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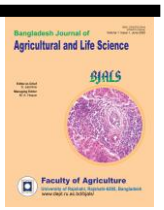

Biochemical and microbiological quality of dried marine fishes collected from local market in Rajshahi city of Bangladesh

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ABSTRACT

The study was conducted in November 2019 to know the quality of marine dried fishes available in Rajshahi city of Bangladesh. Three marine dried fish species namely; Ribbonfish (*Trichiurus lepturus*), Bombay-duck (*Harpadon nehereus*), and Hairfin Anchovy (*Setipinna taty*) were selected to monitor the quality considering the moisture, pH, total volatile base-nitrogen (TVB-N), thiobarbituric acid reactive substance (TBARS), total plate count (TPC), and total fungal count (TFC) at the laboratory. The moisture content and pH for Ribbonfish, Bombay-duck, and Hairfin Anchovy were 20.50%, 24.1%, and 20.34%; and 6.58, 6.57, and 6.26, respectively. Besides, the TVB-N and TBARS values for Ribbonfish, Bombay-duck, and Hairfin Anchovy were 37.95 mg/100g, 108.92 mg/100g, and 44.73 mg/100g; and 3.04 mg MDA/kg, 3.50 mg MDA/kg, and 2.06 mg MDA/kg, respectively. The TPC and TFC for the Ribbonfish, Bombay-duck, and Hairfin Anchovy were 7.36 log CFU/g, 5.67 log CFU/g, and 6.19 log CFU/g; and 3.10 log CFU/g, 2.00 log CFU/g, and 2.74 log CFU/g respectively. Almost all parameters except the pH and TFC of the dried fishes collected from the local market exceeded the acceptable limit; hence the overall quality of the studied dried fishes was unacceptable for human consumption.

Introduction

Sun drying is a traditional processing method practiced in many parts of the world. It is the most convenient and low-cost method of fish preservation (Sachithananthan et al., 1985, Eyo, 1986). Fish drying is one of the most common and traditional cheapest preservation techniques in Bangladesh. Both marine and freshwater fishes are used for drying. Freshwater fish drying activities are generally located in areas where fishes are readily available, such as around *haor* (a bowl-shaped large tectonic depression) and *beel* (a large inland depression) area. Coastal regions play an important role in the marine fish drying in Bangladesh. Among all the coastal regions, Cox's Bazar is the most recognized area for fish drying, and the lion share of dried fish is produced in Cox's Bazar. Fish drying activities generally starts in October and ends in March in the coastal regions. In some seaside villages, it occasionally begins in early September and lasts

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until May (Nowsad, 2005). Therefore, the winter season is the peak season for drying fish.

Dried fish is one of the popular fishery products in Bangladesh due to its flavor. About 20-22% of fish are dried in the coastal areas of Bangladesh. Mainly 8-10 species of marine fishes are commercially taken for drying purposes. Bangladesh has now exported approximately 3,144 metric tons of dried fish during the fiscal year of 2017-2018, whose market value was about 42.59 crore BDT as foreign currency (DoF, 2019). Quality is one of the primary considerations for the consumer to consume any food product. Many consumers are now very much conscious about the quality of dried fish (Nowsad, 2005). The physical and organoleptic quality cannot satisfy the consumers until now. Damages occurring due to flies and insects are of great significance in open sun drying, and this is a severe problem in traditional drying. The major problem during the distribution of fish and fishery products is their susceptibility to spoilage by spoilage microorganisms and contamination by pathogenic microorganisms (Gram & Huss, 1996). The quality of dried fishes also deteriorated by the contamination during different stages of handling and processing. Therefore, it is crucial to maintain the required hygiene during the different phases of fish drying (Relekar et al., 2014).

After drying, dried fish products are usually kept or stored in a damp warehouse. During the rainy season, the weather becomes more humid and contains high moisture and dumps the weather of the warehouse. As a result, the dry fishes get a great chance to absorb moisture so quickly and become suitable for the growth and survival of beetles, mites, and insects. Infestation is also occurred for the fraudulent activity by the producers, such as improper drying in fear of losing weight of dried fishes. For the protection of dried fish from infestation, fish traders use insecticides whatever they are getting in their locality. There are many insecticides sold in the local markets without any brand names. Processors also use many types of pesticides during drying without maintaining the proper doses (Bhuiyan et al., 2008, Flowra et al., 2013).

