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MICROBIOLOGICAL QUALITY OF DRIED BOMBAY DUCK (*HARPODON NEHEREUS*) SAMPLES OBTAINED FROM NORTH-EASTERN BANGLADESH (SYLHET DISTRICT)

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Abstract

This research was selected to assess the microbial and sensory quality of dried Bombay duck (loitta) in Sylhet district. Fourty eight (48) samples of dried Bombay duck (loitta) were collected from eight Upazila dried fish markets in Sylhet district in each of the eight upazilas during September 2022 to February 2023, the study collected 48 samples from two markets in each of the eight upazilas. Microbiological analyses included Aerobic Plate Count (APC), Total Coliform Count (TCC), presence of *Escherichia coli* & *Salmonella* spp., and sensory evaluation. Results revealed that aerobic plate counts showed the highest (log 5.69 ± 0.2) in Gowainghat upazila markets and the lowest (log 3.453 ± 0.2) in Sylhet sadar market. Total Coliform counts (ranged from 21 ± 1 to 80 ± 3 MPN/g) of the samples analyzed remained within permissible limits. The prevalence of *Escherichia coli* was found the highest in Gowainghat (67%) and the lowest in Sylhet Sadar and South Surma (17%), and the prevalence of *Salmonella spp*. was found the highest in Companiganj and Fenchuganj (67%) and the lowest in Sylhet Sadar (17%). Sensory examination favored samples from South Surma. Presence of pathogenic bacteria suggests unsanitary processing conditions. Dried fish quality varied significantly among upazilas, emphasizing the need for improved hygiene and sanitation practices to ensure safe, high-quality dried loitta production.

Key words: Upazila, Dried loitta, *Salmonella* spp., *E. coli*, Health risk

Introduction

Dried fish is popular in Bangladesh coastal and riverine communities, and gradually it becomes popular in other areas in Bangladesh. Dried fish holds cultural significance, being the most consumed fish category in Bangladesh, providing access to year-round nutrition. It is an excellent source of high-quality proteins, and are a unique source of important nutrients & minerals such as iodine, zinc, copper, selenium, and also contains healthful fatty acids, including long-chain omega-3 fatty acids like eicosapentaenoic acid

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(EPA) and docosahexaenoic acid (DHA) (Siddhnath *et al*. 2022). This category of dried fish is particularl important for people who cannot afford fresh fish or meat (Banna *et al*. 2022), and in addition to dietary mainstay dried fish contributes significantly to Bangladesh's economy.

It ranks second globally in dried fish production, exporting around 2339.36 metric tons in 2018-19, earning nearly USD 4 million (FRSS, 2019). This industry is a major employer, particularly for women, boosting foreign exchange reserves through exports.

One prominent fish species is Bombay duck (*Harpodon nehereus*), or "loitta," vital to Bangladesh's fishing sector. Comprising over 10% of marine catches in the Bay of Bengal, it's a favorite among coastal populations and commands strong export value. "Loitta shutki," is widely enjoyed and has gained international acceptance for using in various culinary applications. Dried Bombay duck dried fish (Loitta shutki) contributes significantly to Bangladesh's economy, with an annual export of around 7000 metric tons generating approximately USD 14 million (Mandal, 2021).

However, inadequate sanitation and poor hygiene in Bangladesh's drying processing and practices (i.e. improper handling and storage of dried fish in general) pose risk for microbial contamination (Rana *et al*., 2020) and resulting in compromised food safety, driven by *Escherichia coli* and *Salmonella* spp. (Beuchat and Mann 2011) and becomes public health concern (Rasul *et al*., 2020).

