ABSTRACT

Cephalopods are valuable in fisheries and are consumed as seafood in various cuisines around the world. A study was conducted to know the proximate compositions (protein, ash, fat, carbohydrate and moisture) and minerals (calcium, phosphorus and iron) of cephalopods (octopuses, squids, and cuttlefishes) of Bangladesh. Three species, cuttlefish (*Sepiella inermis*), squid (*Uroteuthis duvauceli*) and octopus (*Cistopus taiwanicus*) were selected on the basis of their availability. The average protein was estimated 16.14%, 15.04%, and 14.68% for Cuttlefish, Squid, and Octopus, respectively whereas the amount of carbohydrate was 0.59%, 0.71%, and 0.53% for the three species. The ANOVA test of the findings of the proximate components showed no significant differences among the three groups (p < 0.05). All the three cephalopods have shown quite the same amount of protein (16.14%, 15.04%, 14.68%) as in commonly consumed fish (rui, catla) and shrimp. The findings suggest that these organisms serve as outstanding sources of protein while containing minimal amounts of fat. The lower amount of fat of cephalopods also proves them leaner than other groups in the comparison. Thus, cephalopods can be a promising source of animal protein in Bangladesh. Both the octopus and cuttlefish showed a good amount of calcium and phosphorus, while the squid proved to be an ideal source of iron. This study proposes to investigate the potential of other cephalopods in the human diet further by undertaking a more thorough analysis that includes all necessary micro and macronutrients.

KEYWORDS: Cephalopod, cuttlefish, squid, octopus, nutrient contents, Bay of Bengal

Introduction

Seafood products belong to the most demanding sustenance commodities in the world that contain higher nutritional value. Due to the population growth and awareness among the consumers’ regarding balanced food items, the seafood trade has drastically increased in the last couple of decades (FAO, 2016). Cephalopods includes various fascinating creatures such as octopuses, squids, cuttlefishes, and nautiluses. They are known for their advanced intelligence among invertebrates and are highly adapted to their aquatic environments. They are undoubtedly an essential seafood source for human consumption. A recent study showed that in the last 60 years cephalopod populations have increased due to the changing ocean environment (Doubleday et al., 2016). The increasing demand for cephalopods in the global market is mostly because of the increased awareness about their nutritional qualities (Lee, 1994; Okuzumi and Fuji, 2000). They have well palatability and high nutritional value (Zlatanos et al. 2006; Ozogul et al. 2008). Japan, Spain, South Korea, Italy, Portugal, Taiwan, China, and Hawaii are the major consumers of cephalopods and their yearly global production exceeded 3.5 million tons (Shenoy, 1988; FAO, 2016). In Bangladesh, cephalopods are exploited in a small quantity as a bycatch of bottom trawl, shrimps trawl and marine set bag net (MSBN) rather than any specialized fishing gear. Proximate compositions and mineral analysis of cephalopods can provide nutritional value information. The cephalopods were accounted to be a rich source of long-chain n-3 polyunsaturated fatty acids, essential amino acids, antioxidants and minerals (Zlatanos et al., 2006). Cephalopods were found to possess a greater protein content, with over 80 percent of their entire body weight being comprised of edible parts suitable for human consumption (Lee, 1994). This has noteworthy implications for the market appeal of cephalopods, particularly when compared to shellfish, which typically have around 40 percent edible content, and teleost, with a maximum of 75 percent (Kreuzer, 1984). An attempt was made to analyze the proximate compositions and mineral of cephalopods (octopuses, squids, and cuttlefishes) of Bangladesh.
Materials and Methods

Sample Collection and identification

The targeted cephalopod specimens were collected from the landing center of Cox’s Bazar (21.400906 N, 91.845397 E) and Chittagong (22.3285 N 91.8482 E and 22.3306 N 91.8433E). The specimens were identified following Roper, 1984 and Ahmed et al., 2008. The selected species were cuttlefish (Sepiella inermis), squid (Uroteuthis duvauceli) and octopus (Cistopus taiwanicus) which are available and largely exploited among cephalopod species.