In the case of marketing of dried fish, owner of drying plants purchase fishes from local landing centers or suppliers and bring those fishes to their processing plant or drying yards. They perform different activities in drying yards to make dried fishes ready to sell. Owners of fish drying plants usually pay commission to the *aratdars* (sale agents) when selling fish through *arat* (Islam et al., 2001). Various marketing channels are exist in marine dried fish sector where producers and consumers are linked together by the presence of wholesalers, *aratdar*, middlemen, and retailers. In most of the processes, intermediaries get more profit, while producers and consumers do not find the product at reasonable prices most of the time (Shamsuddoha, 2007).

There is a frequent complaint about the quality of the dried fishes available in the local market. As the dried products are not packaged in the most cases, there is no information related to packaging such as ingredients, nutritional value, date of manufacture, date of expiry, which can ensure the consumers about the quality and shelf-life of the dried products. Therefore, analysis of the quality of the dried fishery products from the local market is essential, which can explore the actual quality status of the dried fishes selling in the local market. For this purpose, the present study was conducted to know the biochemical and microbial quality of marine dried fishes collected from the local market of Rajshahi city of Bangladesh.

Materials and Methods

Study area and sample collection

Marine dried fishes were collected in November 2019 from a mobile shop in Vodra, Rajshahi city. The shop opens every day only in the evening besides the Vodra rail crossing, Rajshahi. There is no permanent dried fish shop rather a very few mobile dried fish shops operated in Rajshahi city. Dried fish shop in Vodra is one of them. Three species used for the investigation were Ribbonfish (*Trichiurus lepturus*), locally called *churi*; Bombay-duck (*Harpadon nehereus*), locally called *loitta*, and Hairfin Anchovy (*Setipinna taty*), locally called *phasa*. The fish samples were packed species wise in polythene bags tightly and brought to the Quality Control Laboratory of the Department of Fisheries, University of Rajshahi for biochemical and microbiological study. Samples were kept separately species-wise in an airtight plastic pouch at room temperature during the study period. After that different biochemical and microbiological analysis were conducted in the laboratory.

Moisture content

At first 15 g of each species were taken and blend them properly by a blender (Bajaj, India). After that, approximately 3 g of each sample was taken in empty crucibles and weighed them by an electric weighing machine. Then the crucibles were kept in an oven at 105ºC for 24 h. Then, the crucibles were taken out from the oven and kept in a desiccator for 1h. Weighed the crucible again and maintained the data correctly. The result is expressed as the percentage of the sample according to the following formula:

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

pH

At first, 10 g of each sample was taken in a blender and add 50 mL of distilled water and then appropriately homogenized. The homogenate was then transferred to a beaker. Then, the pH of the homogenate was estimated employing an electrode of the digital pH meter (HANNA, USA) that was previously calibrated and kept the reading correctly.

Total volatile base nitrogen (TVB-N)

The TVB-N value was determined according to the EC (2005) method. The flesh of the sliced fish was cut into small pieces from each of the pack and ground carefully using a blender. Exactly ten (10) grams of the ground fish sample was weighed out into a container and mixed with 90 mL of 6% perchloric acid, homogenized for 2 min with a blender (Bajaj, India), and then filtered. Fifty (50) mL of extract was taken in a Kjeldahl flask and added 8-10 drops of phenolphthalein indicator. After placing the flask to the distillation unit, some glass-beads were added, and then 6.5 mL or more of 20% NaOH as per the requirement was poured to the flask. Then, steam distillation was started immediately.

The steam distillation was controlled so that about 100 mL of distillate was produced in 10 minutes. The outflow tube was submerged in the receiver with 100 mL of 3% boric acid solution, in which 3-5 drops of the mixed indicator (2 g methyl-red and 1 g methylene-blue were dissolved in 1000 mL 95 % ethanol) was added. The distillation was ended after around 10 minutes. Then distillation outflow tube was removed from the receiver and washed out with water. The volatile bases contained in the receiver solution were determined by titration with 0.01 (N) HCl solutions. The pH of the endpoint should be 5.0 ± 0.1. A blank test was carried out by the same procedure without using the sample. The TVB-N value was calculated using the following equation:

$$TVB - N\ (mg/100g\ sample) = \frac{(V_1 - V_0) \times 0.14 \times 2 \times 100}{M}$$

Where, V_1 = Volume of 0.01 (N) HCl in mL for sample; V_0 = Volume of 0.01 (N) HCl in mL for blank; M = Weight of sample in g.

