ESTIMATION OF BIOCHEMICAL COMPOSITION OF PURPLE SEA URCHIN (SALMACIS VIRGULATA, L.AGASSIZ AND DESOR, 1846)

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ABSTRACT

The proximate composition, fatty acids, minerals and vitamins contents of purple sea urchin (Salma
cis virgulata) from the spine, viscera, test region of the S. virgulata analysed. Among the different tissues studied, a highest fat, and carbohydrate content of 39.56mg, 23.57mg and 21.94mg was recorded in the test region of the sea urchin which was collected in the Mudasalodai, Southeast coast of India. There were seven different fatty acids (FA’s) were identified from the tissues that were esterified and resulting FA analysis. The analysis showed that the presence of saturated, and higher level of unsaturated fatty acids in particular, alpha linoleic acid (53.26 %). The FA’s were determined as ranging from 1.02% to 53.26%. Nine different vitamins were identified viz., A, D, E, B1, B2, B5, B6, B12 and vitamin C. in the tissues of S. virgulata. The vitamin C was observed as predominant in the tissues. Seven minerals such as Ca, Mg, Zn, Fe, Cu, Na and K were noticed in S. virgulata. However, Na, K and Ca found dominant elements and Cu, Fe, Zn least in utmost all the tissues of the sea urchin.

Key words: Fatty acids, Gas chromatography, HPLC, Nutrients.

INTRODUCTION

Sea urchins support important fisheries in several areas of the world [1]. Unfortunately, many of the wild populations of commercially valuable sea urchins are overexploited and as a consequence, there has been an increased interest in echinoculture of traditionally exploited species and the development of new species for the market [2]. Most lipid storage in sea urchins occurs in the gonads, and several studies have compared FA signatures of urchin’s gonads on kelp diets with those of urchins on a variety of artificial diets in fisheries and aquaculture applications [3-5]. While these studies have shown that FA’s compositions of urchins resemble those of their diets to some extent, the experimental diets were markedly different from each other and bore little resemblance to food normally encountered by urchins in the wild. Animals are known to receive a considerable amount of lipid via their diet [6] and the diet type has been found to alter the FA’s composition of certain herbivorous copepods, for example, Calanus spp. [7]. It has been suggested, therefore, that certain fatty acids, or their ratios, can be used to provide a more precise indication of an organism’s diet than, for example, analysis of the gut contents [8]. This assumption was based, however, on observational studies of feeding habits in situ and unfortunately there have been no studies involving the experimental manipulation of sea urchin diet to support this claim.

The aim of this study was to determine the FA profile, vitamins and minerals present in the entire body of S. virgulata, including the occurrence of biochemical composition in spine, viscera and test were carried out.

MATERIALS AND METHODS

Sample collection

Moribund Sea urchins were collected from Mudasalodai (Lat.11°29’N; Long. 79°29’E), Tamil Nadu, Southeast coast of India in the month of April, and transported to the laboratory packed in ice. The sea urchins were dissected, following organs namely spines, viscera and test were removed, shade dried and coarsely powdered in a grinder.

Preparation of sample

Powdered samples were weighed out (1-10mg) and 50ml 12%NaOCl per mg of powdered sample was added at room temperature. The powder was soaked for 48 h and vortexed after 24 h to ensure complete penetration of the bleaching agent. Then filtered through Whatmann #1 filter paper to remove macro particles. The filtrate was diluted to
25 ml with ultrapure water in a volumetric flask and 1 ml of the resulting liquid was used for the further analysis.

**Biochemical analysis**

**Estimation of total protein**

The Folin-Ciocalteu Phenol method [9] was used for the determination of the total protein from the sample.

**Estimation of total carbohydrates**

The total carbohydrates were estimated by phenol-sulphuric acid method described by Dubosis [10].

**Estimation of total lipids**

Total lipids were quantified through the method proposed by Folch [11].

**Estimation of Fatty acid analysis**

Twenty microliter of the combined hexane extracts were injected manually on a 30 m X 0.25 mm (i.d.) fused silica column with macrogel as stationary phase, with a thickness of 0.25 µm and helium at 20 psi as mobile phase. The column was mounted in Hewlett-Packard 5890A gas chromatograph equipped with a flame-ionization detector (FID). The injector temperature was set at 250°C and the detector temperature at 280°C. The oven was programmed as follows: 90°C for 4 min, 30°C/min to 165°C, then 3°C/min to 225°C, where it was left isothermally for 10.5 min before cooling for the next run. The chromatographic peaks were identified by comparison with a chromatogram of a standard fatty acid mixture.

**Estimation of Vitamins analysis**

The fat soluble vitamins A, D, E and K and the water soluble vitamins B1, B2, B5, B6, B12 and C were analyzed in the HPLC (Merk Hitachi L-74000) following the method of Sadasivam and Manickam [12]. The folic acid was estimated by following the calorimetric procedure of Sethi [13]. The pyridoxine, pantothenic acid and vitamin B12 were estimated by following methods are suggested in USP NF 2000 Asian edition.