The consequences of bacterial contamination are terrible, affecting both health and economics. Deterioration of dried fish quality due to bacterial growth results in spoiled product with reduced shelf life, off-flavors, and altered texture. Pathogenic bacteria like *Vibrio parahaemolyticus, Escherichia coli, and Salmonella* spp. can cause foodborne illnesses, especially, *Salmonella* spp. can cause a range of diseases in humans, collectively referred to as salmonellosis (symptoms like diarrhea, abdominal cramps, fever & vomiting). These typically emerge 12 to 72 hours after bacterial exposure and can persist for about a week. In severe case Salmonellosis can result in high fever, headache, and abdominal pain. It is caused by the *Salmonella enterica* serovar Typhi and is typically contracted through having contaminated food or water (Coburn *et al.* 2007). *Escherichia coli,* is commonly found in - human and animal guts (Bélanger *et al*. 2011). It can be transmitted through contaminated food or water, as well as with infected individuals or animals. Some strains of *E. coli* are associated with outbreaks of foodborne illness and can cause symptoms like diarrhea, abdominal cramps, and fever. In acute cases, *E. coli* infection can lead to complications as hemolytic uremic syndrome (HUS),

which can cause kidney failure and other complications can be life-threatening (Bélanger *et al*. 2011).

This situation underscores the importance of proper handling and storage. Ensuring the correct temperature, adequate drying, appropriate hygienic packaging, and preventing cross-contamination with other foods are essential. Regular testing and monitoring further enhance product safety. Hygiene measures are to be taken for reducing potential loss of nutritional value and health hazards due to microbial contamination.

Despite some existing research on dried fish safety and quality, this study aims to fill the gap of knowledge, evaluating microbial safety specifically for dried Bombay duck. The present research was undertaken to assess bacterial load, total coliform, *E. coli*, and *Salmonella* spp. in dried Bombay duck and to analyze the presence of pathogenic bacteria to evaluate potential health risks associated with consumption. As dried Bombay duck holds cultural, economic, and culinary importance, this study has far-reaching implications for public health, food safety, and local economies.

Materials and Method

Study Area: The study cover eight upazila dried fish markets of the Sylhet district. Two dried fish markets were taken to collect samples randomly from each upazila.

Study period: September 2022 to February 2023

Study place: Laboratory of the Department of Fisheries Technology and Quality Control under the Faculty Fisheries, Sylhet Agricultural University.

Sample size: 48 dried Bombay duck, (*Harpodon nehereus*) samples were collected from the eight Upazila dried fish markets of Sylhet district.

Preparation of Sample: Using the sequential decimal dilution method with pour plates, standard plate counts are computed and represented as Colony Forming Units per gram (CFU/g) of material. A gram of the sample was taken and aseptically homogenized in a sterile mortar. The sample was homogenized with 10 mL peptone water and then transferred to a clean container. Then the samples were diluted with sterile peptone water in test tubes upto 10^{-6}, and so forth.

Procedure of Aerobic Plate Count: The each of diluted sample was transferred onto plate count agar (PCA) plate, spread uniformly and were kept in an incubator for 24 hours at 37°C. After 24 hours of incubation, plates only containing 30 to 300 colonies were

counted. The formula used to compute the number of bacteria per gram of the sample (CFU/g) is as follows:

$$\text{CFU/g} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{Volume of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Procedure of Total Coliform Count: The total number of coliforms was counted by the MPN (Most Probable Number) method, and the results were recorded.

Isolation & Identification of Escherichia coli: Escherichia coli was isolated from the samples using the MacConkey Ager and Violet Red Bile agar (VRB) where pink colour and red coloured colonies of *E. coli* developed, respectively.

Presumtive identification of the *E. coli* isolates was done by growing isolates on EMB agar, where colonies showed metalic sheen colour. EMB, MacConkey, and BGA agar was used as subculture medium for the VRB agar colonies. The *Escherichia coli* strains tested positive for the tests for indole, catalase, motility, and MR, but not for VP.

Isolation & Identification of Salmonella spp.: *Salmonella* sp. was isolated from the samples by using *Salmonella-Shigella* (SS) agar, where colonies of *Salmonella* sp. had a dark foundation and were black on SS agar. Nutrient agar, MacConkey (MC) agar, Brilliant Green agar, Salmonella-Shigella (SS) agar, and Violet Red Bile agar are all used to grow *Salmonella* spp. *Salmonella* colonies were smooth, clear, and opaque on nutrient agar. Colonies on (MC) agar are light or colorless. The colonies on Brilliant Green agar had a cream color. Colonies had a dark foundation and were black on SS agar. Colonies on VRB agar had a light cream color. On TSI (Triple Sugar Iron) slant and SS-agar, *Salmonella* created black hydrogen sulfide (H_2S), which is a product of the bacterial process. *Salmonella* tested positive for the MR, motility, catalase, and TSI tests, while it tested negative for the Indole and VP tests.

