



Determination of microbiological quality of dried jat punti (*Puntius sophore*) collected from Sylhet district, Bangladesh

Md. Ashraf Hussain¹, Md. Abu Sayeed¹, Md. Lutful Kabir¹, Tofael Ahmed Sumon^{2*}, Sangita Das Himu³, Md. Afsar Ahmed Sumon⁴

¹Department of Fisheries Technology and Quality Control, Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh

²Department of Fish Health Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh

³Department of Aquatic Resource Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh

⁴Department of Fish Biology and Genetics, Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh

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*Corresponding Author

Tofael Ahmed Sumon
✉ sumons.h@hotmail.com

ABSTRACT

Dried fish is one of the most popular sources of dietary protein in Bangladesh and play a vital role in providing nutrition for the economically disadvantaged people. Yet there are frequent complaints on the quality of dried fish which are processed by traditional methods. Comparatively a few studies have been conducted on microbiological quality of dried fish produced in Bangladesh. Considering the above context, this study was carried out to investigate the microbiological quality of dried jat punti (*Puntius sophore*) available in Sylhet region of Bangladesh. The samples were collected over a period of five months from December 2014 to April 2015 from two drying yards of Mahtabpur (DY-1) and Toker Bazar (DY-2) and a retail of market of Sylhet district. Control samples were prepared in laboratory condition. Significantly highest and lowest TPC (Total plate count), TFC (Total fungal count) and TCC (Total coliform count) was found in samples of retail market and control, respectively. No significant differences were observed in TPC, TFC and TCC between DY-1 and DY-2. Besides this, 25 dried jat punti samples from each four sources were analyzed to detect *Escherichia coli*, *Salmonella* and *Vibrio cholera*. *E. coli* was found in all the samples (100%) of both drying yards and retail market but 60% in control samples. Highest amount (44%) of *Salmonella* was detected in retail market samples followed by DY-2 (36%) and DY-1 (32%), respectively. Samples of DY-1 (28%), DY-2 (24%) and retail market (40%) were found contaminated with *V. cholera* as well. However, *Salmonella* and *V. cholerae* were not found in control sample. Findings of this study discovered poor microbiological quality of dried jat punti of this region.

INTRODUCTION

Fish is one of the most important sources of animal protein and has been widely accepted as an excellent source of other elements such as vitamins and minerals for the maintenance of healthy body (Ravichandran et al. 2012). In 2013-14 fiscal year, Bangladesh produced 3.548 million MT of fish and it alone supplies 60% of total animal protein of the country (DoF 2014). It is estimated that around 20% of the local artisanal fish catch are sun dried and consumed in the domestic market of Bangladesh (Mazid and Kamal 2005). Sun drying of fishes is a simple, the oldest known and the least expensive method of fish preservation (Balachandran 2001). Dried fish is a very popular and delicious food item in the

coastal, central and north-eastern districts of Bangladesh (Nowsad 2007). An amount of 2,895 MT dried fish were exported (including salted and dehydrated fish) which accounted for 3.74% of total exported fishery products (FRSS 2015). However, the physical and organoleptic qualities of many traditional sun dried products are unsatisfactory for human consumption (Nowsad 2007).

The traditional drying of fish is mainly performed by poor fishermen who are mostly illiterate (Azam et al. 2003). Hence, traditional drying is carried out in open place under direct sunlight without maintaining proper hygiene and sanitation. Besides, during the monsoon, when the humidity is high, proper drying cannot be achieved by

traditional methods where fish can absorb the moisture and it serves as a habitat for microbial population such as bacteria, fungi and insect attack (Azam 2002). Moreover, fish is a reservoir of large number of microorganisms. Some are inherent, coming from where the fish is caught, and others are from contaminations at various stages of handling from the farm to fork. Majority of these microorganisms are non-pathogenic causing only spoilage of the fish, but there are some pathogenic microorganisms which can cause food poisoning (Valsan et al. 1985).

The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. Growth of fungus causes off flavours, soften the flesh and some can produce potentially dangerous mycotoxins under certain circumstances (FAO 1982) which cause considerable decrease in the consumption of dried fish. Apart from this, other common sources of contamination are air and dust in and around fish processing zone, contaminated water and soil and unhygienic handling (FAO 1982; Prabhakaran and Gupta 1990).

