The Quality and Safety Aspects of *Anabas testudineus* (Bloch 1972) and *Oreochromis niloticus* (Linnaeus 1758) Collected from Pond and Open Water Environment, Bangladesh


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Abstract: Study was conducted on the quality attributes of koi (*Anabas testudineus*) and tilapia (*Oreochromis niloticus*). Biochemical analyses showed that moisture, protein, lipid, ash and TVB-N content of *Anabas testudineus* harvested from pond water and open water were 64.03 and 65.02%, 17.03 and 16.95%, 12.13 and 11.82%, 5.87 and 6.30% and 2.24 and 2.63 CFU/g respectively. On the other hand, moisture, protein, lipid, ash and TVB-N content of *Oreochromis niloticus* harvested from pond water and open water were 69.83 and 70.26%, 17.15 and 16.24%, 8.03 and 7.09%, 4.03 and 4.87% and 5.32 and 5.49 CFU/g respectively. Protein contents of both fishes were higher in pond water specimen than open water, which may occur due to the proper feeding in pond culture system. The bacterial load of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from pond water were 2.25×10⁶ CFU/g and 1.84×10⁵ CFU/g whereas in the harvested samples from open water the values were 2.54 × 10⁶ CFU/g and 1.97 × 10⁵ CFU/g respectively. Study found that heavy metal (cadmium and copper) concentrations in *Anabas testudineus* and *Oreochromis niloticus* were higher in the open water than the pond water that may be resulted from pollution in open water environment.

Key words: Biochemical properties • Bacterial load and Heavy metal

INTRODUCTION

The climbing perch (*Anabas testudineus*) or koi is considered an important economic fish species which has high market value and good nutritional profile. It has delicious taste and can be used for patient food. Once, climbing perch or koi was very much abundant in almost all freshwater systems of Bangladesh [1]. The availability of this fish is decreasing from natural system in the recent years. The reasons behind of severe decline of Koi fish are ecological degradation, indiscriminate use of pesticides, destruction of habitats, obstruction of breeding migration and fishing pressure etc. Fisheries biologists are thinking of its cultivation through intensive farming [2]. On the other hand, tilapia is currently having an important impact on poor people in developing countries, both as cultured species in household-management systems and through access to fish produced in informal and formal fisheries [3]. But the culture practice of tilapia varies to a great extent from country to country and even among the different farms. Development of a stimulation model of tilapia production may help researchers as well as farm managers to make decision regarding different inputs use and adoption of management policies for tilapia production in aquaculture ponds. The preservation of aquatic environment and at the same time restoration of heavy metal contaminated water are very essential for sustainable aquaculture development. The present study was undertaken to analyze the proximate composition of *A. testudineus* and *O. niloticus* that were harvested from pond water and open water stock and to evaluate their nutritional quality; to observe the degree of freshness by evaluating the total volatile base nitrogen (TVB-N) and
bacterial counts; and to determine the quality and safety aspects of the fish by determining the contents of heavy metals.

**MATERIALS AND METHODS**

Sampled fishes of tilapia (*Oreochromis niloticus*) and koi (*Anabas testudineus*) were collected from pond water and open water sources. Fish samples were packed tightly in polyethylene bags and stored at low temperature for subsequent studies.

**Proximate Composition:** AOAC [4] method was followed for proximate composition of the *Anabas testudineus* and *Oreochromis niloticus*. Homogeneity of the samples was done by using a blender. All the determinations were made in triplicate. Prior to analysis the *Anabas testudineus* and *Oreochromis niloticus* without any pretreatment were first chopped with large knife in order to make into small pieces.

**Moisture:** Moisture was determined by placing an accurately weighed known amount of ground sample in a pre-weighted porcelain crucible in an electric oven at 105°C for about 24 hours until constant weight was obtained. The loss of moisture was calculated as percent moisture.

\[
\text{Moisture content} (\%) = \frac{\text{Weight of wet material} - \text{Weight of dry material}}{\text{Weight of wet material}} \times 100
\]

**Ash:** About 3-5g prepared sample was taken in pre-weighted porcelain crucible and was placed in muffle furnace at 550°C for 6 hours. Then the crucibles were cooled in desiccators. The average in percentage of each sample of the remaining materials was taken as ash.