Analysis of data: For the initial processing of the unprocessed raw data for dried loitta used in this investigation, the mean and standard deviation were established. These data were used for additional statistical analysis and interpretations using software like Microsoft Excel**.** SPSS (IBM 2020 and Version 16) was used for the statistical analysis. To determine the significance difference at the 5% level of confidence, a one-way ANOVA was conducted. The Chi-square test was applied for group comparison.

Sensory analysis: Dry fish products are evaluated based on sensory qualities that indicate their freshness and acceptability. In this study, sensory analysis was conducted using the Quality Index method, considering seven sensory attributes: color, odor, texture, general appearance, fungus, broken pieces, and insect infestation.

Results and Discussion

This investigation examined the Aerobic Plate Count (APC) of dried loitta fish from different Upazilas shown in Figure 1. The highest bacterial load was found in Gowainghat Upazila, (5.695±0.2), and next Companiganj upazila (5.24±0.1). Conversely, Sylhet Sadar exhibited the lowest bacterial load (3.453±0.2), which is deemed acceptable and complied with the ICMSF (1986). The bacterial load in the remaining samples from other Upazilas also complied with the ICMSF (1986). The study noted a variation in APC among different

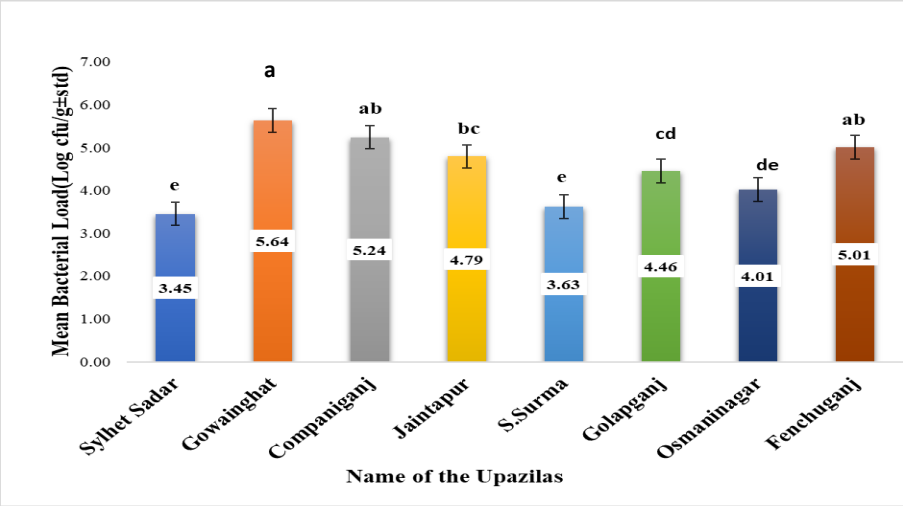

Fig 1. Bacterial load of dried loitta in different Upazilas (Different letters indicates significant differences).

Upazila markets. Statistical analysis confirmed highly significant different Upazila markets. Furthermore, statistical analysis confirmed highly significant results with a p-value <0.05. According to ICMSF, 1986 it may be noted that the aerobic Plate Count of the collected samples of the markets of Gowainghat upazila and Companiganj upazila little bit exceeded the standards. It probably happened due to the unhygienic condition of the markets and poor handling of dried fish. From this statement it may be concluded that due to the partial contamination by the bacterial load, might have some risks of human health through consumption. On the other hand, the samples of all other upazilas were quite ok. Ultimately no health risks through consumption of the said dried loitta. The result is related to the study of Mukilan *et al*. (2022), where they conducted a study in

Parangipettai, Tamil Nadu, focusing on the pathogenic bacterial contamination and quality of various dried fishes obtained from different fish markets.