Furthermore, fishery products are also recognized as a carrier of food borne bacterial pathogens found in the internal and external surfaces of the fishes (Huss 1997) like *Salmonella*, *Vibrio* sp., *E. coli* and *Listeria* sp. (Venugopal et al. 1999; Venugopal et al. 2002). The incidence of pathogenic microbes in dried fishes is also reported (Prakash et al. 2011; Logesh et al. 2012; Sulieman and Mustafa 2012; Immaculate et al. 2013). Therefore, complaints from the consumers about the quality of the dried fish products are quite common. Determination of microbiological quality of such processed fishes is very important for guarding consumer's health and hygiene (Lilabati et al. 1999).

In spite of playing a key role in the production and export, there was no study found addressing the microbiological quality of dried fishes produced in Sylhet region. Henceforth, we felt urge to conduct this study to evaluate the microbiological quality of dried jat punti (*Puntius sophore*) which is one of the most popular and highly produced dried fish in Sylhet.

MATERIALS AND METHODS

Collection of samples

Jat punti (*Puntius sophore*) subjected to drying were collected for a period of five months (December 2014 to April 2015) from two drying yards viz. Mahtabpur drying yard (DY-1) and Toker bazar drying yard (DY-2) and a dry fish retail market of Sylhet district. Collected samples were packed in clean air tight polythene bags and immediately brought to the laboratory. Fresh jat punti were purchased from local markets to prepare control samples in laboratory condition.

Total plate count (TPC)

Enumeration of bacterial load was done using plate count agar (PCA) by spread plate technique. Twenty-five grams of the sample was mixed with 225 ml Butterfield buffered phosphate diluent. Appropriate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24-48 hours and the colonies were counted for total plate count and the count was expressed as cfu/g (Yong, 1992).

Total fungal count (TFC)

Fungal count was carried out by using sabouraud dextrose agar (SDA) to which Chloramphenicol (antibiotics) was incorporated. Twenty-five grams of the sample was blended with 225 ml of 0.05% agar in saline solution (0.85% NaCl) and 0.1 ml of the appropriate dilutions of the sample was spread on the surface of the medium and incubated at room temperature (28±1°C) for 3-5 days and the colonies were counted for total fungal count and the count was expressed as cfu/g (Yamagata, 1992).

Total coliform count (TCC)

The MPN (Most Probable Number) technique was used to determine the level of coliforms in dried fish samples. The samples to be tested were prepared in 10-fold dilution series, and then 1 ml aliquots of three selected dilution (10^{-1} , 10^{-2} and 10^{-3}) was inoculated into each of 3 lauryl sulphate tryptose broth (LSTB) tubes containing Durham's tube. After that LSTB tubes were incubated at

37°C for 48 hours and observed for growth and gas production. Any tube producing gas was considered positive for the presence of coliforms. MPN count/100 g of the sample was calculated using the MPN table (Yong, 1992).

Detection of pathogenic bacteria

E. coli

Dry fish homogenate was transferred to LSTB tubes and incubated at 37°C for 24 hours and observed for growth and gas production. Samples from positive LSTB tubes were transferred to EC broth tubes and incubated at 37°C for 24-48 hours. Samples from positive EC broth was streaked on to eosine methylene blue (EMB) agar plate to confirm the *E. coli*. Black or dark centred colonies with or without greenish metallic sheen were produced by *E. coli* (AOAC, 1998).

Salmonella

In the detection of *Salmonella*, lactose broth (LB) was used as pre-enrichment and tetrathionate broth and selenite cystine broth were used in enrichment. For *Salmonella* isolation, xylose lysine deoxycholate (XLD) was used. Further biochemical tests were done for identification. *Salmonella* exhibited pink colonies with or without black centres (FDA BAM, 2007).

Vibrio cholerae

V. cholerae was detected by the method described by Yong (1992). Fifty gram of the samples was

mixed with 200 ml of alkaline peptone water and incubated at 35°C for 6-8 hours. After the incubation period, a loopful obtained from the pellicle (surface growth) was streaked onto thiosulfate citrate bile salts sucrose (TCBS) agar. Then TCBS agar plates were incubated at 35°C for 24 hours. *V. cholerae* exhibited large, smooth and yellow colored colonies on TCBS agar. Further biochemical tests were done for identification.

