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BACTERIOLOGICAL QUALITY OF RAW, FROZEN AND DRIED TAKI (*Channa punctatus*)

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Abstract

Freshwater snakehead, a commonly available fish species of Bangladesh, popularly known as Taki (*Channa punctatus*) is generally consumed as fresh or after being frozen and dried. In the current investigation, the bacteriological quality of dried, frozen, and fresh Taki was compared. Raw Taki were collected on monthly basis from December to May from local fish markets of Sylhet Sadar and Total Viable Count (TVC), Total Coliform Count (TCC), pathogenic bacteriological status were assessed either for raw (n=18) sample or after subjected to freezing (n=18) and drying (n=18) application. It was found that raw Taki had noticeably greater TVC and TCC than dried and frozen samples. The pathogenic bacteria explicitly, *Salmonella* spp., *Vibrio* spp. and *E. coli* were found abundantly in fresh conditions. On the contrary, *E. coli* was detected in the few samples of frozen and dried Taki. In comparison, dried fish comprised lower bacterial load, TCC and pathogenic bacteria than those of frozen samples but both the samples contained significantly lower values compared to raw fish and complied with ICMSF standard. Based on the findings of the present study, it can be concluded that higher bacterial load of raw sample can be reduced by applying freezing and drying techniques under proper hygiene and sanitary conditions and fish can be stored safe for longer period.

Keywords: Snakehead fish, Frozen and Dried fish, TVC, TCC, *Slamonella*

Introduction

Fish provides a good source of animal protein and other essential nutrients, resulting in it significant for human health (Rasul *et al.,* 2021a; Nie *et al.,* 2022). Although, fish is typically viewed as a safe and nutritious food item, it is recognized as a very perishable food due to the rapid microbial growth that can occur either naturally or as a result of contamination (Nie*et al.,* 2022). It has already known that fishes from both fresh and brackish water can possess many microbes from its habitat (Emikpe*et al.*, 2011). According to previous studies, bacteria from the genera *Aeromonas, Corynebacterium, Myxobacterium, Streptococcus, Pseudomonas* and *Vibrio* are primarily responsible for transmission of infectious diseases in fish (Baten *et al*., 2021) that may take part directly or indirectly in the quality of the harvested fish. The environment, rough handling of caught fish, personnel hygiene, processing and storage equipment and occasionally preservatives like salt or ice, all have also an impact on the microbial contamination of fish (Pal *et al*., 2015). Microbial threat is considered as a prime factor for reduction fish quality, which causes a 25–30% loss of the fish product (Hossain *et al*., 2017). It was observed that microbial activity affects one-fourth of the world's food supply, which causes significant losses to the economy (EEC, 1992). In order to preserve the quality and prolong the shelf life of fish and fisheries products, a variety of preservation techniques include drying, smoking, freezing, chilling, brining, fermenting, and canning are frequently used (Ghaly *et al*., 2010; Rasul *et al*., 2021; Afrin *et al*., 2021). It has known that the growth of microorganism is inhibited at low temperatures (+4 to -1° C) and freezing temperatures (-18 to -30°C) mostly stopped them (Berkel *et al*., 2004) where approximately 10 to 60% of the viable bacterial population are killed during freezing and the rest may gradually grow under unsuitable freezing temperatures (Rahman, 1999). In Bangladesh as well as around the world, drying is another widely used method of fish preservation (Rasul *et al*., 2018). When fish undergoes a drying procedure to lower its moisture level, microorganisms and muscle enzymes stop working. Although freezing and drying techniques similarly halt the growth of microorganisms in fish, the suitability of these methods in maintaining food safety and quality is not yet clearly understood. Moreover, this species is generally marketed as raw and dried condition in Bangladesh and commonly kept at frozen condition before processing for consumption.

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Thus, it is important to know, how the bacterial flora of dried and frozen product vary in accordance to the processing and preservation technique.

Taki, *Channa punctatus*, often known as the freshwater or spotted snakehead, is one of the commercially important small indigenous species in Bangladesh as well as the Indian subcontinent that can grow to a maximum length of 25 cm or 9 inches (Felts *et al*., 1996). It is particularly prevalent in the vast freshwater ecosystems of Bangladesh as ponds, haors and beels, canals, streams, rivers, and flood plains (Hossain *et al*., 2017).

