acid	0.16	0.64
C13:0	Tri decyclic acid	0.18	0.71
C14:0	Myristic acid	1.61	1.91
C15:0	Pentadecyclic acid	1.43	1.92
C16:0	Palmitic acid	10.72	11.63
C17:0	Margaric acid	1.94	1.52
C18:0	Stearic acid	6.22	4.31
C19:0	Nonadecyclic acid	2.51	0.82
C20:0	Arachidic acid	1.55	0.74
C21:0	Heneicosanoic acid	1.01	2.16
C22:0	Pehinic acid	1.22	2.92
C23:0	Tricosanoic acid	1.55	1.94
C24:0	Lignoceric acid	1.61	1.74
Σ of SFAs		31.71	32.96
C14:1ω-3	Cis-3 Myristoleic acid	0.87	0.16
C14:1ω-5	Trans-5 Myristoleic acid	-	0.74
C14:1ω-7	Cis-7 Myristoleic acid	0.96	0.94
C15:1ω-6	Cis-6-Pentadecenoic	1.96	1.33
C16:1ω-5	Cis-5-Palmitoleic acid	1.01	1.06
C16:1ω-6	Cis-6-Palmitoleic acid	6.13	7.12
C16:1ω-7	Trans-7-Palmitoleic acid	0.18	0.71
C16:1ω-9	Trans-9-Palmitoleic acid	1.01	1.33
C17:1ω-7	Cis-7- Heptadecenoic acid	0.55	0.34
C17:1ω-8	Trans-8- Heptadecenoic acid	0.61	0.55
C18:1ω-5	Cis-5-Octadecenoic acid	0.18	0.11
C18:1ω-7	Cis-7-Octadecenoic acid	0.44	0.56
C18:1ω-9	Oleic acid	7.12	6.13
C19:1ω-8	Nonadecenoic acid	0.92	0.74
C20:1ω-5	Cis-5-Eicosenoic acid	-	0.13
C20:1ω-6	Cis-6-Eicosenoic acid	0.16	-
C20:1ω-7	Cis-7-Eicosenoic acid	-	0.12
C20:1ω-9	Trans-9-Eicosenoic acid	0.19	0.31
C22:1ω-7	Trans-7-Docosenoic acid	0.16	0.22
C22:1ω-9	Cis-9-Docosenoic acid	0.72	0.44
C24:1ω-3	Cis-3-Tetracosenoic acid	-	0.72
C24:1ω-6	Cis-6-Tetracosenoic acid	0.64	0.84
C24:1ω-9	Trans-9-Tetracosenoic acid	0.74	0.92
Σ of MUFAs		24.55	25.52
C16:2ω-6	Hexadecenoic	0.13	0.84
C18:2ω-3	Trans-3-linoleic	0.19	0.63
C18:2ω-6	Linoleic	5.11	5.96
C18:3ω-3	Alfalinolenic	0.92	0.82
C18:3ω-6	Gammalinoleinic	1.01	1.33
C18:4ω-3	Stearidonic	0.93	0.84
C19:2ω-6	Octadecenoic	0.11	0.64
C20:2ω-6	Eicosadienoic	0.14	0.84
C20:3ω-6	Dihomogammalinoleic	-	0.33
C20:4ω-6	Arachidonic acid	-	0.14
C20:5ω-3	Eicosapentaenoic	11.10	10.34

C20:5ω-6	Cis-6 Eicosapentaenoic	0.11	0.36
C22:3ω-3	Docosatrienoic	0.74	0.82
C22:4ω-6	Docosatetraenoic	0.63	0.84
C22:5ω-3	Decosapentaenoic	0.83	0.72
C22:6ω-3	Docosahexaenoic	7.12	8.14
Ó of PUFAs		29.07	33.59
C14:oIso		0.17	0.16
C15:oIso		-	0.14
C15:oAnteiso		0.92	0.13
C16:oIso		0.64	0.14
C17:oIso		0.11	0.74
C17:oAnteiso		0.33	0.34
C19:oIso		0.11	0.16
C20:oIso		-	0.72
C20:oAnteiso		-	0.63
Σ of Branched		2.28	3.16
Unknown & Others		12.39	4.77
ω3/ ω6		21.83/7.24	22.31/11.48
Ratio ω3/ω6		2.78	1.97