Data analysis

All data were subjected to statistical analysis using one-way ANOVA (IBM SPSS, Version 20.0). Probabilities of $P < 0.05$ were considered significant. Significance differences between means were evaluated using the Duncan's multiple range test.

RESULTS AND DISCUSSION

TPC

TPC of dried jat punti varied with their sources and months. Highest TPC ($8.58 \pm 0.46 \times 10^6$ cfu/g) was estimated in retail market sample and lowest ($2.20 \pm 0.15 \times 10^5$ cfu/g) in control sample (Table 1). In every month TPC of retail market sample was found significantly ($P < 0.05$) highest and TPC of control sample was observed significantly lowest. TPC of DY-1 did not significantly differed with DY-2 in all months but March. TPC of dried jat punti were found to be higher in the month of April and lower in the month of February.

Table 1
Total plate count of dried jat punti in different months.

Months	TPC (cfu/g)			
	Drying yard-1	Drying yard-2	Retail Market	Control
December'14	$4.85^b \pm 0.31 \times 10^6$	$5.50^b \pm 0.48 \times 10^6$	$6.90^c \pm 0.55 \times 10^6$	$4.58^a \pm 0.43 \times 10^5$
January'15	$3.60^b \pm 0.45 \times 10^6$	$3.07^b \pm 0.25 \times 10^6$	$6.57^c \pm 0.35 \times 10^6$	$2.62^a \pm 0.16 \times 10^5$
February'15	$3.31^b \pm 0.20 \times 10^6$	$3.85^b \pm 0.73 \times 10^6$	$7.10^c \pm 0.26 \times 10^6$	$2.20^a \pm 0.15 \times 10^5$
March'15	$4.97^c \pm 0.45 \times 10^6$	$3.60^b \pm 0.18 \times 10^6$	$7.17^d \pm 0.68 \times 10^6$	$3.03^a \pm 0.20 \times 10^5$
April'15	$5.52^b \pm 0.62 \times 10^6$	$6.10^b \pm 0.20 \times 10^6$	$8.58^c \pm 0.46 \times 10^6$	$5.20^a \pm 0.23 \times 10^5$

Values are means \pm SD of triplicate groups and different superscripts in each row represent significant difference ($P < 0.05$).

Table 2
Total fungal count of dried jat punti in different months.

Months	TFC (cfu/g)			
	Drying yard-1	Drying yard-2	Retail Market	Control
December'14	3.81 ^b ±0.27×10 ³	2.25 ^b ±0.23×10 ³	5.67 ^c ±0.30×10 ⁴	1.98 ^a ±0.35×10 ²
January'15	2.25 ^b ±0.30×10 ³	2.55 ^b ±0.22×10 ³	4.88 ^c ±0.37×10 ⁴	1.15 ^a ±0.10×10 ²
February'15	2.62 ^b ±0.17×10 ³	3.45 ^c ±0.37×10 ³	4.63 ^d ±0.37×10 ⁴	1.25 ^a ±0.13×10 ²
March'15	4.10 ^b ±0.28×10 ³	3.87 ^b ±0.57×10 ³	6.80 ^c ±0.46×10 ⁴	1.82 ^a ±0.40×10 ²
April'15	5.03 ^b ±0.25×10 ³	4.68 ^b ±0.28×10 ³	7.43 ^c ±0.25×10 ⁴	2.47 ^a ±0.21×10 ²

Values are means ± SD of triplicate groups and different superscripts in each row represent significant difference (P<0.05).

Quality levels are based on the plate counts with representative sample unit less than 5×10⁵ cfu/g is considered as good quality while, plate count between 5×10⁵ and 10⁷ cfu/g is marginally accepted quality and plate count at or above 10⁷ cfu/g is considered as unacceptable for human consumption (ICMSF 1986). In the present study, bacterial count of dried jat punti from DY-1, DY-2 and retail market was found in the marginally accepted quality category and bacterial count of control sample was observed in the category of good quality. Patterson and Ranjitha (2009) stated total plate count seemed to be high in the commercially dried fishes than the experimentally dried fishes which is similar with findings of the present study. Logesh et al. (2012) observed highest TPC of 5.3×10⁶ cfu/g in dried fish (*Sardinella longiceps*) of Cuddalore district in India. Similarly, Saritha et al. (2012) reported higher bacterial count such as 2.13×10⁶ cfu/g was observed for the sun dried fish (*Paraupeneus indicus*). Islam et al. (2013) found 2.3×10⁵ cfu/g TPC in dried punti (*Puntius* sp.) from Natore district, Bangladesh. Variation in TPC in different months may be due to the monthly variation of temperature and moisture content in atmosphere (Logesh et al. 2012). The result is also supported by Lilabati et al. (1999) and Prakash et al. (2011) and they reported that there was a direct relationship between the microbial counts and moisture content of the sample.

