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Microbiological risk assessment of frozen fishes in relation to their effects of different processing treatments

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Abstract

In the present experiment, four frozen fishes (Silver Carp, Rui, Hilsha, and Rupchada) were analyzed bacteriologically which were collected from three local markets and a super market, to enumerate total microbial and total coliform count, and detection of *Pseudomonas sp.*, *Staphylococcus sp*. Among the different frozen fish samples the highest total microbial count was found in Silver carp (*Hypophthalmichthys molitrix*, 9.87×10^5 CFU/g) and lowest bacterial count was found in the sample of Rui (*Labeo rohita*, 4.72×10^5 CFU/g). *Pseudomonas* count was highest (12.50×10^5 CFU/g) in Hilsha (*Tenualosa ilisha*) and lowest in Rui (3.11×10^5 CFU/g).*Staphylococci* count was highest in Hilsha(10.70×10^3 CFU/g) and lowest also in Hilsha (3.40×10^3 CFU/g). Coliform count was highest (1100 MPN/g) in Hilsha and lowest in Rupchada (*Stromateus niger*, 15MPN/g). High microbial load in frozen fishes indicate these samples were unacceptable, and the presence of coliform indicated that the raw frozen fish is not safe for consume. Highest percentage of microbial load reduction was documented after washing with acetic acid 5% concentration in Silver Carp while the lowest reduction was observed in Hilsha.

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Introduction

Fish has been wide accepted as a good source of protein and other elements for the maintenance of healthy body. It represents about almost 14% of all animal protein on a global basis (Abolagaba and Melle, 2008; Clucas and Ward, 1996). In Asian countries over 50% of the animal protein intakes comes from fish while Africa, the proportion is17.50% (Williams, *et. al.*, 1988).

Along with consumption of microbiologically spoiled fish, the off-odor and off-taste of the products caused by oxidation of lipids and some other metabolites may largely affect the consumer acceptability (Moini *et al*, 2009; Rostamzad *et al.*, 2010). For maintenance better quality of fish preservation is very necessary. Good preservation retains the nutritional quality of fish. Storage time and temperature are the major factors affecting the rate of loss of quality and shelf life of fish (Whittle, 1997).

Fishes are mainly contaminated by Coliform, *Pseudomonas, Staphylococci*. Coliforms include all aerobic and facultitively anaerobic gram negative non spore forming bacilli which ferment lactose with gas formation within 48 hours at 35 °C. Coliforms include psychotropic type microbes capable of multiplying at 3-10 °C. Thus they can multiply in foods even when refrigerated. *Staphylococcus* are non-motile cocci that are catalase positive and facultatively anaerobic, having both an oxidative and fermentative type of metabolism. In the lab, *S. aureus* produces white to golden colored colonies and is positive for the coagulase test. *Pseudomonas* is a genus of gammaproteobacteria, belonging to the family pseudomonadaceae containing 191 validly described species (Euzeby, 1997). The best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth promoting *P. fluorescens* (Matthijs, *et. al.,* 2007).

Fishery products, which are of great importance for human nutrition and provide clear health benefits, can also act as a source of various food borne diseases (Darlington and Stone, 2001). Export market of Bangladesh is threatened with low quality processed foods which may be contaminated with pathogenic bacteria such as coliform, faecal coliform, *Pseudomonas, Staphylococcus* etc. (Noor, *et al.*, 2013). In the recent time, modern biotechnology has introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life of fish (William and Michael, 2009). Research on fish contamination assessment and the way of preservation is also increasing to maintain quality of fish products (Okoro*et al.* 2010; Begum *et al.* 2010; Prabakaran*et al.* 2011; Anbudhasan*et al.* 2012).

Fisheries sector plays an important role in the national economy as well as in socio-economic development of Bangladesh. It contributes 3.74 % in national GDP, 2.7 % in export earnings, and 22.23 % of the agriculture sector and supplying about 58 % of the domestic animal protein consumption (DoF, 2011). But every year huge fish and fish product rejected from the foreign country because of spoilage by microorganism.

The study was designed to examine the hygienic status of frozen fishes in three local markets and a supermarket. The work reports on the isolation and detection of specific fish borne pathogens, e.g.- coliform, *Staphylococcus sp*. and *Pseudomonas sp*., to assess the bacterial quality of fish for consumption, and to find the way to remove these spoilage microorganisms from Hilsha (*T. ilisha*) fish through different chemical treatments.