This species is well accepted to all kinds of consumers for it deliciousness, high nutritional content and therapeutic properties and even advised as a diet during convalescence (Paray *et al*., 2013). Since bacterial contamination is evident in this species (Sankar *et al.,* 2012), it is necessary to evaluate the bacterial biomass of this species under different processing conditions. Thus present study intended to assess and compare the insight of bacterial biomass of this species under raw, dried and frozen conditions.

Materials and Methods

Study area and collection of samples

Raw Taki fish (*Channa punctatus*) were purchased from three local fish markets of Sylhet town on a monthly basis for 6 months from December to May. These fish were promptly brought to the Fisheries Microbiology Laboratory of the Department of Fisheries Technology and Quality Control at Sylhet Agricultural University. Bacteriological parameters were analyzed monthly as raw (n=18) or after being subjected into frozen (n=18) and dried (n=18) applications.

Preparation of frozen and dried items for analysis

Collected raw fish were dressed, gutted, and washed properly. These fish were immediately processed either for freezing (-18±3 ^{o}C) and sun drying. After 4 days of sun drying, dried Taki were kept at room temperature (18 to 28^{o}C). Total Viable Count (TVC), Total Coliform Count (TCC) and pathogenic bacteriological status were evaluated at every 7 days of interval during 21days of storage period for the frozen and dried samples and thereafter made a comparison with the samples prepared from raw fish.

Preparation of raw, frozen and dried sample for analysis of bacteriological parameters

Pour plate technique followed by consecutive decimal dilution (1:10) was used to calculate standard plate count, which was subsequently expressed as colony forming units per gram (CFU/g) of dried fish. To prepare the samples, 20 g of aseptically collected samples were mixed with 180 mL of alkaline peptone water (30 g of alkaline peptone in 500 mL of distilled water). 1 mL of the sample was then added to a test tube with 9.0 mL of peptone water to obtain 10^{-1}dilution of the original sample. The test tube was then thoroughly shaking using a vortex mixture and similarly other dilutions such as 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5,}$ 10^{-6} and so on was made as per recommended standard (ISO, 1995).

Procedure of Total Viable Count

Using a sterile tip with micropipette, 1 mL of each sample that had been diluted ten times was transferred to petri-dishes containing plate count agar and rotated three times clockwise and anticlockwise to spread the samples throughout the petri-dishes homogeneously. These petri dishes were then incubated in an incubator at inverted position for 24 to 48 hours at 37°C. After that the petri-dishes were taken out, observed carefully and the plates that contained only 30 to 300 colonies were taken into consideration for quantifying bacteria to get accurate results. Using the following formula, the number of bacteria per gram of sample (CFU/g) was counted.

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

Enumeration of Total Coliform Count (TCC)

As per ISO (4831:1991), the dilution of 10^{-1}, 10^{-2} and 10^{-3} were transferred into three separate tubes of Lauryl Sulphate Tryptose Broth (LSTB) containing Durham's tube and incubated for 48 hours at 37 °C. The positive gas production tubes were noted. After that, a loopful of broth media was taken from each positive LSTB tube and inoculated in the Brilliant Green Bile Broth (BGBB) and kept at incubator for 24 hours at 37°C. Following the observation of positive gas production tubes, the results were computed using MPN Chart (FDA, 2011).

Isolation and detection of pathogenic bacteria

In accordance to the Bergeys Manual for Determinative Bacteriology (Al Harbi and Uddin, 2007; Holt et al., 1994), the morphological characteristics e.g. size, shape, arrangement, motility and the colony features were observed. The biochemical parameters such as oxidase, catalase, amylase, gelatinase, lipase, indole, H_2S production and nitrate reduction were also conducted to isolate and detect the pathogenic bacteria. In brief, firstly TVC and TCC were determined by using plate count agar and lactose broth, respectively. Subsequently, Nutrient agar, Eosin Methylene Blue (EMB), Barid Parker Agar (BPA), M-Aeromonas agar, Acetamide agar, MacConkey agar, Salmonella-Shigella (SS) agar, Brilliant Green Agar (BGA) and Thiosulphate Citrate Bile Salt Sucrose (TCBS) were used in subculturing the suspected colony to promote the growth of a particular type of bacteria. Selective media was used to obtain the pure culture. In every step, aseptic condition was highly ensured during the study period. Finally, the findings were carefully assessed the isolation and detection of bacteria.