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

**Crude Protein:** For the determination of crude protein by kjeldahl method first of all, the fish sample was taken and chopped into small pieces and was ground by grinder. Approximately 1g of sample was taken in a clean kjeldhal flask and 4g of digestion mixture was added along with 25ml of conc. H₂SO₄ by swirling the flask. Then the kjeldhal flask is placed in an inclined position on heating device of kjeldhal apparatus and were heated at 70°C for about 1-1.5 hours. The end point of digestion was indicated by a completely clear and of light blue color solution. The content of the flask was cooled at room temperature and 100ml of distilled water and 25ml of Na₂S₂O₃ were continuously added in each flask and were mixed and cooled. A few glass beads were added in each flask to prevent bumping. Then 100-120ml of 40% NaOH was added in each flask to make the solution sufficiently alkaline. The flask was immediately connected to distilling bulb on condenser. A conical flask containing 50ml of 2% H₂BO₃ with 2 drops of mixed indicator was placed under the condenser against kjeldhal flask to collect the distillate. After completion of distillation (about 100ml distillate) the collected distillates were titrated with standard HCl. The end point was indicated by light pinkish color.

Total nitrogen was calculated by using the following formula:

\[
\text{Nitrogen}(\%) = \frac{\text{ml Acid titrated} \times \text{normality of acid titrated} \times \text{milliequivalent of N}(0.014)}{\text{Weight of sample}} \times 100
\]

\%

of crude protein = Nitrogen% \times 6.25

**Lipid:** Lipid content was determined by soxhlet apparatus using acetone as solvent. Accurately weighed samples (2-3g) were taken in thimbles and were dipped in pre-weighted aluminum cups with acetone. At first boiling was done for 15 minutes and then rising for 25 minutes and finally extraction was done for 10 minutes. After extraction, the aluminum cups were taken out from chamber and acetone was placed in an oven at 100°C for 30 minutes. The cups with lipid was cooled in desiccators and weighed again. The calculated value for lipid content was obtained as percentage sample.

\[
\text{Lipid content} (\%) = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times 100
\]

**Total Volatile Base-Nitrogen (TVB-N):** Exactly 10g of ground sample are weighed, mixed with 90ml of 6% perchloric acid and homogenized for 2 minutes with a blender. 100ml of extract with 4-6 drop phenolphthalein was placed in a kjeldhal flask after placing on the distillation on it and distillation should be continued for more or less 15 minutes. The distillate was collected in the conical flask containing 50ml of 3% boric acid and 1 drop mixed indicator. Distillation confirmed through changing in colour of mixed indicator, i.e. violate to greenish. After distillation the collected distillate was titrated with 0.01N HCl and regarding the violet colour of mixed indicator confirms the end point.
The result can be calculated by the following formula:

\[ \text{TVB-N (mg/100 g sample)} = \frac{\text{ml of titrant} \times 0.014 \times \text{Normality of titrant}}{\text{Weight of sample (gm)}} \times 100 \]

**Determination of Microbial Load:** Plate count agar is a commercial preparation (Hi media, India) that was used for enumeration of viable bacterial load in experimental sample. Accurately weighed and suspended 23.5g of media was mixed in 1000ml distilled water and boiled to dissolve the ingredients completely. The media was then sterilized at 121°C for 15 minutes under 15 lbs /inch² pressure in an autoclave.

**Determination of Heavy Metals:** Collected samples were weighed by electronic balance and 5 ml of di-acid mixture (5ml conc. HNO₃: 3ml 60% HClO₄) were added to each sample. The content mixed for overnight. Samples were then digested, initially at 80°C temperature and later on 150°C for 2 hours. The completion of digestion was indicated by almost colorless material. The brown fumes also cease to exist at completion of digestion. The samples were separately filtered by using an ash less filter paper and volume made up to 25ml with 0.5% HNO₃ which were subjected to analysis by Atomic Absorption Spectrophotometer (HG-AAS, PG-990, PG Instrument Ltd. UK) at Professor Mohammad Hossain Central Laboratory, BAU, Mymensingh, followed the method of Clesceri et al. [6]. The wave length of Cd and Cu is 193.7 nm and 217 nm respectively. The concentration of Cd and Cu in fish samples were calculated by the following formula:

\[ \text{Metal concentration} = \frac{\text{mg/g conc. observed} \times \text{final volume of sample in ml}}{\text{Weight of tissues taken in gm}} \]

**RESULTS AND DISCUSSION**

**Biochemical characteristics of Anabas testudineus and Oreochromis niloticus:** The results of proximate analysis Anabas testudineus and Oreochromis niloticus (moisture, protein, lipid and ash) in wet weight basis and TVB-N content of fishes are shown in table 1.

