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Biochemical and Microbiological Quality Evaluation of Marine Fishes and Shrimps Available in the Local Market of Rajshahi City

Mst. Tahsin Farzana, Md. Farukh Abdullah, Isma Khatun Liza, Md. Rashedul Kabir Mondol and Md. Tariqul Islam*

Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh.

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ABSTRACT

The current study was conducted to assess the quality of marine fishes and shrimps available in the local market of Rajshahi City. Four marine fishes; Hilsa (*Tenualosa ilisha*), Rupchanda (*Pampus argenteus*), Skipjack tuna *(Katsuwonus pelamis),* Bombay duck or Loitta *(Harpodon nehereus)* and two marine shrimps; Bagda (*Penaeus monodon*), Harina (*Metapenaeus monoceros*) were selected to investigate biochemical and microbiological parameters such as moisture content, pH, free fatty acid (FFA), thiobarbituric acid reactive substances (TBARS) and bacterial count by aerobic plate count (APC). The highest moisture content was found in Loitta (84.97%) whereas the lowest moisture content was found in Hilsa (73.04%). The pH values were in the range of 6.26 to 7.14 and FFA values ranged from 0.18% to 0.35% in all samples. The highest TBARS value was found 0.55 mg MDA/kg in Loitta fish, and the lowest value was 0.01 mg MDA/kg in Bagda shrimp. APC of Hilsa, Rupchanda, Tuna, Loitta fish, Bagda and Harina shrimps were 5.11, 6.09, 6.82, 7.11, 6.97 and 5.51 log CFU/g, respectively. Based on the assessment, almost all the fishes and shrimps were found within permissible limits, except the Loitta fish, whose APC was high. The overall quality of marine fishes and shrimps available in the local market of Rajshahi city was found fit for human consumption.

Introduction

Fish is one of the most important sources of animal protein and is widely accepted as a good source of animal protein and other essential nutrients for the maintenance of a healthy body (Ravichandran et al., 2012). Fish is the second most important agricultural crop, and its production supports the livelihoods and employment of millions of people in Bangladesh (Belton et al. 2011). Furthermore, 18.1 kg of fish is consumed per person per year, greatly exceeding the frequency of consumption of any other animal-source meal (Belton and Thilsted, 2014). Some essential fatty acids such as eicosapentaenoic acid (EPA or 20:5ω3) and docosahexaenoic acid (DHA or 22:6ω3) are also available in fish (Njinkoué et al., 2002; Rasoarahona et al., 2005), and these amino acids are reported to play an important role in the

***Address of correspondence**
Department of Fisheries
University of Rajshahi, Rajshahi-6205, Bangladesh.
E-mail: tariqul.fish@ru.ac.bd (Md. Tariqul Islam)

human body such as prevention of cardiovascular diseases, rheumatoid arthritis, cancers, and inflammation (Clandinin et al., 1997; Raatz et al., 2013). Fish muscle and bones are good sources of essential minerals (Ersoy and Özeren, 2009; Nurnada et al., 2013). Minerals play an important role in maintaining body functions in humans, such as maintaining acid–base balance, aiding in hemoglobin formation (Duran et al., 2010). It also controls the water balance and bone and teeth structure formation and catalyzes many metabolic reactions (Mendil et al., 2010). In Bangladesh, the fisheries sector provided 3.57% to the national GDP and 26.50% to the agricultural GDP and 1.24% to foreign exchange earnings by exporting fish products in 2020-2021 (DoF, 2022). Fisheries is the second largest employing sector, and about 11.6 million people depend on fishing for their livelihood directly or indirectly, which is more than 11% of the total population of Bangladesh (DoF, 2022).

Fish and fishery products differ from all other foods in terms of harvesting method, product fragility during transport, temperature dependency, and species diversity. To ensure food safety, determining the quality of fish is becoming popular in modern research and development (Cheng, 2015). For a consumer, 'eating quality' of a fish is associated with freshness (Nielsen et al., 1997; Olafsdottir et al., 1997). Therefore, we must ensure the freshness of fish taken from the market.

