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Microbiological Properties of Dry Salted Hilsa, *Tenualosa ilisha* (Hamilton, 1822) Fish of Bangladesh

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ABSTRACT

The present study was conducted to assess microbiological quality of dried salted Hilsa (*Tenualosa ilisha*) samples collected from three different fish retailed markets (Chandpur, Chittagong and Narsingdi) and control samples prepared in laboratory environment. This study revealed that total plate count (TPC) in retail markets and control samples ranged from $5.75\pm0.35\times10^6$ to $8.74\pm0.39\times10^7$ cfu/g and $2.23\pm0.15\times10^5$ to $3.13\pm0.20\times10^5$ cfu/g, respectively. In samples of retail markets total fungal count (TFC) ranged from $5.67\pm0.30\times10^4$ to $7.43\pm0.25\times10^4$ cfu/g, TFC in control samples ranged from $1.15\pm0.10\times10^2$ to $2.47\pm0.21\times10^2$ cfu/g. It also found that total coliform count (TCC) in retail market and control samples ranged from 73.27±16.74 to 94.03±20.14 MPN/g and 20.11±2.39 to 31.45±5.74 MPN/g, respectively. Both samples for all counts, the highest TPC was found in Narsingdi sample and the lowest was in Chandpur sample. Besides this, 25 dry salted Hilsa samples from each three sources were analyzed to detect *Escherichia coli*, *Salmonella* and *Vibrio cholerae*. Maximum *E. coli* was found in Chittagong (100%) and Narsingdi (100%) retail market samples. In Chandpur, 52% of *E. coli* was found in control sample. The highest amount (76%) of *Salmonella* was detected in retail market of Narsingdi samples followed by Chittagong (36%) and Chandpur (32%), respectively. For control, *Salmonella* was detected in Narsingdi. Retail market samples of Chandpur (06%), Chittagong (09%) and Narsingdi (16%) were found contaminated with *V. cholerae* as well. However, *V. cholerae* were not found in control sample. Findings of this study indicated that the retail market and control samples of Chandpur were good quality for consumption, both samples of Chittagong and Narsingdi were more contaminated thus reflects the unhygienic condition of the markets.

INTRODUCTION

Dry fish (shutki in Bengali) is one of the popular food items in Bangladesh. It is the staple source of protein in many areas of our country like Chittagong, Dhaka, Chandpur, Narsingdi, Kuakata, Barisal etc. In Bangladesh salted sun-drying of fish have been used for centuries and dressed fish is mixed with salt to remove the water from fish body and is carried out in the open air using the energy of the sun to evaporate the water and air currents to carry away the vapor. Dried salted fish is an important source of animal protein and people eat dried fish as a tasty dish all over the country. For this reason about 20% of total fish caught are salted dried and mostly consumed in the domestic market annually (DoF, 2011). In Asia, Bangladesh is ranked as third largest aquaculture producing country after China and India (DoF, 2012). Fisheries items are the major protein source of Bangladesh which contributing 58% of the nation's animal protein demands (Ali *et al.*, 2012). The current fish consumption rate is 17.52 kg/people/year whereas the demand is 20.44 kg/people/year and is 29.74 MT per year (Kabir *et al.*, 2012, Minar *et al.*, 2012). Fisheries is the second amongst the top earning export sectors in Bangladesh and provide direct or indirect employment to about 10% of total population of the country (DoF, 2006). Every year Bangladesh earns foreign currency by exporting dried salted fishes. During the year 2008-09, Bangladesh exported 309.35 metric tons dried salted fish and fishery products and earned 119.9 million taka (DoF, 2006). The contribution of dried salted fish and fishery products in national income, employment, health, nutrition

and foreign exchange earnings is increasing (Noor *et al.*, 2013, Antony *et al.*, 2002).

Fish as a food is easy to digest and contains high nutritional value (Leisner *et al.*, 2001). These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria (Adam *et al.*, 1999). Fish is one of the most perishable (Sallam, 2007; Adebayo-Tayo *et al.*, 2012d) and difficult to handle of all foods. Its quick perish ability has been the main hurdle in its preservation (Okoro *et al.*, 2010). Bacterial activity is by far the most important factor influencing fish quality (Gram and Huss, 1996). According to Adams and Moses (2008), the normal bacterial load of the surface slime of fish can range from $10^2 - 10^7$ CFU/cm^2 and the Gills and Intestines can range up to 10^3 and 10^7 CFU/g respectively. Hood *et al.* (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g).