**Moisture:** Moisture contents of Anabas testudineus and Oreochromis niloticus that were harvested from pond water were 64.03% and 69.83% whereas the moisture contents of Anabas testudineus and Oreochromis niloticus that were harvested from open water were 65.02% and 70.26% respectively. The lowest values of moisture were obtained from pond water and the highest values from open water. Chowdhury [7] reported more or less similar result for the same species. He showed an inverse relationship between fat and moisture. The moisture content of the T. mossambica from the four dams ranged between 69.7 to 76.6%. The high moisture content of the fish sample would increase the deterioration level of fish when kept for a long time. This is because the micro-organisms would be highly active with high moisture content [8]. Similar observations have been reported by Abolude and Abdullahi [9], Otitologbon et al. [10] and Balogun and Adebayo [11].

**Protein:** The protein content is the most important element from the nutritional point of view. The protein contents of Anabas testudineus and Oreochromis niloticus that were harvested from pond water were 17.03% and 17.15% whereas the protein contents of Anabas testudineus and Oreochromis niloticus that were harvested from open water were 16.95% and 16.24%. The lowest values obtained from open water and the highest values from pond water Anabas testudineus and Oreochromis niloticus. It was reported that the protein contents in A. testudineus (14.80%), N. notopterus (19.8%), C. punctatus (19.40%), H. fossilis (22.8%), C. batrachus (18.30%), N. nandus (21.30%) and M. vittatus (18.96%). The variation in protein content may be occurred due to habitat, season and sex and or water quality. The protein contents of the present study were more or less similar to the result of Hossain et al. [12].

**Lipid:** The lipid contents of Anabas testudineus and Oreochromis niloticus that were harvested from pond water were 12.13% and 8.03% whereas the lipid contents of Anabas testudineus and Oreochromis niloticus that were harvested from open water were 11.82% and 7.09%.

### Table 1: Biochemical Characteristics of A. testudineus and O. niloticus

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Ash (%)</th>
<th>TVB-N CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koi</td>
<td>pond water</td>
<td>64.03 ± 0.10</td>
<td>17.03 ± 0.10</td>
<td>12.13 ± 0.08</td>
<td>5.87 ± 0.06</td>
<td>2.24 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>open water</td>
<td>65.02 ± 0.12</td>
<td>16.95 ± 0.08</td>
<td>11.82 ± 0.02</td>
<td>6.30 ± 0.05</td>
<td>2.63 ± 0.06</td>
</tr>
<tr>
<td>Tilapia</td>
<td>pond water</td>
<td>69.83 ± 0.16</td>
<td>17.15 ± 0.06</td>
<td>8.03 ± 0.06</td>
<td>4.03 ± 0.10</td>
<td>5.32 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>open water</td>
<td>70.26 ± 0.14</td>
<td>16.24 ± 0.05</td>
<td>7.09 ± 0.05</td>
<td>4.87 ± 0.10</td>
<td>5.49 ± 0.11</td>
</tr>
</tbody>
</table>

*Mean ± SD, n = 4.
The lowest values obtained from open water and the highest values from pond water. Hossain et al. [12] reported the lipid contents of some selected fishes from Mymensingh ranged from 1.87 to 9.55%, the findings of the present study was within the range. Carbonera [13] reported that the fat content issued as a practical criterion for comparisons between different fish species. Thus, this author considered the fish as fat, when the minimum content of lipids is 10%, semi-fat between 2.5and 10% and lean for values below 2.5%. According to the results, we classified the analyzed fishes as lean.

**Ash:** The ash content of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from pond water were 5.87% to 4.03% whereas the ash content of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from open water were 6.30% and 4.87%. The lowest values obtained from pond water and the highest values from open water. In the present study, the value of ash content of *A. testudineus* was lower than the findings of Chowdhury [7] that the highest value of ash content (6.79±1.26%) in *A. testudineus*. The proximate composition varies with the species, nutritional state, seasonality, age and gonadal conditions [14].