**Mineral analysis**

The minerals were estimated by following the method of Guzman and Jimenez [14].

**RESULTS**

The protein, carbohydrate, fat, ash, moisture content of sea urchin *S. virgulata* were analysed in three different tissues namely spine, viscera and test region in the following study were as follows: Protein (20.75mg, 20.09mg, 21.94mg), Carbohydrate (18.78 mg, 22.67mg, 23.57mg), Fat (35.67mg, 36.78mg, 39.56mg), Ash (6.89mg, 10.33mg, 9.62mg), Moisture (2.61mg, 1.46mg, 2.45mg) respectively. A higher quantity of fat recorded in the test region of *S. virgulata* than the viscera and spine as mentioned in Figure 1. The FA profile of sea urchin obtained from the present study Table.2 was compared with the standard FA profile (Table.1) of the purple sea urchin was revealed by the chromatogram.

The chromatogram (Figure 3) revealed the presence of FA’s namely palmitic acid, stearic acid, oleic acid, linoleic acid, alpha linoleic acid and moroctic acid were present in the purple sea urchin. A higher level of (44.40%) alpha linoleic and (32.81%) oleic acid was observed in the tissues of *S. virgulata*. Moreover, 10 vitamins were identified from *S. virgulata*. The vitamin A, D, E, B1, B2, B5, B6, B12, vitamin C, vitamin A and vitamin D were measured by in an international unit (IU). Presence of different vitamins in the tissues of sea urchin were as follows: Vitamin A (22.4 IU), Vitamin D (5.45 IU), Vitamin E (2.95 mg), Vitamin B1 (2.78mg), Vitamin B2 (4.34mg), Vitamin B5 (1.52mg), Vitamin B6 (3.45mg), Vitamin B12 (1.23mg) and Vitamin C (22.3mg). The concentration of different vitamins present in the whole body of *S. virgulata* are showed in the Figure 5.

Seven different minerals were identified from the whole body evaluation of *S. virgulata*. Level of minerals such as Ca, Mg, Zn, Fe, Cu, Na and K were as follows: Ca 34.8mg, Mg 12.3mg, Zn 2.98 mg, Cu 0.72 mg, Na 122.3mg, K 45.9mg. The concentration vitamins recorded in the *S. virgulata* are depicted in the figure 6.

**Table 1. Fatty acids profile of Standard**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Fatty acids</th>
<th>Carbon</th>
<th>RT</th>
<th>Area</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic</td>
<td>C 16</td>
<td>30.5</td>
<td>3456.6</td>
<td>7.55</td>
</tr>
<tr>
<td>2</td>
<td>Stearic</td>
<td>C 18</td>
<td>33.5</td>
<td>1156.4</td>
<td>2.53</td>
</tr>
<tr>
<td>3</td>
<td>Oleic</td>
<td>C 18</td>
<td>35.5</td>
<td>8645.6</td>
<td>18.91</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic</td>
<td>C 18</td>
<td>37.2</td>
<td>7651.6</td>
<td>16.73</td>
</tr>
<tr>
<td>5</td>
<td>Alpha linoleic</td>
<td>C 18</td>
<td>40.2</td>
<td>24356.700</td>
<td>53.26</td>
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<tr>
<td>6</td>
<td>Moroctic</td>
<td>C 184</td>
<td>44.5</td>
<td>454500</td>
<td>1.02</td>
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<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>45724.467</td>
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**Table 2. Fatty acid profile of Sea urchin**

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<tr>
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<th>Carbon</th>
<th>RT</th>
<th>Area</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic</td>
<td>C 16</td>
<td>30.6</td>
<td>133.4</td>
<td>5.79</td>
</tr>
<tr>
<td>2</td>
<td>Stearic</td>
<td>C 18</td>
<td>33.7</td>
<td>167.8</td>
<td>7.28</td>
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</table>
DISCUSSION

In the present study, the protein content of spine, viscera and test were found to be 20.75mg, 20.09mg and 21.94mg respectively. Highest level of carbohydrate content was present in the test (23.57mg) when compared with that of spine and viscera (Figure 1). Hammer et al., [15] reported that the increasing dietary protein and decreasing dietary carbohydrate resulted in increased concentrations of stored protein in the gonad, and drastically decreased concentrations of stored carbohydrates. Generally, female sea urchin contains high protein content and low carbohydrate percentage. Mataix-Verdu [15], reported that

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<tbody>
<tr>
<td>3</td>
<td>Oleic acid</td>
<td>C 18 1</td>
<td>35.7</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic acid</td>
<td>C 18 2</td>
<td>37.4</td>
</tr>
<tr>
<td>5</td>
<td>Alpha linoleic acid</td>
<td>C 18 3</td>
<td>40.4</td>
</tr>
<tr>
<td>6</td>
<td>Behenic acid</td>
<td>C 22 : 0</td>
<td>46.2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>2307.667</td>
</tr>
</tbody>
</table>