TFC

Estimated TFC of dried jat punti is presented in Table 2. Variations in TFC of dried jat punti with

months and their sources were also observed. Uppermost TFC of 7.43 ±0.25×10⁴ cfu/g was determined in retail marker sample, whereas lowermost (1.15 ±0.10×10² cfu/g) was found in control sample. TFC of retail market samples were significantly (P< 0.05) highest and TFC of control samples were significantly lowest in every moths. No significant difference was observed between the TFC of DY-1 and DY-2 in each month except February.

The quality of dry fishes can adversely be affected by occurrence of fungi (FAO, 1982). In this study, monthly variation in the fungal population was observed in all the dried fish samples. Kumar (2008) estimated highest TFC of 1.5×10⁴ cfu/g in dried fish collected from market of Southeast coast of India. Likewise, Saritha et al. (2012) reported highest fungal count of 2.1×10⁴ cfu/g in dry fishes of Cuddalore market. The results of this study are paralleled to the report of Patterson and Ranjitha (2009) where they observed higher TFC in the commercially dried fishes than the experimentally dried fishes. The presence of high fungal count in dried fish may be due to post harvest delay, improper transportation, unhygienic handling and processing during the salting and sun drying process, contaminated working floor, salt and water (Saritha et al. 2012). Additionally, presence of different types of fungi and bacteria in dried fishes has been reported by several researchers (Kumar 2008; Prakash et al. 2011; Logesh et al. 2012; Saritha et al. 2012). The fungus *Aspergillus flavus* is responsible for the production of aflatoxin and it is also found that it cause food borne intoxication which leads to serious health hazards.

Hashem (2011) have studied the mycotoxins from the fishes and recorded that *Aspergillus* is the main genus that commonly involved in the production of mycotoxins. Thus the presences of these fungi are of great significance in view of food safety and quality.

TCC

TCC of dried jat punti is shown in Table 3. Significantly ($P < 0.05$) highest and lowest TCC was observed in the sample of retail market and control, respectively. TCC of retail market sample was ranged from 71.33 ± 6.35 to 96.00 ± 22.64 MPN/g and TCC of control sample was ranged between 20.00 ± 3.60 and 30.00 ± 7.74 MPN/g. TCC of DY-1 and DY-2 did not indicate any significant difference. TCC of dried jat punti from all four sources was found to be highest in April.

Table 3
Total coliform count of dried jat punti in different months.

Months	TCC (MPN/g)			
	Drying yard-1	Drying yard-2	Retail Market	Control
December'14	$57.00^b \pm 12.12$	$60.67^b \pm 16.25$	$81.00^c \pm 10.39$	$28.33^a \pm 6.11$
January'15	$39.67^b \pm 2.89$	$36.67^b \pm 7.09$	$71.33^c \pm 6.35$	$21.00^a \pm 6.00$
February'15	$50.00^b \pm 12.12$	$41.33^b \pm 2.89$	$73.67^c \pm 16.74$	$20.00^a \pm 3.60$
March'15	$60.67^b \pm 16.25$	$64.33^b \pm 18.47$	$87.00^b \pm 10.39$	$28.00^a \pm 7.54$
April'15	$67.67^b \pm 6.35$	$71.33^b \pm 6.35$	$96.00^c \pm 22.64$	$30.00^a \pm 7.74$

Values are means \pm SD of triplicate groups and different superscripts in each row represent significant difference ($P < 0.05$).

Table 4
Status of pathogenic bacteria in dried jat punti.

Sources of samples	Number of analyzed samples	Frequency (Percentage)		
		<i>E. coli</i>	<i>Salmonella</i>	<i>Vibrio cholerae</i>
Drying yard-1	25	25 (100)	8 (32)	7 (28)
Drying yard-2	25	25 (100)	9 (36)	6 (24)
Retail market	25	25 (100)	11 (44)	10 (40)
Control	25	15 (60)	ND	ND

ND- Not detected.