Data analysis

The data were expressed as mean ± standard deviation (m ± SD). One-way ANOVA was done in order to determine whether there was a significant difference among the values. The student t-test was used to compare the samples. Statistics were considered significant at P values of <0.05.

Results

Bacteriological standards of raw, frozen and dried Taki

Log CFU/g ± SD was used to calculate the mean bacterial load of monthly collected raw fish or after frozen or dried fish samples. TVC values for fish evaluated in raw, frozen, and dried conditions were observed as 7.22±174, 5.75±0.098 and 4.28±0.510Log CFU/g ± SD, respectively. Bacterial load of raw Taki was significantly (P< 0.05) higher than those of frozen and dried samples. When compared between frozen and dried state, it has been demonstrated that dried fish contained significantly lower bacterial load than the frozen one (Figure 1).

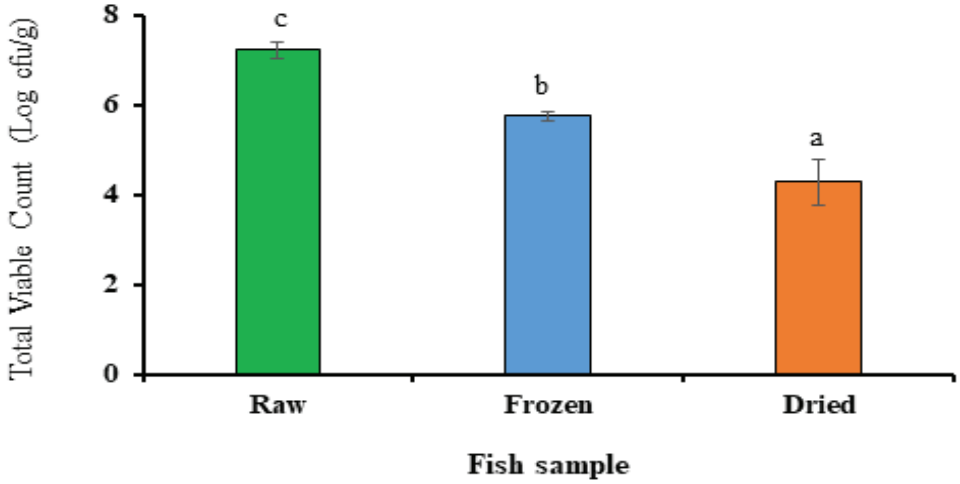

Figure 1. Bacterial load of raw, frozen and dried Taki. Values expressed as mean ± SD. Different superscripts indicates significantly different (P<0.05).

Total Coliform Count (TCC) of raw, frozen and dried Taki

The Total Coliform Counts (TCC) of raw, frozen and dried Taki are shown in Figure 2. It was found that the mean TCC of raw, frozen and dried Taki were 155.67± 44.261, 22.92± 2.884 and 14.89± 1.655 MPN/g, respectively. The mean Total Coliform Count showed that the number of coliform bacteria in raw fish was substantially ($P < 0.05$) higher than that in frozen and dried fish samples. In comparison between frozen and dried fish, the TCC value also significantly differed from each other.

Pathogenic bacteriological status in raw, frozen and dried Taki

Three pathogenic bacteria as *Escherichia coli, Salmonella* spp. and *Vibrio* sp. were detected from raw frozen and dried Taki based on cultural characteristics and morphological feature (Table 1). All these pathogenic bacteria were abundantly observed in raw fishes whereas only *E. coli* were detected in the few samples of frozen and dried fishes (Table 2).