Materials and methods

This experiment has been conducted in the department of Biotechnology and Genetic Engineering, Islamic University, Kushtia during the year 2012-13 in order to assess the bacteriological load of fish and their changes in different treatments.

Study area and sampling

Four different markets near the Islamic University, Kushtia were selected as sampling sites for the

investigation. Four samples were collected in each area. Samples were collected aseptically early in the morning and taken in sterile polyethylene bags with ice and transported immediately to the laboratory.

Sample preparation

About 10 gm of fish sample was cut from the middle and tail region with a sterile knife. This cut sample were crushed into small piece, then it dissolve into 10 ml Ringer solution with 80 ml distilled water.

Preparation of serial dilution

Eight (8) ml of sterile H_2O with 1 ml Ringer solution was poured aseptically into six tubes and 1 ml of the original crushed fish sample was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first test tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. The crushed sample was therefore diluted from 10^{-1} to 10^{-6} for each fish sample.

Culture medium and growth condition

The 0.1 ml of suspension from each dilution of the samples was spread onto Nutrient agar, Staphylococcus medium no. 110 and *Pseudomonas* agar plates for the estimation of TVB, *Staphylococci* and *Pseudomonas*, consecutively. For TVB and Staphylococcal assay, plates were incubated at 37°C for 24 hours. For *Pseudomonas* count plate were incubated at room temperature for 48-72 hours.

Data recording and analysis

After 24 hours of incubation colonies were counted upon visualization and data was recorded. Fecal coliforms were counted by following standard MPN table.

Results and discussion

In order to assess the bacteriological load of fish and their changes in different treatment, different sample from different investigations areas were tested. This test includes the total viable bacterial count, total no. of coliform, and detection of *Pseudomonas*, and *Staphylococcus sp*. After that, this sample were treated with different chemicals of different concentration to reduce the bacterial load of frozen fishes and find out the exact concentration of chemicals by using this concentration the fish should be safe to consume. The pathogens that are normally associated with the contamination of processed sea foods are *Escherichia coli* (Candrian *et. al.*, 1991), *Staphylococcus aureus* (Yang *et. al.*, 1993), and human pathogens such as *Salmonella sp.* (Bej *et. al.*, 1994). It is well known that the spoilage of any food product is attributed to microbial growth due to improper handling, long gap between harvesting and processing and poor storage conditions (Gram and Huss, 2000). *S. aureus* contamination up to 25% has been reported in marine fishes like frozen grouper and mackerels (Adebayo- Tayo *et. al.,* 2012).

Microbial count before washing treatment

Total bacterial count

The term total bacterial count refers to all organisms living and dead. The total count also gives an estimate of the total number of microorganisms to which a substance has been exposed. Among the sample the average highest bacterial count was found at the sample of the Silver Carp (*H. molitrix*,8.18×10^5 CFU/g) and the average lowest bacterial count was found at the sample of Rupchada (*S.niger*,5.61×10^5 CFU/g). A significant number of average total bacterial counts were found in the Hilsha (*T. ilisha*, 6.51×10^5 CFU/g) and Rui (*L. rohita*, 5.80×10^5 CFU/g) collected from different markets as shown in Table 1.

Total coliform count

Coliform bacteria are the indicator organisms whose presence in food in large quantity indicates the probability of having pathogenic bacteria (Shankar, *et. al.*, 2010). Among 16 samples of different area, the average highest coliform count was found at the sample of Rui (*L. rohita*, 603 MPN/g) and average lowest count was found in Rupchada (*S.niger*,20.75 MPN/g)(Table 1). A significant number of average total coliform counts were found in the Hilsha (*T. ilisha*, 542.5 MPN/g) and Silver Carp (*H. molitrix*, 515 MPN/g) collected from different markets.

Pseudomonas count

From the Table 1, it has observed that among 16 samples of different area, the average highest *Pseudomonas* count was found at the sample of Hilsha (*T. ilisha*, 9.65×10^5 CFU/g) and the average lowest *Pseudomonas* count was found at the sample of Rui (*L. rohita*, 3.51×10^5 CFU/g). A significant number of average *Pseudomonas* counts were found in the Silver Carp (*H. molitrix*, 7.93×10^5 CFU/g) and Rupchada (*S. niger*, 5.53×10^5 CFU/g) collected from different markets.