Sample preparation: From fresh samples, 100g muscle of each were taken in a falcon tube and proceeded for nutrient analysis. Only the edible parts of the cephalopods, such as the whole body of cuttlefish and squids (except viscera) and arms of octopus were taken. The analyses were carried out from outsource (Fish Technology Research Section of the Institute of Food Science and Technology, Bangladesh Council of Scientific & Industrial Research). Proximate analysis was carried out according to the standard procedure of The Association of Official Analytical Chemist (William, 2005).

Determination of moisture content

Three grams of the sample were meticulously spread evenly across the dish and subjected to a 3-hour drying period at 105°C. Once the drying process was completed, the dish, along with a partially covered lid, was moved to the desiccator to cool. Finally, the dish with the dried sample was weighted and calculated the weight of sample after drying.

\[
\text{Moisture (\%)} = \frac{\text{weight (g) of the sample before drying} - \text{weight (g) of the sample after drying}}{\text{weight (g) of the sample before drying}} \times 100
\]

Determination of protein content

Indicator solution: Mixture of 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol).

A sample of weighing 0.5 to 1.0 grams was carefully placed into a digestion flask with 5 grams of Kjeldahl catalyst and 200 ml of concentrated \( \text{H}_2\text{SO}_4\). The flasks were then positioned at an incline and gently heated until frothing ceased, after which they were boiled vigorously until the solution became cleared. Once cooled, 60 ml of distilled water was cautiously introduced. Without delay, the flask was connected to the digestion bulb on the condenser, with the condenser’s tip immersed in standard acid and 5-7 drops of a mixed indicator in the receiver. The flask was rotated to ensure thorough mixing before being heated until all \( \text{NH}_3 \) was distilled. Subsequently, after removing the receiver, the condenser’s tip was washed, and the excess standard acid distilled was titrated using a standard NaOH solution.

\[
\text{Protein (\%)} = \frac{(A-B) \times N \times 14.007 \times 6.25}{W}
\]

Where A = volume (ml) of 0.2 N HCl used sample titration
B = volume (ml) of 0.2 N HCl used in blank titration
N = Normality of HCl
W = weight (g) of sample
14.007 = atomic weight of nitrogen
6.25 = the protein-nitrogen conversion factor for fish and its by-products

Determination of ash content

Crucible and its lid underwent a process of heating in the furnace at 550°C overnight and cooled in a desiccator for approximately 30 minutes. Approximately 5 grams of the sample were carefully placed into the crucible, and the ensemble was heated at 550°C overnight with the lid partially covered. The final step involved cooling the crucible and its contents in the desiccator. The ash, along with the crucible and lid, was then weighed when the sample had transformed to a gray color.

\[
\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100
\]

Determination of fat content

Fat content was determined following Soxhlet method (McNichol et al., 2012). Approximately 3-5 grams of the sample were weighed onto a paper filter and transferred into an extraction tumbler and placed into a Soxhlet apparatus. About 250 ml of petroleum ether was filled into the bottle, which was then heated at 105°C on a mantle. The Soxhlet apparatus was connected, and the sample underwent heating for approximately 14 hours with a heat rate of 150 drops per minute. After this drying process, the bottle with a partially covered lid was transferred to a desiccator to cool. After reweighing the bottle and its dried content weight of sample was found subtracting empty tumbler.

\[
\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100
\]

Determination of Carbohydrate content

The content of total carbohydrates was determined after calculating the rest of the parameters of proximate analysis.

\[
\text{Carbohydrate (\%)} = 100 - (\% \text{moistures} + \% \text{ashes} + \% \text{lipids} + \% \text{proteins})
\]

Determination of Minerals component

The presence of iron, calcium and phosphorus per 100mg were evaluated. To obtain this, firstly the samples were washing and the ash was dissolved with deionized water and concentrated hydrochloric acid in a standard flask. Then the solution was analyzed for detecting the composition of iron and calcium by Atomic Absorption Spectrophotometer (Shimadzu type AA 7000). The determination of Phosphorus was conducted by using a colorimetric spectrophotometer, which was described by Reitz et al., 1987.