In Bangladesh, the Bay of Bengal is the main source of marine fish and supports multi-species of marine fisheries, including 475 species of fish and 24 species of shrimp (Murshed-E-Jahan et al., 2014). The fisheries sector contributes 3.57% of the national GDP and one-fourth (26.50%) of the total agriculture GDP of Bangladesh (DoF, 2022). Hilsha (Ilish), shrimp and prawn, Bombay duck (Loitta), and Jewfish are the most dominant species among other captured fish species from the Bay of Bengal (DoF, 2019). Four shrimp species are also exported to different countries, and ten species are commercially exploited (Mondal, 2013).

The majority of the nation's estuarine and marine catch fisheries are based on artisanal fishing, which is thought to account for around 95% of all marine fish production (DoF, 2002). Quality fish and fishery products are important for domestic consumption as well as for export to the international market. Unfortunately, nearly one-third of caught freshwater or marine fishes and shrimps are lost before they reach consumers. In addition to the physical loss, fish quality also deteriorates by 15 per cent (FAO, 2021). Studies showed a very high level of post-harvest loss when fishery products were being prepared for processing, storing and transportation (Nowsad, 2005, 2006). Loss of quality can be caused by a variety of reasons, including inadequate packaging, improper handling during loading or unloading, wrong processing methods, and processing delays. In the case of marine fishes, the average post-harvest loss per metric ton of marine fish was 11.67%, and the highest weight loss per metric ton was found for Bombay duck (15.55%) (Nowsad, 2004). About 20 per cent of the marine fish and shrimp that were landed in Cox's Bazar were degraded by up to 80 per cent of their original quality before being put on the truck for long-distance transportation due to local losses in the wet fish distribution chain and processed products (Nowsad, 2004).

There have been complaints about the marine fish and shrimp offered at the local market, particularly in the market far from the coastal region. As the raw marine fishes and shrimps are not packaged properly, there are no information regarding packaging, nutritional value, manufacturing date or expiration date that may assure customers about the product's quality and shelf life. Therefore, analysis of the quality parameters of the marine fishes and shrimps from the open market is essential to satisfy the consumers for buying the marine fishes and shrimps. Considering the above-mentioned details, this research work was undertaken to study the biochemical and microbiological quality of marine fishes and shrimps available in the local open market of Rajshahi City. This study can be beneficial for promoting marine fishes in the market and increasing their demand among people by providing nutritional security.

Materials and methods

Sample Collection

Four marine fish species; Hilsa (*Tenualosa ilisha*), Rupchanda (*Pampus argenteus*), Skipjack tuna (*Katsuwonus pelamis),* Bombay duck (*Harpodon nehereus*) and two shrimps; Bagda (*Penaeus monodon*), Harina (*Metapenaeus monoceros*) were collected from the open fish

market of Saheb Bazar, Rajshahi city, Bangladesh. Iced marine fishes and shrimps were bought from the Saheb Bazar fish market. The samples were packed tightly in polyethene bags separately and brought under icing conditions to the quality control laboratory in the Department of Fisheries, University of Rajshahi, Bangladesh.

Biochemical and Microbiological Analysis

The biochemical parameters; moisture content, pH, Free Fatty Acid Value (FFA) Thiobarbituric Acid Reactive Substance (TBARS) and microbiological parameters; Aerobic Plate Count (APC) were evaluated in the laboratory to know the quality parameters of four marine fishes and two marine shrimps.