Fig. 1. The colonial ascidian collection site

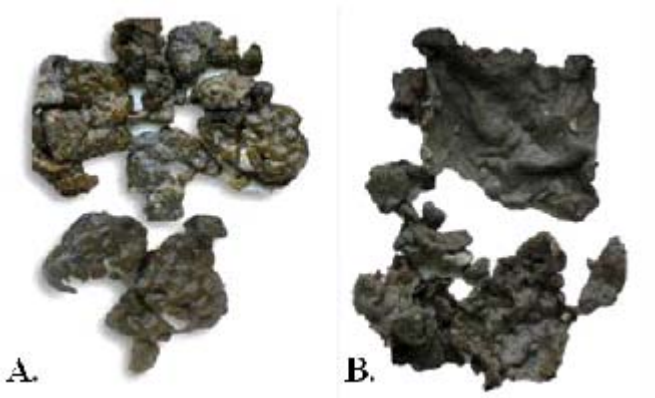

Fig. 2. Collected colonial ascidian (A.) *E. viride* and (B.) *D. psammathodes*

certain parts of the Mediterranean have realised and appreciate the food value of both *Polyclinum* sp. and *P. nigra* because of its low calorific value and high content of proteins. This corresponds to the higher amount of cellulose and crude fiber. The amount of carbohydrate, protein, lipid and minerals such phosphorous and calcium contents in these ascidians previously reported and found that the concentration of total carbohydrate was higher in their body. The protein recorded the maximum level of all the biochemical components in the mantle body (23 %) which is followed by lipid, cellulose, carbohydrate and fiber. A total of eight amino acids *viz.* alanine, arginine, aspartic acid, glycine, leucine, lysine, methionine and tyrosine were found to occur. A high concentration of free amino acids has been found in the mantle body of the study animals as compared to the test. In addition to the amino acid profile, some B-complex vitamins such as riboflavin and thiamin were also estimated to understand the nutritive value (Rajesh and Ali, 2008).

In the present investigation ascidians *E. viride* and *D. psammathodes* were found high amount of protein, carbohydrate, amino acid, lipid and fatty acids content which shows high nutrient value and thus surpass many marine food sources in terms of value added marine food. The protein content 3.78 and 3.62 µgmL^{-1}, carbohydrate contents 2.15 and 2.2 µgmL^{-1} and crude fiber content 9.2 and 7.9 µgmL^{-1} showed in ascidian *E. viride* and *D. psammathodes* tissues. The values reported here are comparable with those reported by Kowalke *et al.* (2001). Carbohydrates constitute only a minor percentage of total biochemical composition. The high level of insoluble proteins occurs in body components of antarctic solitary ascidian, *Cnemidocarpa verrucosa* and likely reflects the contribution of insoluble protein to structural materials including connective tissue (Koh *et al.*, 2000). Madin *et al.* (1981) investigated North Atlantic salp (Tunicata: Thaliacea) species and found proteins to be the major contributor which corresponded to 26.6% of the dry weight. The higher protein concentration measured by Madin *et al.* (1981) is likely to have resulted from lower lipid content in the salps. Biological value of protein is obviously reflected upon its essential amino acids concentration. As far as total essential amino and non-essential amino acids were concerned they constituted 27.221 and 3.994% respectively and were comparable with the earlier reports by Choon-Kyu *et al.* (1991) in solitary ascidians. In the present study total free amino acid content showed 3.2 and 3.9 µgmL^{-1} in *E. viride* and *D. psammathodes* tissues. Totally 15 essential and non-essential were analysed from that leucine, lysine, threonine, methionine, histidine and arginine shows high amount in *E. viride* and *D. psammathodes* tissues. The total lipid content of this ascidian shows 0.23 and 0.32 mgmL^{-1} respectively. The lipid content was very low as compared to protein and carbohydrate. The lower value of lipids in ascidians was reported by Choon-Kyu *et al.* (1991). In our study the ascidian *E. viride* and *D. psammathodes* shows 31.71 and 32.96 µg/g of total saturated fatty acids (Σ of SFAs), 24.55 % and 25.52 % of total mono unsaturated fatty acids (Σ of MUFAs) and 29.07 % and 33.59 % of total poly unsaturated fatty acids (Σ of PUFAs). The saturated fatty acids (SFAs), palmitic acid (C16:0) shows high amount 10.72 % and 11.63 %. In mono unsaturated fatty acids (MUFAs), cis-6-palmitoleic acid (C16:1ω-6) show high amount of 6.13 % and 7.12 %. In poly unsaturated fatty acids (Σ of PUFAs), eicosapentaenoic (C20:5ω -3) show high amount of 11.10 % and 10.34 % g in ascidian *E. viride* and *D. psammathodes*.

Our result is very similar to the findings of Nanton and Castell, (1999) who found significantly higher content of PUFA in harpacticoid copepods. At intermediate temperature (15°C), the PUFA were the lowest whereas MUFA were at their highest levels in both *Amonardia* and *Tisbe*, perhaps making up for the lower levels of PUFA. Lipids of edible ascidian *Halocynthia roretzi*, very popular in Japan and Korea, have also been studied (Jeong *et al.*, 1996). These tunicates contained only trace amounts of PUFA, which are usually predominant in this phylum (Viracaoundin *et al.*, 2003). PUFA represent the most important class, accounting for around 50%. The tunicates *Eudistoma bituminis* and *Cystodytes violatinctus* from the Indian Ocean were investigated for their phospholipid FA content. In both cases, the most abundant FA was the saturated ones (C10 to C18). *Cystodytes violatinctus* contained high amounts of oleic acid (20%). Both *E. bituminis* and *C. violatinctus* contained phytanic acid and É10 FA, which had

not previously been found in such organisms. The results from this study may help in developing appropriate diets for human nutrition and especially ascidian culture which is popular in Japan and Korea.

CONCLUSION

In the present study ascidians *E. viride* and *D. psammathodes* were showed high amount of protein, carbohydrate, amino acid, lipid and fatty acids content which shows high nutrient value and thus surpass many marine food sources in terms of value added marine food.

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