Statistical analysis

The expressed results represent the average of three measurements, and the data were displayed as mean ± standard deviation. To assess variations among the species, a one-way ANOVA was conducted, with significance level at P < 0.05.

Results and Discussion

The present study provided proximate compositions and the quantity of three vital minerals (Calcium, Phosphorus and Iron) of three commercially important Cephalopod species available in the Bay of Bengal. The species were Cuttlefish Sepiella inermis, Squid Uroteuthis duvauceli and Octopus Cistopus taiwanicus. Edible muscle from these species was utilized to conduct this study.
Proximate Composition
From time immemorial, Cephalopods have been considered as a dietary component due to their nutritive value. The proximate analysis of our study has shown a promising value in every parameter that is similar to the proximate analysis of cephalopods by Zlatanos et al., (2006). The average protein was found 16.14%, 15.04%, and 14.68% where ash was 0.45%, 0.38%, and 0.73% for cuttlefish, squid, and octopus, respectively. In terms of fat composition, cuttlefish, squid, and octopus exhibited small amounts at 1.6%, 1.73%, and 2.4%, respectively. The carbohydrate content (%) on the other hand, was determined as 0.59%, 0.71%, and 0.53% for cuttlefish, squid, and octopus, respectively. The moisture content (%) for the three cephalopods was 81.19%, 82.14%, and 81.66%, respectively. There was no significant differences in the proximate parameters among the three groups through one-way ANOVA test (p<0.05). All the three cephalopods have shown quite the same amount of protein as three commonly consumed fish and one shrimp species. The findings suggest that these organisms serve as outstanding sources of protein while containing minimal amounts of fat. The lower amount of fat of cephalopods also proves them leaner than other groups in the comparison. Thus, cephalopods can be a promising source of animal protein in Bangladesh.

Mineral Composition
Minerals are an essential part of the diet of any living organism, as it is required by the body to carry out various important metabolic functions. Humans are not indifferent in this case. Along with other vital minerals, calcium, iron, phosphorus act as the active components of hormones, enzymes and proteins in every age group and physical condition of a human body. Minerals have undoubted roles to coordinate healthy metabolic systems by ensuring a strong immune system and keeping the vital organs active and well-nourished. Deficiency of these minerals disrupts the basic function of the body, expresses deficiency syndromes, hinders growth and activity even poses the risk of life-threatening issues in an individual. Being an active marine predator, cephalopods deposit a pleasant amount of minerals in their body incorporated through their diet and direct absorption from saline water through body surface and gills (Lall, 2002). Three essential mineral elements were measured (Table 1). Cuttlefish, squid, and octopus exhibit calcium levels of 433.73 mg, 384.69 mg, and 470.27 mg per 100g, respectively. In terms of phosphorus and iron content, cuttlefish, squid, and octopus contain 413.7 mg, 407.2 mg, and 387.28 mg of phosphorus, and 54.25 mg, 98.58 mg, and 25.12 mg of iron, respectively, per 100g. Both the octopus and cuttlefish showed a good amount of calcium and phosphorus, while the squid proved to be an ideal source of iron.

Calcium: Calcium is a vital micronutrient that needs to maintain good bone and teeth health, for muscle movement and nerve signaling between the brain and every body part. In addition, calcium is used to help blood vessels move blood throughout the body and aids to release hormones and enzymes to carry out the different metabolic functions in the human body (Lall, 2002). According to the National Institute of Health (NIH), depending on the age the dietary need for calcium ranges from 1000-1300 mg/day in mature human beings. Our study finds that the average amount of Ca was 429.56 mg/100 gram of sample.