Moisture Content

The moisture content was determined using the AOAC (1980) standard procedure. The known weight of each sample (10 ± 0.5 g) was placed in a separate crucible and oven dried at 105°C until a constant weight was attained. To express the result as a percentage of the sample, the following formula was utilized.

$$\text{Moisture content (\%)} = \frac{(\text{Sample wt.} - \text{Dry sample wt.})}{\text{Sample wt.}} \times 100$$

pH Value

The pH of the samples was measured using a digital pH meter (HANNA, USA). At first, pH meter was calibrated using pH buffer solutions (7.00 ± 0.01 and 4.01 ± 0.01 at room temperature). For this measurement, 10 g of minced sample was mixed with 50 mL of distilled water. The homogenate's pH was measured using a pH meter.

Free Fatty Acid (FFA) Value

The free fatty acids (FFA) were determined according to the method of Karim et al. (2017). At first, 2g of each sample was weighed, then homogenized with 40 mL of chloroform and filtered with Whatman filter paper no 541. The final volume was made up to 40 mL by adding chloroform. Then, 25 ml of chloroform extract was mixed in a conical flask with 25 ml of neutral ethanol and 1 mL of phenolphthalein (1%) solution. With repeated shaking, the mixture was titrated with NaOH to the desired endpoint, and the volume of NaOH was recorded. The following formula was used to express the result as a percentage of the oleic acid of the sample.

FFA (% oleic acid) = V×N×0.282×100/W; Where, V= titration (mL) of NaOH, N=Normality of NaOH and W= fish sample (g) in 25 mL sample.

Thiobarbituric Acid Reactive Substance (TBARS) Value

TBARS values were calculated using the Park et al. method (2007). A homogenizer was used to combine 20 grams of the sampled fish with 50 mL of 20% trichloroacetic acid (in 2M phosphoric acid) at 10000× *g* for two minutes. The resulting slurry was diluted to 100 mL with HPLC grade water and then homogenized and filtered through Whatman no. 1. Five mL of filtrate was then taken in a test tube, followed by 5 mL 2-thiobarbituric acid (0.005 M in HPLC grade water). The test tube was thoroughly shaken and left at room temperature for 15 hours in the dark. Using a spectrophotometer (UV-visible spectrophotometer, UV-1601, Shimadzu, Japan), the reactive chemicals were detected at 530 nm. TBARS values were calculated as follows: TBARS value (mg malonaldehyde/kg) = optical density (OD) ×5.2

Aerobic Plate Count (APC)

APC reported as colony forming units (CFU/g) was calculated on plate count agar using the serial dilution procedure described by APHA (1992) method. Decimal dilution sample was prepared using peptone physiological saline (PPS). Using a micropipette, 1 mL of prepared, well-shaken, diluted sample was first transferred aseptically to empty plates, and then warm agar (44 ±1 °C) was poured onto the plates. Colonies formed after 48±2 hours of incubation at 35°C, and only the plates with 30-300 colonies were counted using a colony counter. Microbiological data were transformed into logarithms of the number of colony forming units (log CFU/g). The following formula performed the result-

$$\mathrm{N} = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

Where, N = number of colonies per mL or g of product (CFU/g); ∑ C = sum of all colonies on all plates counted; n_1 = number of plates in first dilution counted; n_2 = number of plates in second dilution counted; d = dilution from which the first counts were obtained

Statistical Analysis

All values were expressed as Mean ± SD. For laboratory experiments, differences among species were estimated using one-way ANOVA