Pathogenic or indicator bacteria may not be present sufficiently in large numbers in water or food to be detected by plating methods. In such cases, MPN methods are used, where large volumes of samples can be used for inoculation. MPN is only a statistical approximate measure on the test bacteria in the given sample and not the actual number. MPN method is used to detect the coliform bacteria in water or food (Surendran et al. 2006). Prakash et al. (2011) measured the total coliforms of dried fish 30-65 MPN/100g, 45-115 MPN/100 g and 65-150 MPN/100g in summer, monsoon and post-monsoon, respectively. Total coliform group can be sub grouped as faecal and

non-faecal coliforms. The faecal coliforms are derived from faeces of human and other warm-blooded animals such as cows, sheep, poultry etc. The non-faecal subgroup is frequently found on vegetation and in the soil; some are plant pathogens (ICW 1972). The presence of faecal coliform organisms indicate recent and possibly hazardous faecal pollution. The most common faecal coliform species is *E. coli* (Kabler and Clark 1960).

Detection of pathogenic bacteria

All the samples of DY-1, DY-2, retail market and 60% of control samples were found contaminated with *E. coli*. *Salmonella* was detected in 44% samples of retail market. It was also identified in 32% samples of DY-1 and 36% of DY-2. Meanwhile, it was observed that 28%, 24% and 40% samples of DY-1, DY-2 and retail market contaminated with *V. cholerae*, correspondingly. However, control samples were recorded devoid of *Salmonella* and *V. cholerae*.

Patterson and Ranjitha (2009) observed higher *E. coli* in commercial dried fish than experimental dried fish which is concurred with findings of the present study. Prakash et al. (2011), Saritha et al. (2012) and Immaculate et al. (2013) mentioned that they found all the analyzed dried fishes contaminated with *E. coli*. The presence of *E. coli* indicates the dried fish samples contaminated with total and faecal coliforms. Normally coliforms are normal flora of human and animal intestine (Kakatkar et al. 2010). Faecal contamination in the landing canter, washing the catches in polluted water with the disposal of sewage, reused water and improper disposal of faecal materials are the possible sources for coliform contamination in dried fish samples (Saritha et al. 2012).

Salmonella are widely distributed in nature and they survive well in a variety of foods. There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia 2008). *V. cholerae* is the causative agent of cholera (an enteric diarrheal disease) in humans and continues to be a worldwide health concern (Kaper et al. 1995). Cholera has been categorized as one of the emerging and re-emerging infections in developing countries (Satcher 1995) and is classified as Category B bioterrorism by Centre for Disease Control and Prevention (WHO 2008).

Presence of *Salmonella* and *Vibrio cholerae* species contamination in dried fishes was observed by enriching the samples and plating them on selective plates. Samples were considered positive when typical colonies appeared on selective plates. In recent years, contamination of fish and fishery products with *Salmonella* and *Vibrio* sp. has been reported by many researchers in different parts of India (Prakash et al. 2011; Logesh et al. 2012;

Immaculate et al. 2013) and Bangladesh (Sultana et al. 2010; Mrityunjy et al. 2013). Though, Azam et al. (2003) and Saritha et al. (2012) observed the absence of the spoilage organisms *Vibrio* sp. and *Salmonella* sp. in all the dry fish samples. Incidence of pathogens in the sample of fish market may be attributed to external contamination (Iyer and Shrivastava 1989) and poor handling at ambient temperature (Jedah et al. 1998). In some of the cases, the food borne illness such as scombroid poisoning is observed in dry fishes mainly due to the chemical agent (histamine) and it is also known as histamine poisoning; *E. coli* is responsible for the production of histamine in the dried fishes (Logesh et al. 2012). In rare cases, *Salmonella* and *Staphylococcus* species produce histamine residue (Huang et al. 2010). So safety measures should be taken to reduce the contaminations of *Salmonella* and *V. cholerae* to ensure the food safety.

CONCLUSION

Results of the present study revealed poor microbiological condition in dried jat punti which may be caused by the unhygienic handling, poor processing, improper storage and inadequate packaging of the products. Therefore, control measures such as ensuring scientific method of fish drying (e.g. use of good quality raw material, good quality salt, hygienic handling practices, potable water, good quality packaging material), training of the fisher folks and increasing the awareness of mass people about food safety should be taken.

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