Biochemical profile and nutritional quality of Indian squid, *Uroteuthis duvauceli*

KR Remyakumari, J Ginson, KK Ajeeshkumar, KV Vishnu, KK Asha and M Suseela

Abstract

This study was designed to find out the biochemical and nutritional profiling of Indian squid. Proximate composition of *Uroteuthis duvauceli* showed a content of 80.47% for moisture, 17.5% for protein, 0.52% for fat and 1.13% for ash, respectively. Amino acid analysis showed a higher content of glutamine, followed by aspartine, tryptophan, leucine, alanine and glycine. Higher concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA), total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) were observed. In case of fatty acids, saturated fatty acids, palmitic and stearic acid contributed highest quantity; whereas, DHA, EPA and arachidonic acid were the major unsaturated fatty acids in the sample. Among the macro minerals, potassium showed highest content followed by sodium and calcium. As in the case of micro minerals, magnesium content showed highest proportion and copper showed least quantity. Commendable quantities of biochemical and nutritional content in *U. duvauceli* signify the appropriateness of this moderately exploited resource as an essential nutrients for nutritionally deprived population.

Keywords: *Uroteuthis duvauceli*, proximate composition, amino acids, fatty acids, minerals

1. Introduction

Squids were widely accepted seafood commodity because of its peculiar palatability, sensory properties and better yield percentage of meat for consumption. Marine lipids were mostly accepted by the consumers because of their high content of omega-3 fatty acids and low content of omega-6 fatty acids (Steffens, 1997) [44]. Significant content of omega-3 polyunsaturated fatty acids in squids were essential for growth and maintenance of the body (Ozyurt et al., 2001) [31]. Moreover, it had the potential to develop various value added products such as squid gel, surimi and seafood analogues (Sánchez-Alonso et al., 2007) [37]. Generally, squids were marketing as fresh, dried (Kugino et al., 1993) [39], chilled (Hurtado et al., 2001) [25] and frozen forms (Paredi and Crupkin, 1997) [31]. However, quality changes especially browning were the major concern for the marketing of squid which was greatly affected by biochemical composition (Calli et al., 2006) [19]. Biochemical profiling of squid had remarkably different from fishes. Spitz et al., (2010) [43] suggested that ecosystem had significant role for the nutritional quality of an organism. Similarly, metabolic activity had greatly influencing the biochemical composition and body mass of an organism (Schmidt-Nielsen, 1984) [39]. Most of the squids usually showed isometrical metabolism (Seibel, 2007) [40]. According to Roper et al., (1984) [34], squids are beneficial to elderly population because of low fat and high proteins content. Bano et al., (1992) [7] observed high protein and low fat content in squid species. Bano et al., (1992) [3] analyzed and compared the protein and amino acid content of *Sepiella inermis* and *Symplectoteuthis oualaniensis* and found better protein content in *S. oualaniensis*; whereas, homogenous amino acids content was observed. Amino acids play vital role for the growth and metabolic functions of humans. Amino acid composition and its score determine the nutritional quality of protein (Iqbal et al., 2006) [23]. Generally, seafood are a good source for essential amino acids and aromatic amino acids (Adyeeye, 2009) [3]. Presence of aspartic acid, lysine, glutamic acid, leucine, serine, arginine, cystine, histidine and tryptophan were found in the protein of squid (Bano et al., 1992) [7]. Ali (1987) [4] noticed significant quantity of lysine in squid, which is essential for

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Correspondence
KR Remyakumari
Senior Research Fellow, Biochemistry and Nutrition Division Central Institute of Fisheries Technology, Cochin, Kerala, India

J Ginson
Assistant Professor, St. Albert’s college, Ernakulam Kochi, Kerala, India

KK Ajeeshkumar
Senior Research Fellow, Biochemistry and Nutrition Division Central Institute of Fisheries Technology, Cochin, Kerala, India

KV Vishnu
Senior Research Fellow, Biochemistry and Nutrition Division Central Institute of Fisheries Technology, Cochin, Kerala, India

KK Asha
Senior Research Fellow, Biochemistry and Nutrition Division Central Institute of Fisheries Technology, Cochin, Kerala, India

