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Nutritional Values of Solitary Ascidian *Microcosmus exasperatus* Heller, 1878 (Ascidiacea: Pyuridae)

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Abstracts: The aim of this study was to demonstrate the nutritive value of solitary ascidians *Microcosmus exasperates* at Palk Bay region (Southeast coast of India) during March 2009. Analysis of protein, carbohydrate and lipid analysis were carried out. These ranged from 23.5±2.65 -17.3±2.11%, 14.11±2.42 - 11.15±2.12% and 2.67±0.21-2.01±0.41%, respectively on mussels in test a dry matter (DM) basis. Totally, 18 essential and non essential amino acids were reported. Among these, 10 essential and 7 non essential amino acids are reported in ascidians mussel. The maximum recorded level of essential amino acid (567.3mg) was leucine and the minimum level of non essential amino acids (0.212mg) was aspartic acid. The contents of saturated fatty acids (SAFA), mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranged from 1.664-0.110, 1.565-1.034 and 1.786-1.293mg of total fatty acids, respectively. These results indicated that the biochemical composition and subsequently the nutritional value of these ascidians are not only genetically determined but also influenced by its maturity stage and type of ingested food.

Key words: *Microcosmus exasperates* · Protein · Carbohydrate · Lipid · Amino acids · Fatty acids

INTRODUCTION

Marine invertebrates are being widely used as food and feed supplement around the world. The spatial and temporal patchiness of food in nature, periods of food deprivation are common in the lives of many animals [1]. Particularly in small ectothermic animals with high metabolic intensity and low scopes for activity, periods of food deprivation are expected to require tight metabolic control [2]. Seafood products are currently in high demand as they are considered healthy and nutritional [3-6]. Ascidians are conspicuou and important members of shallow benthic communities [7-11]. Therefore, information on their growth, production and biochemical and energetic composition [12-14] is important in modelling the flow of materials and energy within marine benthos. Marine ascidians have been shown to be a very rich source of unique and biologically active secondary metabolites that have attracted the interest of both chemists and pharmacologists, [15]. It is considered as a highly nutritious seafood. Consumption just after harvest is favoured 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. Ascidians are typically feed on meiobenthic and detritus that they filter from the seawater. They are characterized by a tough outer "tunic" made of the polysaccharide tunicin [16]. Defenses of ascidians may include physical attributes such as skeletal elements (spicules) or a tough outer tunic that sometimes incorporates grains of sand [17, 18]. Moreover, low nutrient levels could make some ascidians unattractive prey [19], while chemical defences (acids, heavy metals, secondary metabolites) may make tissues distasteful to predators [20-30] and inhibit the settlement of fouling microorganisms [31-33]. In the present study, we report the nutritional quality (biochemical and energetic composition) of solitary ascidians of *M. exasperatus* in order to evaluate them as a potential trophic resource.

MATERIALS AND METHODS

The ascidians, *M. exasperatus* were collected from Gopalapattinam (Lat. 09°57' N: Long. 79°11' E), Palk Bay region Southeast coast of India. The study was

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conducted in March 2009. The collected ascidians were thoroughly rinsed with sea water to remove all the epiphytes and then washed with distilled water to remove the salts and extraneous materials. They were brought to the laboratory and acclimatized to the laboratory conditions. Only for mature organisms (60 to 80 gm) of mussels and test were taken for analysis. The mussels were removed from test or body. Ascidians mussels and test were shade dried for few days. The dried mussels were made into powder for biochemical analysis such as protein, carbohydrate, amino acid, lipids and fatty acid.

Estimation of Protein: The total protein was estimated using the Biuret method [34].

Estimation of Carbohydrate: The total carbohydrate was estimated by following the Phenol-sulphuric acid method [35].

Estimation of Amino Acid: The amino acid were determined by an amino acid analyzer (Shimatzn-high performance Liquid chromatography, company-HITACHI, Detector-SPD 10 A VP, serial number: C_{20} 994111453 LP pump LC-10AT VP). Twenty micro litters of the filtered derived amino acid sample injected in to single column and analyzed using sodium buffer system [36].

Estimation of Lipids: The extraction of lipid was done by the chloroform-methanol mixture [37].

Estimation of Fatty Acid: Fatty acid analysis, the samples were homogenized with chloroform-methanol (2:1 V/V) mixture and extracted [38]. After fat extraction, they were esterified with 1% H_2SO_4 and fatty acid methyl esters were prepared by following the procedure of association official Analytical Chemist [39]. The identification and quantification of fatty acid was done using a Gas Chromatography (AGILENT TECHNOLOGIES, 6890'N, Net work GC systems).

RESULTS

The present study shows that the biochemical composition (%) of the solitary ascidian, *M. exasperatus* in the dry weight basis and biochemical constituents like protein, carbohydrate and lipids were 24.7±3.65, 14.97±2.82 and 2.64±1.11, respectively (Table 1). Totally 18 essential and non essential amino acids were reported.