According to ICMSF (1986) standards, plate counts below 5.69 log CFU/g indicated good, counts between 5.69 and 7 log CFU/g indicated marginally acceptable quality, and counts at or above 7 log CFU/g are considered unacceptable in terms of quality. Coliform bacteria are a type of microorganism that serves as an indicator of the presence of diseases, parasites, viruses & sanitary bacteria *(E. coli)* in a given sample. The present study showed that the highest value of the Total Coliform Count (TCC) was 80±3 MPN/g in the samples of Companiganj upazila. On the other hand, the lowest value of TCC was 21±1 MPN/g was in the samples of Sylhet Sadar (Figure 2). But all the value of TCC from the samples of all upazila markets were within the limit. Statistically it was observed that coliform counts vary significantly in different markets of different upazilas

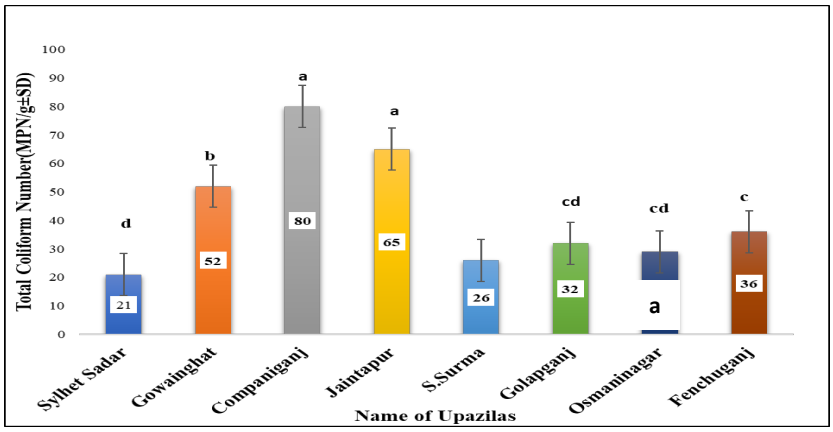

Fig 2. Total Coliform Count of dried loitta in different Upazilas (Different letters indicates significant differences).

($P<0.05$). The probable cause of the presence of coliform is poor hygiene throughout the drying process, which may have involved using dirty processing equipment and handling the dried fish carelessly. These led to the presence of coliforms in such levels. According to ICMSF (1982) guidelines, the acceptable limit for total coliform in dried fish is< 100 MPN/g. The findings of this study are comparable to the findings of Belgische (2012) who reported the highest total coliform count (132 MPN/g) in several samples from local markets, while the lowest count (68 MPN/g) was observed in Adjuevan, a traditional Ivorian fermented fish. This study identified pathogenic bacterial species, including *E.*

coli and *Salmonella sp*., in dried loitta fish, were also found almost in all upazilas which pose risks to human health. The presence of *E. coli* and *Salmonella spp.* in this dried fish samples indicated that poor hygienic conditions were adopted during the processing of dried fish. Factors such as the use of low-quality raw fish, inadequate hygiene practices during processing, improper packaging and storage, moisture absorption during drying from the environment, and mishandling during different stages of the marketing chain can contribute to high microbial counts (Nayeem and Pervin 2010). The highest occurrence of *E. coli* was found in Gowainghat Upazila, (67%) and the lowest in Sylhet sadar (17%), and South Surma (17%) of the analyzed samples of dried loitta (Table 1). The presence of *E. coli* in most of the examined samples indicated poor hygiene and sanitary conditions, which aligns with the findings of Fratamico *et al*., 2016.

Table 1. Upazila wise prevalence of *E. coli* in samples studied.

Name of the Upazila	No of total tested samples	No of positive samples	% of the *E. coli*	Chi square ($\chi 2$)	P value
Sylhet Sadar	6	1	17		
Gowainghat	6	4	67		
Companiganj	6	3	50		
Jaintapur	6	3	50	5.48	.601
Golapganj	6	2	33		
South Surma	6	1	17		
Osmaninanagar	6	2	33		
Fenchuganj	**6**	**2**	33		
Overall			37.5		

Salmonella, another pathogenic bacterium, was also isolated and identified from the dried fish samples. In the case of *Salmonella spp*., the highest occurrence was found was observed in both Companiganj and Fenchuganj Upazilas (67%), and the lowest in Sylhet sadar (17%) of the analyzed samples of dried loitta (Table 2). The presence of *Salmonella* in fish was also reported by Hatha and Lakshmanaperumalsamy (1997). The overall prevalence rates were 37.5% for *E. coli* (Table 1) and 43.75% for *Salmonella* (Table 2). Statistical analysis did not reveal any significant difference. Several factors may contribute to these statistical findings, but one possible explanation is that the source of the dried fish did not have a significant impact on the samples. Instead, the prevalence of

Salmonella and *E. coli* can be attributed to unhygienic market conditions, poor storage facilities, lack of handling knowledge, and failure to wear gloves, among other factors.