M Suseela
Senior Research Fellow, Biochemistry and Nutrition Division Central Institute of Fisheries Technology, Cochin, Kerala, India

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growth and development. Alanine, glutamic acid and aspartic acid were the major amino acid in the gelatin of splendid squid (L. formosana); whereas tyrosine, phenylalanine, histidine and lysine noticed at lower level (Nagarajan et al., 2012) [27]. There is some report for the biochemical profiling of squid whereas, lack information for nutritional composition of Indian squid (Uroteuthis duvauceli). Hence, this study was designed to find out the biochemical and nutritional profiling of Indian squid.

2. Material and materials

2.1 Raw material

Indian squid (U. duvauceli) was collected from the fish landing centre at Fort Cochin, Kerala, India. The samples were transported to the laboratory in iced condition. Raw material had an average length of 570±14 mm and weight 445±15 g. Squid mantle was dissected and used for the analysis.

2.2 Chemicals

All reagents and solvents used in this study were of analytical grade. Standards of fatty acid methyl esters, amino acids were purchased from Sigma Aldrich GmbH (Steinheim, Germany).

2.3 Determination of moisture

Moisture was determined according to the AOAC (2000) [5] method by drying. A clean and dry petridish was cooled in desiccators and weighed (W1). Approximately 10-20g of the finely homogenized samples was evenly spread and weighed (W2). Petridish with the sample was dried in an oven at 105 °C, cooled in a desiccators and weighed (W3). The process of heating and cooling were repeated to get a constant value. Results were expressed as percentage of wet weight.

\[ \text{Moisture (\%)} = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \]

2.4 Determination of protein

Total protein content in the homogenized samples (0.2 g) was determined using Kjeldahl method (AOAC, 2000) [5] and the protein content was calculated by the following methods,

\[ \text{Protein content (g/100g)} = \frac{X \times 0.14 \times 6.25 \times 100}{1000 \times V_1 \times W} \]

Where,

\[ V = \text{Total volume of the digest} \]
\[ V_1 = \text{Volume of the digest for distillation} \]
\[ W = \text{Weight of sample for digestion} \]

2.5 Amino acids analysis

Total amino acid composition was determined using Shimadzu amino acid analyzer (Ishida et al., 1981) [28]. The results were expressed in g/100 g of protein.

2.6 Determination of tryptophan

About 200-250 mg of sample was hydrolyzed with 10 ml of 5% NaOH at 110 °C for 24 hours in a sealed tube filled with pure nitrogen. The hydrolysate was neutralized to pH 7.0 with 6 N HCl using phenolphthalein indicators. The volume was made up to 100 ml with distilled water. The solution was then filtered through whatman filter paper No.1 and filtrate was used for estimation. To a test tube containing 4 ml of 50% H2SO4, 0.1 ml of 2.5% sucrose and 0.1 ml of 0.6% thioglycolic acid were added. These tubes were kept for 5 min in water bath at 45-50 °C and cooled. The sample was then added to the test tubes. A set of (0.1 to 0.8) standard tryptophan (10µg/ml) was treated similarly. The volume was made up to 5 ml with 0.1 N HCl and allowed to stand for 5 minutes. The absorbance was measured using Shimadzu-UV spectrophotometer at 500 nm. The concentration was obtained by drawing standard graph

![Formula](https://example.com/formula.png)

2.7 Estimation of quality of dietary protein

Essential amino acid score was calculated using the following formula (FAO/WHO, 1973) [13].

\[ \text{Amino acid score} = \frac{\text{Amount of amino acid per test protein (mg/g)}}{\text{Amount of amino acid per protein in reference pattern (egg) (mg/g) \times 100}} \]

Determination of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e., (TEAA/TAA); total sulphur amino acid (TSAA); percentage of cysteine in TSAA (% Cys/TSAA); total aromatic amino acid (TArAA), etc.; the Leu/Ile ratios were calculated while the predicted protein efficiency ratio (P-PER) was determined using one of the equations developed by Alsmeyer et al.,1974), i.e., P-PER = -0.468 + 0.454 (Leu) - 0.105 (Tyr).