Thiobarbituric acid reactive substance (TBARS)

TBARS values were estimated by the method of Witte et al. (1970). Twenty (20) grams of ground dried fishes were homogenized with 50 mL of 20% trichloroacetic acid prepared with 2 M phosphoric acid at 10000×g for 2 minutes using a homogenizer (IKA T18 digital ULTRA TURRAX, Staufen, Germany). The resulting mixture was then taken into a 100 mL beaker. The mixture was diluted to 100 mL with HPLC grade water and homogenized again with homogenizer. After filtration of about 50 mL through filter paper (Whatman No. 1, 110 mm), 5 mL filtrate was taken into a test tube, and 5 mL of 2-thiobarbituric acid (0.005 M in HPLC grade water) was also added. After well shaken, the test tube was kept in the dark for 15 hours at room temperature. Then the reactive substances were measured at 530 nm using a UV-Visible Spectrophotometer (UV-1601PC, Shimadzu, Japan). Two replicates of 20 g samples were taken for the measurement. TBARS values were calculated as follows: TBARS value (mg malonaldehyde (MDA)/kg) = optical density (O.D) ×5.2

Total plate count (TPC)

Total plate count (TPC) of the representative samples were determined by a standard plate count method on plate count agar (Sigma-Aldrich, USA) following the serial dilution technique described by APHA (1992). TPC is expressed as colony forming units (CFU/g). This total bacterial count was transformed into logarithm of the number of colony forming units (log CFU/g). For this purpose, dried fish samples were first chopped using a sterile knife to make it into small

pieces and weighed about 25 g. The chopped sample was placed in a sterile stomacher bag and peptone physiological saline (PPS) (0.1% Peptone and 0.85% NaCl with distilled water) at ten times of the sample was then added to it. The samples were then homogenized for one minute in the stomacher paddle blender (SJIA Lab, China). Thus, a sample of 1:10 dilution was obtained. One (1) mL of diluted sample was taken into a test tube containing 9 mL of PPS solution with a sterile pipette tip, and the test tube was vortexed properly. Several ten-fold dilutions were made up to the desired level using the same way.

At first, 1 mL of prepared, vortexed sample was transferred to empty plates using a micropipette. Samples were pipetted out and aseptically taken to the plates. Then the required amount of prepared plate count agar (cooled to 45°C ± 1°C using water bath) was poured to the plates. At least three dilutions were enumerated for all cases. All the plates were inoculated in duplicate. After solidifying the agar, the plates were incubated at 35°C in an inverted position in an incubator (Poleko, Poland). After 48±2 hour of incubation, colonies were developed, and only the plates having 30-300 colonies were counted by using colony counter (Labotronics, India). The result was performed by the following formula:

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

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 the 1stdilution counted; n_2 = Number of plates in the 2nd dilution counted; d = Dilution from which the first counts were obtained

Total fungal count (TFC)

Total fungal count also expressed as colony forming units (CFU/g) of the representative samples were determined by a standard plate count method on potato dextrose agar (Sigma-Aldrich, USA) following the serial dilution technique described by APHA (1992). For this purpose, fish samples were prepared by the same procedure used for TPC. In this case, agar plates were prepared with potato dextrose agar using the required amount of lactic acid according to the manufacturer instruction. At first, 0.1 mL of prepared, well shaken diluted sample was taken aseptically to prepared potato dextrose agar plates using a micropipette. The pipetted samples were spread over the whole surface of the media in a petri dish by using triangle-shaped glass spreader until the samples dried completely. All the plates were inoculated in duplicate. The plates were then incubated in an inverted position at 30°C in an incubator (Poleko, Poland). After 48 hours of incubation, colonies were developed. Later the colonies developed only the plates having 30-300 colonies were counted. The result was performed from the same formula used for total plate count.

Statistical analysis

The differences among dried fish species were estimated by one-way ANOVA with the application of the Tukey test using SPSSVersion-20. The 'p' values <0.05 were considered significant.

Results and discussion

The present study is focusing on the monitoring the moisture content, TVB-N value, TBARS value and the growth of bacteria and fungus in dried fishes collected from the local open mobile market of Rajshahi city stored at atmospheric temperature. These parameters were observed to determine the overall quality and shelf life of dried fishes at atmospheric temperature.

Moisture content

Moisture content is an important quality indicator of dried fishes. In the present study, it was found that the average moisture content of dried Ribbonfish, Bombay-duck, and Hairfin Anchovy were 20.50%, 24.19%, and 20.34 %, respectively. Statistically, there were no significant differences (p>0.05) among three dried fishes. However, the highest moisture content (24.19%) was observed in dried Bombay-duck, and lowest (20.34%) in Hairfin Anchovy fish (Table 1). This result indicated that Bombay-duck contains a higher amount of moisture than other two species. The moisture content of all dried fishes exceeds the optimum level that is 15%. Frazier and Westhoff (1978) indicated that generally, most of the microorganism could not grow in dried products having a moisture content below 15%. In a previous study, Islam et al. (2006) found moisture content at 13% for Ribbonfish, and 15% for Bombay-duck fish and both were freshly dried

fishes collected from low-cost solar tunnel dryer in Cox's Bazar.