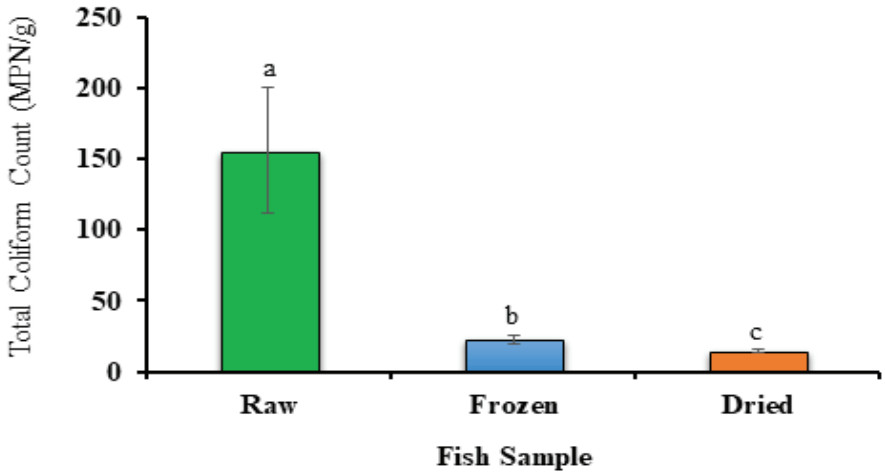

Figure 2. Total coliform count of raw, frozen and dried Taki. Data presented as mean± SD. Different superscript letter showing statistically different ($P<0.05$).

Table 1. Biochemical test for identification of pathogenic bacteria in raw, frozen and dried Taki.

Detected Species	*Color*	Shape	Motility	Gram's reaction	Indole	Catalase	TSI	MR	VP
Escherichia coli	Metallic green	Rod	+	-	+	-	Yellow	+	-
Salmonella spp.	Black	Rod	+	-	-	-	Black	+	-
Vibrio sp.	Yellow/ green	Curve	+	-	+	+	Black	+	-

Table 2. Pathogenic bacteriological status in raw, frozen and dried Taki.

Fish sample	No. of sample analyzed	Detected bacteria		
		E. coli	*Salmonella* spp.	*Vibrio* sp.
Raw	18	15	14	18
Frozen	18	3	0	0
Dried	18	2	0	0

Changes of bacterial load in frozen and dried Taki at different storage interval

The mean Total Viable Count (TVC) of frozen and dried Taki at different storage period is shown in Figure 3. The higher bacterial load was observed at start of the experiment which found to decrease within 7 days of storage period in case of both the samples. The bacterial load further decreased with the increase of preservation time although the bacterial load did not change significantly both in frozen and dried Taki after 14 days.

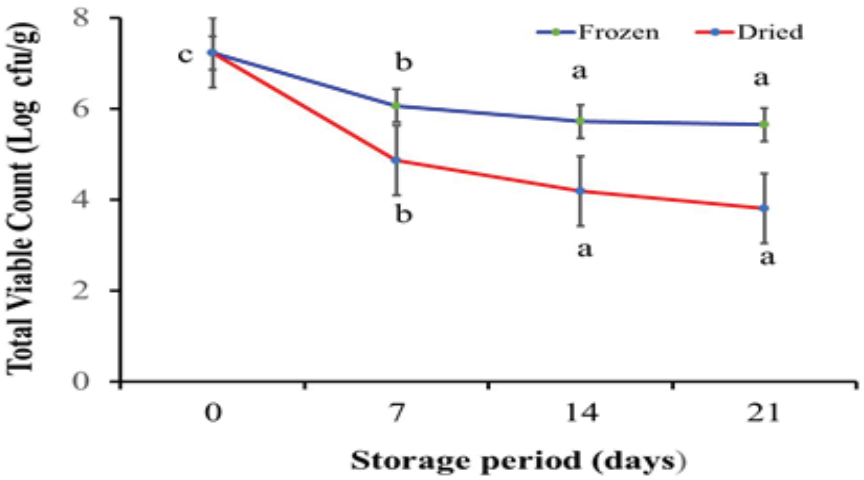

Figure 3. Changes in bacterial load of frozen and dried Taki at different interval of storage period. Data presented as mean ± SD. Different letter showing statistically different (P<0.05).

Discussion

Most often, microorganisms from the aquatic environment and post-harvest handling are carried in fish(Al-Sheraa et al., 2018, Rasul et al., 2021) which may remain even after processing and can make the fish and fishery products unsafe for the consumer's (Akter et al., 2018). In this study, the insight of bacterial population of raw, dried and frozen *Channa punctatus* was evaluated and found that the values varied significantly depending on the preservation process applied.