2.8 Determination of crude fat (Soxhelet method)

The estimation of crude fat content was carried out by continuous extraction of fat with petroleum ether according to AOAC (2000) [5]. 2 g (W1) of dried sample was weighed into a thimble and a cotton plug was kept on top of it. Thimble was placed in a Soxhelet apparatus and extracted with petroleum ether for 16 hours. The apparatus was cooled and the solvent was filtered in to a pre-weighed conical flask (W2). The flask of the apparatus was washed with small quantities of ether and the washings were added to the above flask. The ether was removed by evaporation and the flask with fat was dried at 80-100 °C, cooled in a desiccator and weighed (W3).

\[ \text{Fat content (g/100g)} = \left( \frac{W_2 - W_3}{W_1} \right) \times 100 \]

2.9 Extraction of total lipids

The total lipid content of the tissues was estimated by the method of Folch et al., (1957) [15]. A weighed amount of the samples was minced well and subjected to lipid extraction using chloroform-methanol mixture (2:1). The extraction was repeated twice with fresh aliquot of chloroform-methanol.
mixture. The lipid extracts were transferred to a separating funnel and added 20% of water into it and left overnight. Next day the lipid extracts were drained through filter paper containing anhydrous sodium sulphate and was collected in round bottom flask and was made up to 10 ml by using chloroform. From this 1.0ml was taken into a pre-weighed vial and allowed to dry in warm temperature to constant weight and total lipid content were calculated from the difference in weight. Sample made up to 10 ml was used for the estimation of various lipid components viz, cholesterol (total and free) triglycerides, free fatty acids and phospholipids after evaporating the solvent in air at room temperature.

2.10 Fatty acid analysis
Fatty acids methyl esters (FAMES) were analyzed by the modified method of Gershbein & Metcalfe (1966) [16]. Individual fatty acids were expressed as a percentage of total fatty acids.

2.11 Determination of ash
Ash content was determined by heating sample for 6 h in a furnace at 600 °C (AOAC, 2000) [3]. Results were expressed as percentage of wet weight.

\[ \text{Ash content (\%)} = \frac{(W_3 - W_4)}{(W_2 - W_1)} \times 100 \]

2.12 Determination mineral analysis
The minerals were analyzed by dissolving the ash (obtained in ash determination) in diluted HCl (6 N) and estimated using atomic absorption spectrophotometer (Spectra AA 220, AAS VARIAN), with Deuterium background correction, acetylene and air supplied in constant ratio for flame and hollow cathode lamp. The wavelengths (nm) of light used for analyzing different minerals are 285.2 for magnesium, 213.9 for zinc, 766.5 for potassium, 328.4 for copper, 279.5 for manganese, 248.3 for iron, 240.7 for cobalt, 670.8 for lithium, 228.8 for cadmium, 217 for lead.

3. Result and Discussion
3.1 Proximate composition
Proximate composition of seafood determines the nutritional and edible characteristics in terms of energy units. Moisture, protein, crude fat and ash content of the sample are presented in Table 1. Proximate composition of U. duvauceli showed a content of 80.47% for moisture, 17.5% for protein, 0.52% for fat and 1.13% for ash, respectively. Similar finding was observed in the tentacles of European squid (L. vulgaris) (Atayeter and Ercoşkun, 2011) [9], Santosho et al., (2013) [38] investigated the proximate composition of Japanese common squid (Todarodes pacificus) and reported the content of 44.0 g/100 g of fat, 13.5 g/100 g of protein and 2.11 g/100 g of ash in dry matter. Relatively high moisture content was observed in U. duvauceli as compared to its total fat content and also revealed better content of protein and ash. Roper et al., (1984) [35] suggested that low fat content and high protein concentrations in cephalopods make them appropriate for human consumption especially for elderly population. Bano et al., (1992) [7] analyzed and compared the protein content of Sepiella inermis and Symplecotenellus oualaniensis and found better protein content in S. oualaniensis. The present findings were in corroboration with the findings of Thanonkaew et al., (2006) [48]. Authors suggested that low fat and high protein content were beneficial in the formulation of protein supplements for targeted populations. Lipid was also essential in diet, which absorb fat soluble vitamin A, D, E and K from food and also regulate cholesterol metabolism. Studies had shown that proximate composition of cephalopods varies with habitat, age of maturation, food and feeding, species and season etc. (Ozogul et al., 2008) [29].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U. duvauceli</td>
<td>80.40±0.23</td>
<td>17.50±0.06</td>
<td>0.52±0.08</td>
<td>1.31±0.15</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for three replicates, ND, not detected