Table 1. Average value of microorganisms in each fish.

Sample	Total microbial count (CFU/g)	Total Coliform MPN/g	*Staphylococci* (CFU/g)	*Pseudomonas* (CFU/g)
Hilsha	6.51×10^5	542.5	7.72×10^3	9.65×10^5
Rupchada	5.61×10^5	20.75	–	5.53×10^5
Silver	8.18×10^5	515	–	7.93×10^5
Rui	5.80×10^5	603	5.35×10^3	3.51×10^5

*MPN (Most Probable number), CFU (Colony forming unit), (–) sign indicates not detected.

Table 2. Comparison of total microbial count of Hilsha *(T. ilisha)* fish before washing and after washing with different concentrations of reagents.

Area	Total microbial count (CFU/g)					
	Before washing	After washing with				
		Distilled H_2O	1% NaCl	2% NaCl	1% Acetic acid	5% Acetic acid
Sheikhpara bazaar	7.04×10^5	6.32×10^5	3.11×10^5	2.32×10^5	1.25×10^5	–
Kushtia super market	5.10×10^5	4.30×10^5	2.91×10^5	1.43×10^5	$^*1.10\times10^5$	–
Madhupur bazaar	6.41×10^5	5.20×10^5	3.10×10^5	2.12×10^5	1.12×10^5	–
Bittipara bazaar	$^*7.50\times10^5$	6.40×10^5	3.25×10^5	2.05×10^5	1.41×10^5	–

CFU (Colony forming unit), (–) sign indicates not detected. The highest total microbial count was 7.50×10^5 and the lowest count was 1.10×10^5($P<0.005$).(*) indicates significance value $P<0.005$.

Staphylococci count

The contamination of fish particularly by pathogens such as *Salmonella sp.*, *Staphylococcus aureus*, *Escherichia coli* may occur prior to harvest, during capture, processing, distribution and storage. Among 16 samples of different area, the average highest *Staphylococci* count was found at the sample of Hilsha (*T. ilisha*, 7.72×10^3 CFU/g) and the average lowest *Staphylococci* count was found at the sample of Rui (*L. rohita*, 5.35×10^3 CFU/g) collected from different markets (Table 1).

Microbial count after washing treatment

Fish are washed with different chemicals (Distilled water, Sodium chloride, and Acetic acid) at different concentration. After washing with these chemicals, the microbial count of different fishes were observed. Abolagba*et al.*, (2011) stated that microbial count after smoking treatment has decreased significantly.

It has been revealed here that there were significant changed in the microbial count after washing treatment.

Table 3. Comparison of total coliform count of Hilsha *(T. ilisha)* fish before washing and after washing with different concentrations of reagents

Area	Total coliform count (MPN/g)					
	Before washing	After washing with				
		Distilled H_2O	1% NaCl	2% NaCl	1% Acetic acid	5% Acetic acid
Sheikhpara bazaar	1100*	290	150	75	43	–
Kushtia super market	460	210	160	93	53	–
Madhupur bazaar	150	120	93	53	28*	–
Bittipara bazaar	460	150	93	64	35	–

MPN (Most Probable number), (–) sign indicates not detected. The highest total coliform count was 1100and the lowest count was 28 ($P<0.005$). (*) indicates significance value $P<0.005$.

Table 4. Comparison of total *Pseudomonas* count of Hilsha *(T. ilisha)* fish before washing and after washing with different concentrations of reagents

Area	Total *Pseudomonas* count (CFU/g)					
	Before washing	After washing with				
		Distilled H_2O	1% NaCl	2% NaCl	1% Acetic acid	5% Acetic acid
Sheikhpara bazaar	11.98×10^5	9.37×10^5	4.02×10^5	2.98×10^5	1.32×10^5	–
Kushtia super market	5.01×10^5	4.22×10^5	2.76×10^5	1.53×10^5	$^*0.80\times10^5$	–
Madhupur bazaar	9.10×10^5	8.67×10^5	4.91×10^5	2.85×10^5	1.43×10^5	–
Bittipara bazaar	$^*12.5\times10^5$	11.45×10^5	6.88×10^5	3.73×10^5	1.67×10^5	–

CFU (Colony forming unit), (–) sign indicates not detected. The highest total *Pseudomonas* count was $12.5\times10^5$and the lowest count was 0.80×10^5($P<0.005$). (*) indicates significance value $P<0.005$.