Table 1. Biochemical quality parameters of three marine dried fishes collected from the local market of Rajshahi city

Dried fishes	Moisture (%)	pH	TVBN (mg/100g)	TBARS (mg MDA/kg)
Ribbonfish	20.50±0.42[a]	6.58±0.07[b]	37.95±5.27[a]	3.04±0.08[b]
Bombay-duck	24.19±0.15[a]	6.57±0.06[b]	108.92±13.7[b]	3.50±0.09[c]
Hairfin Anchovy	20.34±2.82[a]	6.28±0.08[a]	44.73±10.6[a]	2.06±0.15[a]

Superscript letters in the same column represent significant difference (mean value ± SD) among the fishes ($p<0.05$)

In the case of drying, water removal usually prevents the growth of spoilage microorganisms and reduces many of the water-mediated deteriorative reactions (Kilic, 2009). Water activity is a term directly related to the water content, which affects the efficacy in preventing microbial growth in dried fish. The final a_w values of the quality dried fish products should be below 0.95.

The relative humidity is high almost throughout the year in Bangladesh as like other tropical countries, where the dried products absorb moisture from the environment. As a result, the water activity increases, and there is a chance of bacterial spoilage in each stage of marketing, particularly during storage. As the experiment was done in October with dried fishes from last season (December to March), the experimental dried fishes could absorb moisture from the air during this long period of storage.

pH

pH value is also an important indicator for the assessment of the quality of many foods. In the present study, the average p^H value was determined as 6.58, 6.57, and 6.28 in dried Ribbonfish, Bombay-duck, and Hairfin Anchovy, respectively. A higher value was observed in Ribbonfish, and lowest value in Hairfin Anchovy fish (Table1). There were no significant differences ($p>0.05$) observed between Bombay-duck and Ribbonfish. However, significantly ($p<0.05$) lower pH value was found in Hairfin Anchovy fish compared to that of other species. All the dried fishes were slightly acidic, which is less favourable for bacterial growth.

Total volatile base nitrogen (TVB-N)

Total volatile base nitrogen (TVB-N) is an important indicator for the quality assessment of fish and fishery products. TVB-N is the totality of the amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish and is commonly used as a spoilage indicator and has been widely used as a freshness index of fish (Wu and Bechtel, 2008). In general, the TVB-N and other volatile basic nitrogenous compounds associated with seafood spoilage. As per European Union directive on fish hygiene, if there is any doubt on the organoleptic examination for the freshness of fish, TVB-N must be used as a chemical check by the inspector (Castro et al., 2006).

In the present study, the average TVB-N values of dried Ribbonfish, Bombay-duck, and Hairfin Anchovy fish were 37.95 mg/100g, 108.92 mg/100g and 44.73 mg/100g, respectively. The highest TVB-N value (108.92 mg/100g) was observed in the case of Bombay-duck and the lowest (37.95 mg/100g) in Ribbonfish (Table 1). There was no significant difference ($p>0.05$) on TVB-N values between Ribbonfish and Hairfin anchovy. However, significantly ($p<0.05$) higher TVB-N value was observed in Bombay-duck compared to that of Ribbonfish and Hairfin anchovy. According to Kimura and Kiamakura, (1934), the TVB-N value of 35 - 40 mg/100 g is regarded as the upper acceptable limit for dried fish, and above that level, the fishery products are considered to be unfit for human consumption. Therefore, the TVB-N values dried Bombay-duck and Hairfin anchovy fish were not within the acceptable limit. However, the TVB-N value of Ribbonfish was within an acceptable limit. Islam et al. (2006) found a lower level of TVB-N values of 27.3 and 30.9 mg/100 g for Ribbonfish and Bombay-duck, respectively collected from low-cost solar drier in Cox's Bazar. The higher TVB-N values observed in the present study indicate that the quality of dried fishes deteriorated during this more prolonged period.

Thiobarbituric acid reactive substance (TBARS)

TBARS is a vital indicator assessment of the quality of many foods. In the present study, the average TBARS value of dried Ribbonfish, Bombay-duck, and Hairfin Anchovy fish were 3.04,

3.50, and 2.06 mg MDA/kg, respectively. The highest value (3.50 mg MDA/kg) was observed in Bombay-duck, and the lowest value (2.06 mg MDA/kg) in Hairfin Anchovy fish (Table 1). There was a significant difference ($p<0.05$) in the TBARS value among three dried fish species. The acceptable limit of TBARS value is 2 mg MDA/kg fish sample. Beyond this limit, an objectionable odor and taste develop in fish (Connell, 1990). The TBARS values in all samples exceed the acceptable limit. Due to higher TBARS values, the dried fishes of the present study produced secondary oxidative product resulting in rancidity that created the bad smells of all samples. This rancid odor could be used as an indicator of spoiled dried fishes for general customers.