3.2 Amino acid composition
Amino acids play vital role for growth and metabolic functions of humans. Amino acid composition and its score determine the nutritional quality of protein (Iqbal et al., 2006) [23]. Total amino acid content in U. duvauceli was 12.46 g/100g and amino acid profile of the sample is presented in Table 2. Giménez et al., (2009a) [15] and Gómez-Guilén et al., (1998) [19] revealed 16 to 17% of amino acid in giant squid (Dosidicus gigas) gelatin. Present study showed a better content of glutamine, followed by aspartine, tryptophan, leucine, alanine and glycine. Glutamic acid and aspartic acid were the acidic amino acid occupied highest concentrations compared to other amino acids. Nagarajan et al., (2012) [27] reported better content of alanine, glutamic acid and aspartic acid in the gelatin of splendid squid (L. formosa); whereas tyrosine, phenylalanine, histidine and lysine noticed at lower level. In the present study leucine and tryptophan were found to be highest essential amino acid (EAA) content in the sample. Nevertheless, amino acid such as phenylalanine, proline, threonine, serine and isoleucine were also observed in commendable quantity. Lysine at a content of 0.15 g/100g was noticed in the sample. Ali (1987) [4] reported significant quantity of lysine in squid, which was essential for growth. Glutamic acid considered as one of the vital amino acid for metabolic activities especially for the synthesis of nucleic acid. Bano et al., (1992) [7] reported the presence of aspartic acid, lysine, glutamic acid, leucine, serine, arginine, cystine, histidine and tryptophan in the protein of squid. Similar findings were reported by Konosu et al., (1958) [25]. Present study was better correspondence with Adayeey (2009) [3] who reported good source of essential (leucine), non essential (glutamic acid) and aromatic amino acids (phenylalanine) in fishes. This indicates the similarities of amino acid composition of cephalopods with fishes. Aromatic amino acids (tyrosine and phenylalanine) were essential for the synthesis of peptide hormones especially epinephrine and thyroxine (Robinson, 1987) [33]. Glutamic acid, glycine and aspartic acid possess wound healing ability (Chyun and Griminger, 1984) [10]. Aromatic amino acids (i.e., tyrosine, histidine, and phenylalanine) and hydrophobic amino acids (i.e., valine, alanine, proline and leucine) were able to scavenge free radicals (Suetsuna et al., 2000) [46]. Among the nutrients, supplementation with amino acids, and branched chain amino acids in particular, could reduce fat accretion and maintain lean body mass, and therefore had a fundamental role in maintaining insulin sensitivity and counteracting obesity-induced metabolic syndrome (Giuseppe D Antona, 2014) [58]. Sulfur-containing amino acids play indispensable roles in biological activities including protein synthesis, methylation, biosynthesis of polyamines and glutathione (Nozaki et al.,...
Bano et al., (1992) compared amino acid content of Sepiella inermis and Symplocoteuthis oualaniensis and found no significant difference for amino acids. However, nitrogen content in the mantle of I. argentines varies with sex and maturity (Clarke et al., 1994). Table 3 shows the concentrations of total amino acid (TAA), total essential amino acid (TEAA), total acidic amino acid (TAAA), total neutral amino acid (TNAA), total sulphur aromatic amino acid (TSAA) and total aromatic amino acid (TarAA). Content of TEAA with histidine in L. duvauceli was 272.00 mg/gcp and it was reasonably compare with egg reference protein (566 mg/gcp) (Paul et al., 1980). This nutritional profile of sample was par with Zonocerus variegates, 351 mg/gcp (Adeyeve, 2005) and S. budgetti, 389 mg/gcp and H. fasciatus, 394 mg/gcp (Abdullahi and Abolude, 2002).