In case of the processed fish, significantly ($P<0.05$) higher bacterial load was observed in frozen Taki (5.75±0.098 Log CFU/g) than those of dried (4.28±0.510Log CFU/g) sample although both were complied with ICMSF standard (ICMSF, 1986).Low temperature preservation has been known to decrease the bacterial load which is evident in case of frozen Hilsha (Saeed, 2003), Chapila (Quaiyum et al., 2012), Pabda (Hossain et al., 2016) and Tilapia (Uddin et al., 2017). The lowering trend of bacterial load in dried Taki was similar to the study of dried *Channa striatus* and *Glossogobius giuris* (Majumdar et al., 2017).It has also been reported that sun-dried salted *C. punctatus* significantly improved the quality of fish by reducing microbial load (Farid et al., 2017). Although both freezing and drying technique are found effective in prompt declining of the bacterial load, our study revealed that drying technique is more suitable in minimizing the bacterial load in the studied species. This might happen due to maintain appropriate hygiene and sanitation in laboratory conditions during processing of raw materials for obtaining final product. It has also previously been observed that commercially prepared dried fish contain higher bacterial load than laboratory made dried fish (Patterson and Ranjitha, 2009; Rasul et al., 2018, 2020, 2021).

The existence of coliform bacteria revealed the risk of pathogenic bacteria probability in the studied samples that can be regarded as a food safety hazard (Hasan et al., 2013; Rasul et al., 2020). Significantly higher TCC was calculated in raw (155.67± 44.261 MPN/g) fish whereas lesser number was shown in frozen (22.92 ± 2.884 MPN/g) and dried (14.89± 1.655 MPN/g) Taki, respectively. Except raw Taki, frozen and dried Taki were complied with ICMSF (1986) standard. It was reported that the unfrozen pabda contains higher coliform bacteria than frozen one (Hossain et al., 2016). The presence of pathogenic bacteria in the harvested fish including *Bacillus species, Salmonella species, Shigella species, E. coli, Aeromonas species, Vibrio species, Pseudomonas species, Staphylococcus aureus* etc. can increase the risk that the fish might get contaminated from the environment and ultimately become unsafe for human consumption(Pal and Mahendra, 2015). In this study, three pathogenic bacteria such as *E. coli, Salmonella spp., Vibrio sp.* were identified from raw Taki, whereas only *E. coli* detected in frozen and dried *C. punctatus*. It was evident that *Vibrio* sp. was isolated from diseased *C. punctatus* (Sankar et al. 2012). Preservation by freezing method can prevent the growth of *Salmonella* spp., *Vibrio* sp. (Sanjee and Karim, 2016; Hossain et al., 2016).*E. coli* and *Salmonella* spp. were noted in sun dried *Mastacembelus armatus* (Akter et al., 2018). In this study, a very few fish was found to possess *E. coli* under dried conditions which although remained in acceptable range (ICMSF, 1986). Salted dried Taki remained within acceptable microbial range up to 24 months (Farid et al., 2017). However, due to improper packaging, faulty storage, and prolonged exposure in the retail market, the dried fish products have the potential to regain a significant quantity of moisture from the surrounding environment during the storage period, especially in high humid conditions that could increase the water activity ($a_{w)}$ resulting higher bacterial growth to the dried fish (Majumdar et al., 2017). Even though the current study only assessed the bacteriological load for shorter storage duration under both frozen dried circumstances, it would be advantageous to examine these bacteriological quality parameters under longer storage period.

In summary, present study provides information about bacteriological status of raw frozen and dried Taki prepared in laboratory conditions. Based on the findings, it can be mentioned that dried Taki contained lower bacterial load, Total Coliform Count (TCC) and pathogenic bacteria than the frozen sample that also complied with ICMSF standard. On the contrary, the bacteriological quality of raw Taki fish was unsatisfactory because of higher bacterial load (TVC), unacceptable limit of TCC and also the existence of pathogenic bacteria. The abundance of *E. coli* in frozen and dried Taki was very low. The bacterial load, total coliform and existence of pathogenic bacteria gradually decreased with the increasing of preservation times. Therefore, it can be assumed that higher bacterial load from the raw fish can be reduced by using suitable processing techniques as freezing or drying with proper hygiene and sanitation practices.

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