Phosphorus: Phosphorus is another essential nutrient element that plays a role as the structural component of bones, teeth, DNA, RNA, cell membrane, and ATP. In addition, phosphorus plays key roles in the regulation of gene transcription, activation of enzymes, maintenance of normal pH in extracellular fluid, and intracellular energy storage (Lall, 2002). The Recommended Dietary Allowance (RDA) of phosphorus is 700-800 in adult. The average amount that has been concluded in this study was 402.73 mg/100g of the sample which meets the 57.53% of RDA. Any food item having a Ca/P ratio of 0.5 is considered as standard (Chakraborty and Joseph, 2015). In our study, the Cuttlefish, Squid and Octopus have shown a Ca/P ratio of 1.05, 0.94 and 1.21 respectively. On average, it was 1.07 and thus might be considered a good source of these essential minerals.

Iron: Iron is an essential element of both hemoglobin and myoglobin. These proteins support muscle metabolism and maintain healthy connective tissue. It is crucial for physical growth, cellular function, neurological development, and the synthesis of some hormones. In 2002, the WHO characterized Iron deficiency Anemia as one of the 10 leading risk factors for disease around the world. Insufficient iron intakes during pregnancy increase an infant’s risk of low birth weight, premature birth, low iron stores, impaired cognitive and behavioral development (WHO, 2008). The amount of iron recommended per day is 8.7 mg for men over 18 and 14.8 mg for women aged 19 to 50, while we found 54.25 mg in cuttlefish, 98.58 mg in squid and 25.12 mg calcium per 100g of Octopus.

### Table 1. Mean proximate and mineral composition of three cephalopods and comparison with Hilsa, Catla, Rohu and Harina Shrimp from Bogard et al., 2015.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cuttlefish</th>
<th>Squid</th>
<th>Octopus</th>
<th>Hilsa</th>
<th>Catla</th>
<th>Rohu</th>
<th>Harina shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)*</td>
<td>16.14</td>
<td>15.04</td>
<td>14.68</td>
<td>16.4</td>
<td>14.9</td>
<td>18.2</td>
<td>17.6</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.45</td>
<td>0.38</td>
<td>0.73</td>
<td>1.4</td>
<td>1.0</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>1.6</td>
<td>1.73</td>
<td>2.4</td>
<td>18.3</td>
<td>0.7</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Carbohydrate (%)*</td>
<td>0.59</td>
<td>0.71</td>
<td>0.53</td>
<td>3.7</td>
<td>14.2</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>81.19</td>
<td>82.14</td>
<td>81.66</td>
<td>60.2</td>
<td>84.1</td>
<td>77.0</td>
<td>79.5</td>
</tr>
<tr>
<td><strong>Mineral contents (mg/100g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>433.73</td>
<td>384.69</td>
<td>470.27</td>
<td>220</td>
<td>210</td>
<td>51</td>
<td>550</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>413.7</td>
<td>407.2</td>
<td>387.28</td>
<td>300</td>
<td>260</td>
<td>210</td>
<td>290</td>
</tr>
<tr>
<td>Iron</td>
<td>54.25</td>
<td>98.58</td>
<td>25.12</td>
<td>1.9</td>
<td>0.83</td>
<td>0.98</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* No significant differences among the three groups (ANOVA test, p < 0.05)
The result of nutrient composition from our study was compared to three commonly consumed fish and one shrimp species in Bangladesh. The species included in this comparison is Hilsa (Tenualosa ilisha), Catla (Gibelio catla), Rohu (Labeo rohita) and Harina shrimp (Metapenaeus monoceros) from Bogard et al. (2015) (Table 1).

The amount of protein present in cephalopods are almost similar to fish and shrimps, while the fat content is quite lower. In the context of minerals, the data from table 1 exhibits that the calcium content in cephalopods is two to eight times larger than fishes on average. Even the phosphorus and iron value are also on the higher level.

The findings of this nutritional analysis study are poised to offer practical insights for the seafood industry, potentially prompting regulatory bodies to advocate for the inclusion of cephalopod species as a viable and nutritious food source. Promising results from the comprehensive analysis, encompassing all essential micro and macronutrients, to further explore the potential of cephalopods in the human diet.

References