Table 2: Amino acid composition (g/100g of meat) of U. duvauceli

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>U. duvauceli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>1.87±0.05</td>
</tr>
<tr>
<td>Thr</td>
<td>0.78±0.12</td>
</tr>
<tr>
<td>Ser</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>Glu</td>
<td>2.92±0.125</td>
</tr>
<tr>
<td>Pro</td>
<td>0.75±0.018</td>
</tr>
<tr>
<td>Gly</td>
<td>1.01±0.043</td>
</tr>
<tr>
<td>Ala</td>
<td>1.19±0.072</td>
</tr>
<tr>
<td>Cys</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Val</td>
<td>0.18±0.025</td>
</tr>
<tr>
<td>Met</td>
<td>0.18±0.031</td>
</tr>
<tr>
<td>Ile</td>
<td>0.92±0.045</td>
</tr>
<tr>
<td>Leu</td>
<td>1.54±0.01</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>Phe</td>
<td>0.76±0.06</td>
</tr>
<tr>
<td>His</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>Lys</td>
<td>0.15±0.072</td>
</tr>
<tr>
<td>Arg</td>
<td>ND</td>
</tr>
<tr>
<td>Try</td>
<td>1.6±0.013</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for three replicates, ND, not detected.

3.3 Fatty acid composition
Fatty acid profile of U. duvauceli is shown in Table 4. Among saturated fatty acids, palmitic and stearic acid contributed highest quantity; whereas, DHA, EPA and arachidonic acid were the major unsaturated fatty acids in the sample. Suzuki et al., (1992) reported better concentration of DHA in the integument of squid Ommastrephes bartrami. Studies showed that cephalopod from Mediterranean Sea was rich in biologically beneficial PUFA (Sinanoglu and Miniaidis-Meimoroglou, 1998) and DHA and EPA were the dominant PUFA (Sinanoglu and Miniaidis-Meimoroglou, 1998). Significant level of essential fatty acids was observed in the present study (Table 5). Occurrence of significant quantity of fatty acid content in U. duvauceli, L. valgaris (Salman et al., 2007) and Sthenoteuthis oualaniensis (Wang et al., 2008) was reported. According to Ozyurt et al., (2006) squids had commendable quantity of nutritional elements including omega-3 polyunsaturated fatty acids, which were essential for growth and maintenance of body. DHA, EPA, arachidonic acid and palmitic were the dominant fatty acid in cephalopods (Stowasser et al., 2006). Fatty acid profile of U. duvauceli had striking similarity with Loligo vulgaris (Salman et al., 2007) and egg of Sthenoteuthis oualaniensis (Wang et al., 2008). Generally, fat content of squid species was low; however, majority of fat contains nutritionally important long polyunsaturated fatty acids.PUFA were nutritionally vital for growth and development integral part of metabolic activities in higher animals and their consumption was well appreciated (Haliloglu et al., 2004). Prostaglandins and thromboxanes were potent arachidonic derivatives play significant role in blood clotting and healing process (Bowman and Rand, 1980). EPA and DHA had crucial role in preventing cardiovascular problems and side effect caused due to prostaglandin mediated inflammation (Connor, 2000). Long chain fatty acids were essential for growth and development of infant. Omega -3 PUFA like DHA plays vital role in developmental stages of brain and retina during pre and post pregnancy (Rogers et al., 2013). Result of the present study reveals the significance of fatty acid profile of U. duvauceli.