Table 1: Proximate composition in *M. exasperates* (%) (SD)

S.No	Ascidians	Protein	Carbohydrate	Lipid
1.	*M. exasperatus*	24.7±3.65	14.97±2.82	2.64±1.11

Table 2: The essential amino acid contents in *M. exasperatus* (mg/g)

S.No	Essential amino acid	Mussels of ascidians (mg/g)
1.	Valine	124.5
2.	Methionine	178.5
3.	Threonine	356.7
4.	Isoleucine	345.6
5.	Leucine	645.5
6.	Histidine	134.4
7.	Lysine	434.5
8.	Tryptophan	67.6
9.	Arginine	345.5
10.	Phenylalanine	89.3
	Total	2722.1

Table 3: The non essential amino acid contents in *M. exasperatus* (mg/g)

S.No	Non essential amino acid	Mussels of ascidians (mg/ g)
1.	Asparagine	24.5
2.	Aspartic acid	T
3.	Glutamtic acid	0.121
4.	Alanine	T
5.	Glycine	0.112
6.	Serine	T
7.	Crystine	87.5
8.	Tyrosine	287.4
	Total	399.633

*T (Trace) = <0.1

Table 4: Fatty acid profile of solitary ascidians of *M. exasperates* (gms)

Fatty acid	Name	*M. exasperatus* (gms)
[a]C14:0	Myristic	0.412
[a]C16:0	Palmitic acid	0.131
[a]C17:0	Margaric	0.201
[a]C18:0	Stearic acid	1.612
[b]C18:1w 9c	Olecic acid	1.304
[b]C18:1w 7c	Octadecenoic	1.546
[c]C18:2	Linoleic acid	1.732
[c]C18:3	Alpha linolenic acid	1.021
[c]C18:4	Moroctic acid	T
Total	[a]Saturated fatty acids	2.356
Total	[b]Monounsaturated fatty acids	2.85
Total	[c]Polyunsaturated fatty acids	2.753

*T (Trace) = <0.1

Among these, 10 essential and 7 non essential amino acids are reported in ascidians mussels. Amino acid content varied from 0.121 to 645.5 mg. Maximum level of essential amino acid (645.5mg) was recorded in Leucine and the minimum level of non essential amino acid (0.121 mg) was recorded in Glycine (Table 2).

For fatty acid analysis only medium sized ascidians were taken into consideration. The total amount of saturated fatty acids was 2.356 mg. Among total 4 individual saturated fatty acids reported, the Stearic acid was maximum 1.612mg and palmitic acid was reported minimum 0.131mg. Olecic acid (1.304gm) and Octadecenoic (1.546mg), two monounsaturated fatty acids were recorded (Table 3). The total amount of polyunsaturated fatty acid was recorded maximum in Linoleic acid (1.732mg) and minimum in Moroctic acid (trace) (Table 4).

DISCUSSION

The protein content of ascidians showed great variation, these values are comparable with those previously reported [19,39,40]. In the present estimation, the protein content was found to be the major biochemical composition in the solitary ascidians (mussles and test), *M. exasperatus*. Similarly, high levels of protein occur in body (test) components of the Antarctic solitary ascidians *Cnemidocarpa verrucosa* and likely reflect the contribution of insoluble protein to structural materials including connective tissue [1,13]. The protein content was positively correlated with salinity of the water [41]. In the present observation, the carbohydrate content was slightly higher than the lipids and lower than that of protein, Carbohydrates constitute only a minor percentage of total biochemical composition. Carbohydrates in fishery products contain no dietary fibber but only glucides, the majority of which consist of glycogen. They also contain traces of glucose, fructose, sucrose and other mono and disaccharides [42]. In general, lipid content has been very low in the ascidians test than that in mussel and as compared to protein and carbohydrate. The lower value lipids of solitary ascidians (test) have been already reported [39].

For comparison, the sum of ash, proteins, lipids, carbohydrates and chitin in crustacean zooplankton typically accounts for 90-95% of the DW [43]. Madin *et al.* [44] investigated North Atlantic salp (Tunicata: Thaliacea) species and found the proteins to be the major contributor (82%) to the TO. This corresponded to 6.6% of the DW. This value is substantially higher than obtained in this study, e.g. 40-50% of the TO for aggregates and solitaries. This corresponded to 4.4% of the DW (Table 2). Much higher protein contribution to the TO measured by Madin *et al.* [44] likely resulted from lower lipid content in the salps when compared to our values. Biological value of protein is obviously reflected upon its essential amino acids concentration. In general, the shellfish have a balanced distribution of all essential amino acids required for an adult per day. There are 20 amino acids found in fish proteins. Some of these are listed as essential amino acids (EAA), *i.e.* arginine, histidine, isolucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine because these are not synthesized in the body. The essential amino acids are required for maintenance of life, growth, synthesis of vitamins and reproduction. The lowest level of any one of these essential amino acids in a protein source, which limits the utilization of that protein, makes it the "First limiting amino acid" [45]. 18 amino acids were reported, among these 10 essential amino acids (Leucine > lysine > threonine > isoleucine> arginine > Methionine> histidine> valine> phenylalanine> Threonine) and 8 non essential amino acids (Tyrosine > Crystine > Asparagine > Glutamtic acid > Glycine > Aspartic acid > threonine> Serine) are reported in ascidians mussels and test. As far as total essential amino and non essential amino acids were 2616.0-1366.5mg and 356.89-250.42 mg in mussels and test, respectively. The amino acid composition in solitary ascidians (mussels and test) was similarly reported [39,46, 47].

In the present study, totally 9 fatty acids are reported among these 4 saturated fatty acid (stearic acid > myristic > palmitic acid > margaric), 2 Monounsaturated fatty acid (Octadecenoic > Olecic acid) and 3 Polyunsaturated fatty acids (Linoleic acid > Alpha linolenic acid > Moroctic acid) in ascidians mussels and test. Totally, the maximum value of polyunsaturated fatty acids (3.109 mg) was in mussels and a minimum value was in saturated fatty acid (1.254mg). This result showed close similarity with the previous finding of Nanton and Castell [48] who found significantly higher content of PUFA, but at intermediate temperature (15°C), it was lowest, whereas, MUFA were at their highest levels at 15°C in both Amonardia and Tisbe, perhaps making up for the lower levels of PUFA at this temperature.

In conclusion, this study provides biochemical analyses of the solitary ascidians, *M. squamiger* species captured from the Southeast Coast of India in relation to their potential value in human nutrition. These results can help in developing appropriate diets for culturing ascidians species.

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