Table 2. Upazila wise prevalence of *Salmonella* spp. in samples studied

Name of the Upazila	No of total tested samples	No of positive samples	% of the *Salmonella* spp.	Chi square ($\chi 2$)	P value
Sylhet Sadar	6	1	17		
Gowainghat	6	3	50		
Companiganj	6	4	67		
Jaintapur	6	2	33	5.33	0.619
Golapganj	6	3	50		
South Surma	6	2	33		
Osmaninagar	6	2	33		
Fenchuganj	6	4	67		
Overall			43.75		

A lower sensory score indicated higher freshness and acceptability of dried loitta fish is shown in Table 3.

Table 3. Grading of dried fish acceptance according to sensory score (Bremner 1985).

Sl. No	Grade	Average defect point	Degree of acceptance
1	A	<2	Excellent, Highly acceptable
2	B	2 to <5	Good/ acceptable
3	C	5	Rejected

The dried loitta from South Surma received the most acceptable score of 1.11±0.247, indicating its high quality. On the other hand, the highest score was found in Gowainghat Upazila, which was 2.8±0.712, considered good and acceptable (Figure 3). However, no significant differences were observed among the samples. These findings are in line with a study conducted by Mithun *et al*. 2021 where they assessed the quality of dried Bombay Duck and found the highest score to be 2.42.The sources of the collected samples from Gowinghat, Companiganj & Fenchuganj upazila indicated that the quality

of these samples were not quite good i.e, moderately good this occurred due processing or handling in a less hygienic condition.

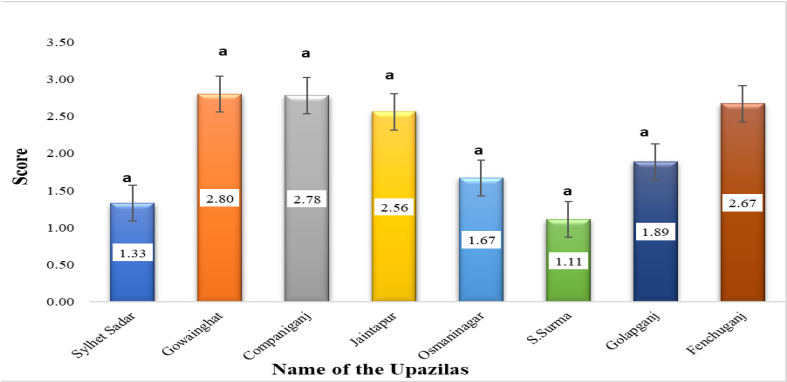

Fig. 3. Sensory scores of dried loitta in different Upazila markets

Conclusions

Dried Bombay duck, known for its high nutrition, is a favorite among Sylhet's people due to its protein-rich content. Also called marine lizard fish, it's abundant in calcium too. However, research highlights concerning microbial conditions, including Total coliform, *E. coli*, and *Salmonella*. Unhygienic handling, inadequate storage, and processing methods contribute to this issue, urging a focus on safety standards. While the sensory scores of studied dried fish were acceptable, enhancing processing, hygiene, and storage is vital to meet growing demand and maintain market reputation. Authorities must implement prevention measures such as training producers, modern drying tech, quality materials, proper packaging, and consumer education on food safety. These actions can ensure safe, nutritional dried Bombay duck in Sylhet's markets and beyond, reducing health risks. Although Sylhet's residents prefer dried loitta so they should have them as cooked not in raw because raw loitta have the presence of pathogenic bacteria. The presence of harmful bacteria raises concerns, urging further study on handling, transport, and processing methods.

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