Table 4: Fatty acid composition (% of fatty acids in terms of total fatty acids) of U. duvauceli

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>U. duvauceli</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:0 (Myristic acid)</td>
<td>2.25±0.13</td>
</tr>
<tr>
<td>C 16:0 (Palmitic acid)</td>
<td>21.63±0.02</td>
</tr>
<tr>
<td>C 18:0 (Stearic acid)</td>
<td>5.24±0.15</td>
</tr>
<tr>
<td>C 20:4 (Arachidonic acid)</td>
<td>1.1±0.02</td>
</tr>
<tr>
<td>C 22:5 (Eicosapentaenoic acid)</td>
<td>30.12</td>
</tr>
<tr>
<td>C 20:5 (Eicosapentaenoic acid)n-3</td>
<td>1.39±0.09</td>
</tr>
<tr>
<td>C 22:6 (Docosahexaenoic acid)</td>
<td>0.59±0.12</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>1.98</td>
</tr>
<tr>
<td>C 20:4(Arachidonic acid) n-6</td>
<td>7.31±0.03</td>
</tr>
<tr>
<td>C 20:5(Eicosapentaenoic acid)n-3</td>
<td>11.96±0.07</td>
</tr>
<tr>
<td>C 22:6(Decosahexaenoic acid)n-3</td>
<td>48.53±0.16</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>67.9</td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for three replicates.

Table 5: Fatty acid n-3/n-6 and polyunsaturated/saturated fatty acid ratio of U. duvauceli

<table>
<thead>
<tr>
<th>Species</th>
<th>n-3/n-6 ratio</th>
<th>P/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>U. duvauceli</td>
<td>8.27</td>
<td>2.24</td>
</tr>
</tbody>
</table>

P/S, polyunsaturated/saturated fatty acid ratio. n-3 -omega 3 fatty acid n-6- omega 6 fatty acid.
3.4 Mineral composition

Minerals were necessary for physicochemical processes as well as metabolic activities of the body (Soetan et al., 2010) (42). Macro and micro minerals profile of the sample is presented in Table 6 and 7, respectively. Among the macro minerals, potassium showed highest content followed by sodium and calcium. Sodium and potassium ATPase was actively involved in sodium and potassium transport across the cells. Calcium and phosphorous were essential for energy metabolism, signal transduction and bone homeostasis (Ha and Bhagavan, 2011) (20). As in the case of micro minerals, magnesium content showed highest proportion and copper showed least quantity. Santos et al., (2013) (38) reported better quantity of macro minerals (sodium, potassium, magnesium, and calcium) and trace minerals (iron, zinc, cadmium, and copper) in Japanese common squid (Todarodes pacificus). However, trace minerals content in seafood depends on various factors such as nourishment sources, biological differences, seasonal factors and environmental conditions (Fallah et al., 2009) (14).

Table 6: Macro minerals profile (wet basis) (g/100g) of U. duvauceli

<table>
<thead>
<tr>
<th>Macro mineral</th>
<th>U. duvauceli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.102±0.05</td>
</tr>
<tr>
<td>K</td>
<td>0.189±0.03</td>
</tr>
<tr>
<td>Ca</td>
<td>0.015±0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for three replicates

Table 7: Micro minerals profile (wet basis) (mg/100g) of U. duvauceli

<table>
<thead>
<tr>
<th>Micro minerals</th>
<th>U. duvauceli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>2.88±0.8</td>
</tr>
<tr>
<td>Zn</td>
<td>1.03±0.09</td>
</tr>
<tr>
<td>Mg</td>
<td>163.01±16.02</td>
</tr>
<tr>
<td>Cu</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for three replicates

4. Conclusion

Biochemical profiling of Indian squid (U. duvauceli) revealed significant composition of essential nutrients such as proximate contents (80.47% of moisture, 17.5% of protein, 0.52% of fat and 1.13% of ash), essential amino acids (leucine and tryptophan) and essential minerals (sodium, calcium, magnesium and potassium). Generally, essential fatty acids had great role for the determination of the level of nutritional quality of seafood. In this study essential fatty acids such as DHA, EPA and arachidonic acid were observed at significant level. Moreover, amino acids such as essential amino acid, acidic amino acid, neutral amino acid, sulphur amino acid and aromatic amino acid also indicates its nutritional significance. Commendable quantities of biochemical and nutritional content in U. duvauceli signify the appropriateness of this moderately exploited resource as an essential nutrients for nutritionally deprived population.

5. Conflict of interest statement

The authors declare that there are no conflicts of interest.

6. Acknowledgements

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