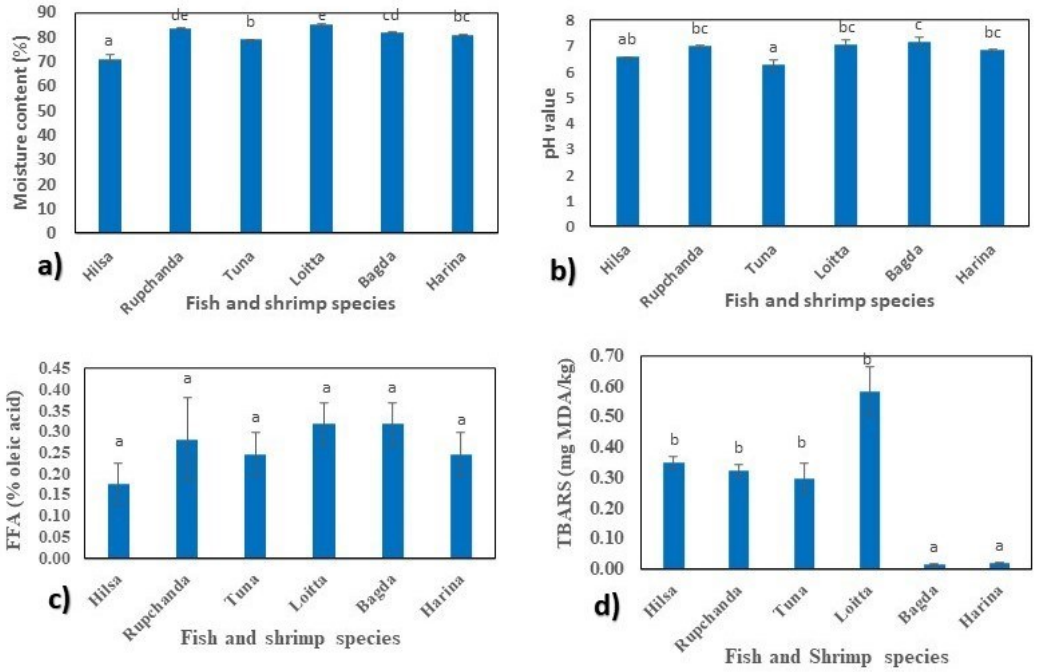

Fig. 1. a) Moisture content (%), b) pH value, c) Free fatty acid (FFA; % oleic acid) and d) Thiobarbituric acid reactive substance (TBARS; mg MDA/kg) of fish and shrimp species collected from the local market in Rajshahi City.

with the application of the Tukey test using SPSS 20. P values of 0.05 or lower were considered significant.

Results and Discussion

The current study aimed to evaluate the biochemical and microbiological quality of iced marine fish and shrimp procured from Rajshahi's local fish market. To determine the overall quality, the following parameters were observed.

Moisture content

Moisture content is one of the essential quality indicators for marine fish and shrimp. Microbial activity in food is influenced by its composition, including the amount of water and nutrients, as well as by physical factors like temperature and the environment (Gram et al., 2002). The number of bacteria in food products and moisture content is closely related. Fish and shrimp are excellent substrates for the growth and reproduction of microorganisms. In the present study, the moisture content of Hilsa, Rupchanda, Tuna and Loitta fish were 73.04%, 83.38%, 79.01% and 84.97%, respectively and for Bagda and Harina shrimp, it was 81.73% and 80.98%, respectively (Fig. 1a). Statistically, there were significant differences ($p<0.05$) between four marine fishes and two marine shrimps. However, the highest moisture content (around 85%) was observed in collected Loitta and the lowest in Hilsa fish (73.04%). This finding suggested that marine fish loitta contain more moisture than other species.

According to Suseno et al. (2015), the moisture content of fresh Skipjack tuna was 71.76%, whereas Matsumoto et al. (1984) found 70.40%, which is lower than that of the present study. Generally, these marine fish are stored for 1-2 weeks to be sold in the local market from the catch area (the Bay of Bengal) to retail consumers. Differences in moisture content may be induced by species, biota age, ambient variations, and organism freshness level (Ayas and Ozugul, 2011). If fishery products are handled properly, high moisture levels might prevent them from decaying quickly. Rupsankar (2010) reported that Bombay duck or Loitta has a high moisture content (89%), and Hossain et al. (2019) also reported higher moisture content in fresh loitta fish ranging from 88.51% to 90.65%. These values

are higher than that of the present study in Loitta fish. On the other hand, Azam et al. (2004) reported that the moisture content of Hilsha was 67.21%, which is lower than the value of the present study.