Bacteria are considered to play a dominant role in fish contamination (Huss *et al.*, 1974). Micro flora found in fish is related to various factors such as developmental stage of the fish, digestive tract structure, water temperature, site, food availability, and physiological state of the organism (Sugita *et al.*, 1985). *Salmonella* species are motile, rod-shaped and gram negative bacteria. Contamination of fish with *Salmonella* is due to growth in polluted waters and poor handling, hygiene and sanitation standards after harvesting and is a major public health concern. Evidence from other studies indicates that from 1 to 10 cells may constitute an infectious dose in some circumstances (D'Aoust *et al.*, 1985; Kapperud *et al.*, 1990). *E. coli* is a gram-negative, non-spore-forming short rod-shaped bacterium capable of growth and gas production at 45.5ºC (except when testing water, shellfish, and shellfish harvest water, which use 44.5ºC) in lactose-containing medium (Kornacki and Johnson, 2001). Mead and Griffin (1998) reported doses as low as 50 cells can be infectious and in one reference the FDA has indicated that as few as 10 cells may be adequate to cause illness (FDA, 2001).

Hilsa (*Tenualosa ilisha*) is the most important species among the other Toil shads or Chinese herrings. As fish are highly perishable food item so they start to spoil as soon as they are harvested (Balachandran, 2001). Deterioration of the fish flesh is caused by the action of enzymes, by micro-organism and by chemical action. During transportation of fish, there is a great chance to be contaminated by bacteria (Clucas and Ward, 1996). In the past Hilsa was the only species in Bangladesh that is commercially processed by salting. However, to the best of our knowledge, there are no such reports related to microbiological properties of dried salted Hilsa. Therefore, the objectives of this study are to assess microbiological quality of dried salted Hilsa (*Tenualosa ilisha*) fish samples collected from different fish retailed markets and control dried salted Hilsa fish samples prepared in laboratory environment.

MATERIALS AND METHODS

Collection of sample

The dry salted Hilsa (*Tenualosa ilisha*) fish were collected from the Local Fish Market of Chandpur (23.25° N, 90.8333° E), Chittagong (22.4875° N, 91.9633° E) and Narsingdi (24.00° N, 90.8333° E) of Bangladesh. The collected Hilsa were transported to the Laboratory in the Department of Fisheries Technology, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University (24.0379° N, 90.3996° E) with the help of clean airtight polyethylene bags. Fresh fishes were purchased from those local markets to prepare control samples in laboratory condition. The samples were stored in freezer at -20°C for subsequent analysis.

Total plate count (TPC)

Determination of bacterial load was done using plate count agar (PCA) by spread plate technique. Ten grams of the sample was mixed with 10 fold volume of physiological saline (0.85% NaCl). Appropriate dilutions of fish homogenate were spread on plate count agar and incubated at 37°C for 24 hours and the colonies were counted for total plate count and the count was expressed as cfu/g (APHA, 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/100g of the sample was calculated using the MPN table (Yong, 1992).

Presence of Pathogenic Bacteria

Escherichia 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).

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.

Salmonella

In the detection of *Salmonella*, lactose broth (LB) was used as pre-enrichment and tetrathionate broth and seletinecystine 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).

Data analysis

Analysis was performed in triplicates and the results expressed as mean ± standard deviation using Statistical Package for the Social Sciences (SPSS) 16.0 for windows (SPSS, SAS Institute Inc. Cary, USA).

RESULTS AND DISCUSSION

Total Plate Count

Total plate count (TPC) of dried salted Hilsa, *Tenualosa ilisha* varied with their different sources is presented in Table 1. The highest TPC ($8.74\pm0.39\times10^7$ cfu/g) was estimated in retail market sample and the lowest ($2.23\pm0.15\times10^5$ cfu/g) in control sample (Table 1). In every regions, TPC of retail market sample was showed significantly ($P < 0.05$) the highest and TPC of control sample was observed significantly also the lowest. On the other hand, comparatively lower TPC was found in Chandpur and Chittagong.