Total plate count (TPC)

Microbial activity in food depends on its nutritional compositions along with the physical parameters, for instance, temperature and surrounding atmosphere (Gram et al., 2002). Dehydration of products prevents the growth of spoilage microorganisms and minimizes many of the water-borne deteriorative reactions (Kilic, 2009). Uncontrolled growth of microorganisms in such dried products may lead to severe consequences in keeping the quality and safety of the product (Abraham et al., 1993).

Table 2. Microbiological quality parameters of three marine dried fishes collected from the local market of Rajshahi city

Dried fishes	TPC (log CFU/g)	TFC(log CFU/g)
Ribbonfish	7.36 ± 0.21^{b}	3.10 ± 0.20^{b}
Bombay-duck	5.67 ± 0.17^{a}	2.00 ± 0.15^{a}
Hairfin Anchovy	6.19 ± 0.79^{ab}	2.74 ± 0.12^{b}

Superscript letters in the same column represent significant difference (mean value ± SD) among the fishes ($p<0.05$)

In this study, the average total plate count (TPC) of Ribbonfish, Bombay-duck, and Hairfin Anchovy fish were 7.36 log CFU/g, 5.67 log CFU/g, and 6.19 log CFU/g, respectively. Comparatively higher bacterial count (7.36 log CFU/g) was observed in Ribbonfish fish and lowest (5.67 log CFU/g) in Bombay-duck fish (Table 2). There was no significant difference ($p>0.05$) on TPC values between Ribbonfish and Hairfin Anchovy and also no significant difference ($p>0.05$) between Bombay-duck and Hairfin Anchovy. However, significantly ($p<0.05$) higher TPC was observed in Ribbonfish compared to that of Bombay-duck. In the present study, the total plate counts exceeded 5 log CFU/g in all dried fish samples, which is considered as the upper acceptability limit for dried fishes (ICMSF, 2002). Therefore, all dried fish samples of this study were unacceptable based on the microbial count. In a previous study of Islam et al. (2006), lower level of bacterial counts observed for Ribbonfish and Bombay-duck fish collected from a low-cost solar dryer in Cox's Bazar which were 4.87 and 4.93 log CFU/g, respectively. As the sellers collected the dried fishes last drying season (December to March) and sold in November in an open place, the dried fishes got chance to be contaminated with environmental microorganisms during this long period.

Total fungal count (TFC)

Fungal growth and production of fungal toxin in the fish favoured by the hot and humid climate, moisture content of >16% and insect damage (Hamblin, 2000). In this study, the average total fungal count (TFC) of Ribbonfish, Bombay-duck, and Hairfin Anchovy fish were 3.10 log CFU/g, 2.00 log CFU/g, and 2.74 log CFU/g, respectively. Comparatively higher fungal count (3.10 log CFU/g) was observed in Ribbonfish and lowest (2.00 log CFU/g) in Bombay-duck fish (Table 2). There was no significant difference ($p>0.05$) on TFC values between Ribbonfish and Hairfin Anchovy. However, significantly ($p<0.05$) lower TFC was observed in Bombay-duck compared to that of the other two fishes. In the present study, the total fungal counts did not exceed the 4 log CFU/g in all dried fish samples, which is considered as the upper acceptability limit for dried fishes according to Sri Lanka Standard Institute. Since the sample stored for an extended period, and moisture content was suitable for fungal growth, the fungal attack was observed in all samples. Islam et al. (2006) found no fungus growth in Ribbonfish and Bombay-duck fishes collected from a low-cost solar dryer in Cox's Bazar. The fungal count of the present study indicates the improper packaging and storage in unhygienic condition.

Conclusion

This study introduces an assessment scheme for better evaluation of the various

microbiological and biochemical quality of dried fishes collected from the local market of Rajshahi city. Considerable post-harvest losses occurred at different stages of handling, transportation, storage and marketing due to lack of knowledge of the people engaged in various stages of processing and place. Nowadays, consumers are becoming more health-conscious and showing interest in convenient food products. With the change of lifestyle, the buying behavior is also changed of the customers. Consumers now insist that the product should be acceptable concerning both quality and safety. To ensure the safety and quality, improved fish drying method, as well as proper packaging system, should be practiced throughout the country which will eventually increase the consumers' acceptance of dry fish.

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