pH value

The pH value is a crucial indicator for determining how fresh a variety of foods are. In the current research, the estimated pH value was below or close to neutral pH. The average pH value was determined as 6.59, 6.99, 6.26 and 7.06, 7.14 and 6.84 for Hilsa, Rupchanda, Tuna and Loitta fish, Bagda and Harina shrimp (Fig. 1b). A greater pH value was seen in Bagda shrimp and lower value in Tuna fish (Fig. 1b). The acceptable pH value range is 6.8-7 (Metin et al., 2001). There were also significant differences ($p<0.05$) found between four marine fishes and two marine shrimps. Reza et al. (2009) found pH value of 7.2 in iced Ribbon fish and 7.98 in iced Big Eye Tuna, whereas Duarte et al. (2020) found a pH value 6.57 in iced Sardine and 7.27 in iced Indian Mackerel. On the other hand, Hossain et al. (2019) found that the pH value increased gradually from 6.2 to 7.1 in Bombay duck or loitta during ice storage.

Free Fatty Acid Value (FFA)

Free Fatty Acid (FFA) is a good indicator of fish quality. FFA are produced by the hydrolysis of oils and fats. Thus, FFA is a crucial component that is connected to the effectiveness and worth of oils and fats as products. FFA values of Hilsa, Rupchanda, Tuna and Loitta fishes were estimated as 0.18%, 0.35%, 0.25%, 0.32%, respectively and for Bagda and harina, it was 0.32% and 0.25%, respectively (Fig. 1c). All of the values were within the acceptable limit of 5% oleic acid (Bimbo, 1998). Because oils and fats are exposed to a variety of conditions, such as storage, processing, heating, or frying, the level of FFA varies with time, temperature, and moisture content. Due to fish's fatty nature, there may be significant rancidity hazards under long-term storage circumstances (Horner, 1997).

According to Khodanazary (2019), the FFA value was found to be 12.07% in iced *Metapenaeus affinis*. In some previous studies, because of the hydrolysis of phospholipids and triglycerides during ice storage, *Metapenaeus affinis* gradually produced more FFA (Rostamzad et al., 2011). In the current study, no significant differences ($p<0.05$) were observed between four marine fishes and two marine shrimps.

TBARS value

Thiobarbituric acid reactive substance (TBARS) is a crucial tool for evaluating the quality of numerous foods. In the current study, the average TBARS values found for Hilsa, Rupchanda, Tuna and Loitta fish were 0.35, 0.32, 0.30 and 0.55 mg MDA/kg, respectively and for Bagda and Harina shrimps, it was 0.01 and 0.02 mg MDA/kg, respectively (Fig. 1d). Significantly ($p<0.05$) higher TBARS value were observed in marine fishes compared to that of shrimps. In the previous study, Duarte et al., (2020) found the TBARS value 2.66 mg MDA/kg in iced Sardine and 0.85 mg MDA/kg in iced Horse Mackerel. On the other hand, Khodanazary (2019) found TBARS value 2.04 mg MDA/kg in iced *Metapenaeus affinis*. The TBARS values were very high in iced Sardine and iced *Metapenaeus affinis* and slightly low in iced Horse Mackerel, but all are higher than the iced Hilsa and Rupchanda of the present study.

According to Connell (1995), 2 mg MDA/kg is the maximum permissible TBARS value for fish and fish products. The TBARS value of iced fishes and shrimps was within the acceptable limit. Significant differences ($p<0.05$) were observed between marine fishes and shrimps. Beyond this point, fish develop an unpleasant taste and odour (Connell, 1990).

Aerobic Plate Count (APC)

Food's intrinsic qualities, like water content and nutrients and its physical characteristics, including temperature and the environment, impact how many microorganisms are present in it (Gram et al., 2002).