Table 1: Total plate count of dried salted Hilsa in different regions

Regions	Total Plate Count (cfu/g)	
	Retail market	Control
Chandpur	$5.75\pm0.35\times10^6$	$2.23\pm0.15\times10^5$
Chittagong	$7.17\pm0.68\times10^6$	$2.62\pm0.16\times10^5$
Narsingdi	$8.74\pm0.39\times10^7$	$3.13\pm0.20\times10^5$

Quality levels are based on the plate counts with representative sample unit less than 5×10^5 cfu/g is considered as good quality while, plate count between 5×10^5 and 10^7 cfu/g is marginally accepted quality and plate count at or above 10^7 cfu/g is considered as unacceptable for human consumption (ICMSF, 1986). In the present study, bacterial count of dried salted Hilsa from retail market of Chandpur and Chittagong was found in the marginally accepted quality category and bacterial count of all control samples were observed in the category of good quality. Only the retail market sample of Narsingdi exceeds the acceptable limit. 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 5.3×10^6 cfu/g in dried fish (*Sardinella longiceps*) of Cuddalore district in India. Similarly, Saritha *et al.* (2012) reported higher bacterial count such as 2.13×10^6 cfu/g was observed for the sun dried fish (*Paraupeneus indicus*). Islam *et al.* (2013) found 2.3×10^5 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.

Total Fungal Count

Estimated total fungal count (TFC) of dried salted Hilsa, *Tenualosa ilisha* is presented in Table 2. Variations in TFC of dried salted Hilsa, *Tenualosa ilisha* with various sources were also observed. Uppermost TFC of $7.43\pm0.25\times10^4$ cfu/g was determined in retail market sample of Narsingdi, whereas lowermost ($1.15\pm0.10\times10^2$ cfu/g) was found in control sample of Chandpur. TFC of retail market samples were significantly ($P < 0.05$) the highest and TFC of control samples were significantly the lowest in every regions.

Table 2: Total fungal count of dried salted Hilsa in different regions

Regions	Total Fungal Count (cfu/g)	
	Retail market	Control
Chandpur	$5.67\pm0.30\times10^4$	$1.15\pm0.10\times10^2$
Chittagong	$4.88\pm0.37\times10^4$	$1.82\pm0.40\times10^2$
Narsingdi	$7.43\pm0.25\times10^4$	$2.47\pm0.21\times10^2$

The quality of dry fishes can adversely be affected by occurrence of fungi (FAO, 1982). In this study, regional variation in the fungal population was observed in all the dried fish samples. Kumar (2008) estimated the highest TFC of 1.5×10^4 cfu/g in dried fish collected from market of Southeast coast of India. Likewise, Saritha *et al.* (2012) reported the highest fungal count of 2.1×10^4 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.

Total Coliform Count

Total coliform count (TCC) of dried salted Hilsa, *Tenualosa ilisha* is shown in Table 3. Significantly ($P < 0.05$) the highest and the lowest TCC were observed in the sample of retail market and control, respectively. TCC of retail market sample was ranged from 73.27±16.74 to 94.03±20.14 MPN/g and TCC of control sample was ranged between 20.11±2.39 and 31.45±5.74 MPN/g. TCC of dried salted Hilsa from all sources was found to be the highest in retail market samples.

Table 3: Total coliform count of dried salted Hilsa in different regions

Regions	**Total Coliform Count (MPN/g)**	
	Retail market	**Control**
Chandpur	73.27±16.74	20.11±2.39
Chittagong	81.83±10.19	28.33±6.11
Narsingdi	94.03±20.14	31.45±5.74

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/100g 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 presences 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

For retail market and control samples, the highest *E. coli* was found in Narsingdi sample and the lowest was found in Chandpur sample (Table 4). *Salmonella* was detected in 36%, 44% and 76% retail market samples of Chandpur, Chittagong and Narsingdi, respectively. On the other view, no *Salmonella* was found in control sample of Chandpur, Chittagong except Narsingdi (4%). Meanwhile, it was observed that 6%, 9% and 16% retail market samples of Chandpur, Chittagong and Narsingdi contaminated with *V. cholerae*, correspondingly. However, control samples for all regions were recorded devoid of *V. cholerae*.

Table 4: Status of pathogenic bacteria in dried salted Hilsa (*Tenualosa ilisha*)

Regions	**Number of analyzed samples**	***Escherichia coli* (%)**		***Salmonella* (%)**		***Vibrio cholerae* (%)**	
		Retail Market	**Control**	**Retail Market**	**Control**	**Retail Market**	**Control**
Chandpur	25	19 (76)	13 (52)	09 (36)	ND*	06 (24)	ND
Chittagong	25	25 (100)	16 (64)	11 (44)	ND	09 (36)	ND
Narsingdi	25	25 (100)	18 (72)	19 (76)	04 (16)	16 (64)	ND

* ND- Not detected

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; Mrityunjoy *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 that both retail market and control samples of Chandpur showed best quality among the others samples of Chittagong and Narsingdi. It also exposed that poor microbiological condition in dried salted Hilsa (*Tenualosa ilisha*) 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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