**TVB-N:** The TVB-N content of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from pond water were 2.24 mg/100g and 5.32 mg/100g whereas the TVB-N content of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from open water were 2.63 mg/100g and 5.49 mg/100g respectively. The lowest values obtained from pond water *Anabas testudineus* and *Oreochromis niloticus* and the highest values from open water. De and Hazera Nazrul [15] reported that the freshwater fish *Anabas testudineus* (Climbing Perch) have TMA content of 1.5-5.2mg TMA-nitrogen/100g flesh and storage for 7 days at a temperature of 41ºF causes an increase of TMA-nitrogen unassisted by any increase of TVB nitrogen. They also indicated that TVB-nitrogen is not suitable quality index in assessing the freshness quality of freshwater fish of Bangladesh. TVB-N contents of *Oreochromis niloticus* showed slow increase during the early stages of storage. At later stages, the level of increase rate more rapidly during ambient storage. TVB-N value for ice-stored samples increased from 5.51mg/100g to an acceptable value of 22.53 mg/100g in 15 days and finally to a rejection value of 38.75 mg/100g at the end of 21days storage period. The value of 30-35mg TVB-N/100g is recommended for fresh fish acceptability [16]. Connell [17] found the results corroborate those of organoleptic assessment and microbial assessment in which *Oreochromis niloticus*, were in acceptable condition for 15 days in ice. Acceptability was about 9 hrs. at ambient temperature. Increase in TVB-N with the lapse of storage, particularly towards the end of storage period may be attributed to bacterial spoilage after the bacterial population has grown. TVB-N is normally low during the edible storage period, increased levels were found in fish near rejection levels. TVB-N might be considered as a good indicator of freshness in ice.

**Bacteriological Characteristics of *Anabas testudineus* and *Oreochromis niloticus***: The total aerobic plate count expressed as colony forming unit in one gram of sample (CFU/g) of the representative samples of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from pond water and open water determined by standard plate count method on plate count agar media. The bacterial load of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from pond water were 2.25 × 10^6 CFU/g and 1.84 × 10^5 CFU/g whereas the microbial load of *Anabas testudineus* and *Oreochromis niloticus* that were harvested from open water were 2.54 × 10^7 CFU/g and 1.97 × 10^6 CFU/g respectively (Table 2). The lowest value obtained from pond water *Anabas testudineus* and *Oreochromis niloticus* and the highest value from open water *Anabas testudineus* and *Oreochromis niloticus*. *Anabas testudineus* was proved to be sensitive to *A. hydrophila* as shown by their mortality to 100%, at a dose of 6.7 × 10^6 CFU/fish and 60%, at a dose of 6.7 × 10^5 CFU/fish. Post infection days of mortality were observed to be from 3-9 days and 5-14 days respectively [18].

![Fig. 1: Cadmium and copper concentrations in koi and tilapia (pond and open water)](image_url)
Detection of Heavy Metals in *Anabas testudineus* and *Oreochromis niloticus*: The results of heavy metal (cadmium and copper) concentrations in koi (*Anabas testudineus*) and tilapia (*Oreochromis niloticus*) that were collected from pond water and open water sources are shown in figure 1. The results of heavy metal concentrations in *Anabas testudineus* and *Oreochromis niloticus* from pond water were Cadmium 0.39µg/g and 0.37µg/g and Copper 0.40µg/g and 0.36µg/g respectively, the concentrations of heavy metal in *Anabas testudineus* and *Oreochromis niloticus* from open water were Cadmium 0.77µg/g and 0.96µg/g and Copper 0.58µg/g and 1.87µg/g respectively, which were within the acceptable level for human consumption. WHO (µg/g) suggests the maximum limits of Mn, Cu, Zn, Pb and Cd for fish are 1µg/g, 30µg/g, 100µg/g, 2µg/g, 1µg/g and FAO suggests the maximum limits of Cu, Zn, Cd for fish are 10µg/g, 100µg/g and 0.2µg/g [19].

**CONCLUSION**

The quality aspects of two species of commercially important fish *Anabas testudineus* and *Oreochromis niloticus* of pond and open water were evaluated by examining the physical and organoleptic properties, chemical composition, total volatile base-nitrogen (TVB-N), total bacterial load and heavy metal of the samples. On the basis of the objectives and achievement of the study and other related consideration, conclusion can be made as the protein contents of koi and tilapia were minimum and moisture contents were high. The lipid and ash contents of these products were within the limit; total volatile base-nitrogen (TVB-N) and the bacterial count of pond and open water of *A. testudineus* and *O. niloticus* were within acceptable level; and values of heavy metal concentrations in *A. testudineus* and *O. niloticus* obtained from pond and open water showed an acceptable level for human consumption.

**REFERENCES**