In the present study, APC were 5.11, 6.09, 6.82, 7.11 log CFU/g for Hilsa, Rupchanda, Tuna and Loitta fish, respectively and 6.97 and 5.51 log

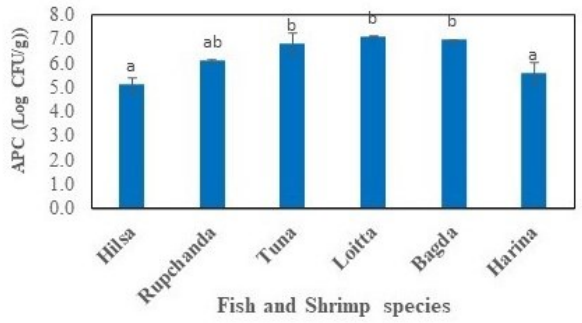

Fig. 2. Aerobic plate count (APC; Log CFU/g) of fish and shrimp species collected from local market in Rajshahi city.

CFU/g for Bagda and Harina shrimps, respectively (Fig. 2). Comparatively higher bacterial count was observed in loitta fish (7.11 log CFU/g) and lowest in Hilsa (5.11 log CFU/g) fish. In the present study, the total plate counts didn't exceed the acceptable limit of 7 log CFU/g (ICMSF, 2002) in most of the samples except the loitta fish. Significantly ($p<0.05$) lower APC value was observed in Hilsa fish compared to that of other marine fishes, whereas significantly ($p<0.05$) higher APC was observed in Bagda shrimp in comparison to Harina shrimp. As a result, based on the microbiological count, all samples of this study except loitta fish were acceptable.

In a previous study, Reza et al. (2009) found that APC of loitta was acceptable after 10 days of storage compared to big eye tuna and Rupchanda. According to Reza et al. (2009), the APC of iced Ribbon fish was 7.36 Log CFU/g, and in Big Eye Tuna the value was 9.63 Log CFU/g. Duarte et al. (2020) observed the APC of iced Sardine 7 Log CFU/g. The APC of Ribbon fish and Sardine was higher than that of iced Hilsa and Rupchanda in that study. Nilla et al. (2012) found TBC (total bacterial count) ranged between $4.2 \pm 0.45 \times 10^6$ and $1.3 \pm 0.25 \times 10^8$ CFU/g in Indian white shrimp *(Penaeus indicus)* from local markets of Dhaka city and the highest TBC for the cultured source was observed to be $5.83 \pm 0.12 \times 10^7$ CFU/g in aquacultured *P. indicus* from Satkhira. Total viable bacterial count (TVBC) was estimated between 6.4×10^4 and 7.0×10^6 CFU/g in case of freshwater Giant prawn (*Macrobrachium rosenbergii*) sold in local markets Nayem et al. (2011). On the contrary, Yousuf et al. (2008) recorded TBC to be 1.2×10^3 CFU/g in Black Tiger Shrimp. Majumdar et al. (2014) found the TBC of $5.98 \pm 4.35 \times 10^7$ in lotta fish and $1.38 \pm 1.23 \times 10^8$ in hilsa shad collected from the different markets of Noakhali district which are higher than that of the present study.

Conclusion

Marine fisheries resources are one of the major protein sources for the people in Bangladesh. This study aimed to evaluate the various microbiological and biochemical parameters of marine fishes and shrimps bought from open markets in Rajshahi City. Although the marine fishes and shrimps were caught far from the study site, we found those fishes and shrimps under acceptable conditions according to biochemical and microbial study and, thus, perhaps safe for human consumption. The findings of this study will aid the fishmonger in bringing the marine fish to the distance market, which will increase the consumption of marine fish and shrimp throughout the country. However, the storage and transportation systems are not good enough which could significantly negatively affect the freshness of sold fish and shrimps, but there are many scopes of improvement to maintain the quality.

Authors' contribution

Conceptualization, MTI and MRKM; Methodology, MTI, MTF, MFA and IKL; Investigation, MTF, MFA and IKL; Writing—original draft preparation, MTF and MTI; Writing—review and editing, MTI and MRKM; Supervision, MTI and MRKM. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interests.

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