Fig 1. Proximate composition of *S. virgulata*

Fig 2. Fatty acids profile of standard chromatogram

Fig 3. Fatty acid profile *S. virgulata* chromatogram

Fig 4. Fatty acid profile of *S. virgulata*

Fig 5. Vitamins composition of *S. virgulata*

Fig 6. Minerals composition of *S. virgulata*
the composition of the protein contents of sea urchin and other shellfish were lower than that of prawns and crabs but higher than in oysters and mussels. Hammer [16] stated that the growth of adult *L. variegates* fed a 20.23% protein: carbohydrate diet was comparable to that of urchins fed a 31:12% diet. Somatic growth of *S. droebachiensis* was maximized at dietary protein levels of 19-20% [17-18].

Sea urchin growth can be described relatively well by the Tanaka growth model [19-20], which comprised of slow initial growth followed by a period of experimental growth and then a period of slow but constant growth. It is reasonable to assume that sea urchins may require different levels of dietary protein and carbohydrate at different life stages. Hammer *et al.*, [21] reported that juvenile sea urchins should be in the exponential growth phase and thus may have a higher requirement for dietary protein.

In this present study, fat contents were higher in spine 35.67mg, viscera 36.78mg and test 39.56mg compared than protein and carbohydrate composition (Figure 1). Phillips *et al.*, [22] reported the total FA’s concentration in gonads from sea urchins fed diet 1 was insignificantly (p<0.05) higher than in gonads from sea urchins collected from the wild. Gonads from sea urchins fed diet 2 had a significantly higher (p<0.05) total FA’s concentration than those from the wild.

In this present study six fatty acids were observed in *S. virgulata*. Alpha linoleic acid and oleic acid were present in high concentration (Table 3) (Figure 4). In addition, the proportion of certain fatty acids in the gonads of the sea urchin, *Psammechinus miliaris* was affected by diet. This suggests that fatty acid composition in *P. miliaris* could be used as an indicator of diet. For instance the sea urchins fed with salmon feed resulted in a large proportion of the lipid that was composed of 22:6 n23 in the gonads. Analysis of the gonads from the sea urchins fed with Atlantic salmon also revealed higher levels of 22.6 n23 in the lipid fraction. This fatty acid is a major PUFA in fish oils [23] and it has been associated with a predominantly carnivorous diet in benthic organisms [24-25]. Tagaki *et al.*, 1986 found that the *Echinolamas sternopetala* contained high amounts of 22.6 n23, which was attributed to its feeding upon the carcasses of marine animals [26].

In *S. droebachiensis*, *P. miliaris* and *P. lividus*, the lipid was also the dominant component of the gonads, among which the neutral lipid, especially triacylglycerol, was the major lipid fraction [27-30]. Shankaratal *et al.*, 2013 reported sea urchin shell had a good source of amino acids [31].

Totally nine vitamins were observed in *S. virgulata*. Vitamin A (22.4 IU) and vitamin C (22.3mg) were maximum and vitamin B12 (1.23mg) was found minimum in the test sample (Table 4) (Figure 5). Rodriguez-Bernaldo de Quiros *et al.*, 2001 reported that the Ascorbic acid (Vitamin C) levels in fresh and canned sea urchin gonad after spiking samples were same amount and ranged from 97.82% to 99.74% [32]. In controversy to that, the Mean Ascorbic acid (Vitamin C) levels were 26.57mg per 100g in fresh samples and 14.25mg per 100g in canned samples. St.Aubin *et al.*, 1980 reported that the Adrenal glands of various marine mammals are very rich sources of vitamin C [33].

In general, sea foods are abundant source of mineral compounds. In this present study minerals viz., sodium 122.3mg, potassium 45.9mg and calcium 34.8mg were found at maximum level (Table 5) (Figure 6). Vignesh and Srinivasan, 2012 recorded the minerals concentration of Calcium (56.7%, and 67.76%) and Sodium (34.67% and 29.11%) in head and bone region of *O. mossambicus* respectively [34]. Kumaren *et al.*, 2012 reported the Mugil cephalus flesh had rich quantity of Na (68mg/100g), Ca (48mg/100g) and Mg (28mg/100g) [35]. This difference may be due to the different feeding types of various fishes. Kuhnlein [36,37] reported Calcium is also very prominent in many fish species, e.g. sculpins (429mg/100g) and skin from Arctic char (268mg/100g). Potassium present in polar bear muscle (552 mg/100g). Furthermore, shrimps and clams (475-858mg/100g) and blue mussel (325mg/100g) have a high content of sodium.

**CONCLUSION**

It is an urgent need to protect the diversity of sea urchin worldwide. It is evident from the present study that they are comprised of high fatty acid content and also holds a considerable amount of vitamins and minerals. It strongly suggests the proportions of certain vital FA’s in the *S. virgulata* along with its amino acid it could be used as an alternative feed to aquaculture and poultry industries.

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