Total bacterial count

After washing with distilled H_2O the amount of total bacterial count was decreased in a very little amount. Sample collected from Shekhpara bazaar; total bacterial count of Hilsha fish before washing was 7.04×10^5 CFU/g and after washing with distilled H_2O total bacterial count is 6.32×10^5 CFU/g (Table 2). After washing with 1% NaCl, amount of total bacterial decrease into 3.11×10^5 CFU/g (Table 2). When the concentration of NaCl increased into 2% then the total bacterial count decrease to 2.32×10^5 CFU/g. After that 1% acetic acid was used, and then the total bacterial count found was 1.25×10^5 CFU/g. After the concentration of acetic acid increase into 5% no bacterial count was obtained. Sample collected from Kushtia super market; total bacterial count of Hilsha

fish before washing was 5.10×10^5 CFU/g and after washing with distilled H_2O total bacterial count is 4.30×10^5 CFU/g. After washing with 1% NaCl, amount of total bacterial decrease into 2.91×10^5 CFU/g. When the concentration of NaCl increased into 2% then the total bacterial count decrease to 1.43×10^5 CFU/g. After that 1% acetic acid was used, then the total bacterial count found was 1.10×10^5 CFU/g. After the concentration of acetic acid increase into 5% no bacterial count was obtained (Table 2).

Table 5. Comparison of total *Staphylococc*i count of Hilsha *(T. ilisha)* fish before washing and after washing with different concentrations of reagents.

Area	Total *Staphylococc*i count (CFU/g)					
	Before washing	After washing with				
		Distilled H_2O	1% NaCl	2% NaCl	1% Acetic acid	5% Acetic acid
Sheikhpara bazaar	10.0×10^3	8.02×10^3	4.00×10^3	2.34×10^3	1.32×10^3	–
Kushtia super market	3.40×10^3	3.10×10^3	2.42×10^3	1.12×10^3	$^*0.80\times10^3$	–
Madhupur bazaar	6.76×10^3	6.12×10^3	4.76×10^3	2.53×10^3	1.56×10^3	–
Bittipara bazaar	$^*10.7\times10^3$	9.92×10^3	6.54×10^3	4.01×10^3	2.65×10^3	–

CFU (Colony forming unit), (–) sign indicates not detected. The highest total *Pseudomonas* count was $10.7\times10^3$and the lowest count was 0.80×10^3($P<0.005$). (*) indicates significance value $P<0.005$.

Total coliform count

After washing with distilled H_2O the amount of total coliform count decreased in a very little amount. Sample collected from Shekhpara bazaar; coliform count of Hilsha fish before washing was 1100 MPN/g and after washing with d. H_2O coliform count is 290 MPN/g (Table 3). After washing with 1% NaCl, amount of this coliform decrease into 150 MPN/g (Table 3.7). When the concentration of NaCl increased into 2% the coliform count decreased to 75 MPN/g. After that 1% acetic acid was used and the bacterial count was 43 MPN/g. No coliform count was obtained when the concentration increased in to 5% (Table 3).

Pseudomonas count

After washing with distilled H_2O the amount of *Pseudomonas* count decreased in a very little amount. Sample collected from Shekhpara bazaar; *Pseudomonas* count of Hilsha fish before washing was 11.98×10^5 CFU/g (Table 4) and after washing with dH_2O *Pseudomonas* count is 9.37×10^5 CFU/g. After washing with 1% NaCl, amount of this *Pseudomonas* decrease into 4.02×10^5 CFU/g. When the concentration of NaCl increased into 2%, the *Pseudomonas* count decreased to 2.98×10^5 CFU/g. After that 1% acetic acid was used, then the *Pseudomonas* count found 1.32×10^5 CFU/g. No bacterial count was obtained when the concentration increased in to 5% (Table 4).

Staphylococci count

After washing with distilled H_2O the amount of *Staphylococci* count decreased in a very little amount. Sample collected from Shekhpara bazaar; *Staphylococci* count of Hilsha fish before washing was 10.0×10^3 CFU/g (Table 5) and after washing with distilled H_2O *Staphylococci* count was 8.02×10^3 CFU/g. After washing with 1% NaCl, amount of this *Staphylococci* decrease into 4.00×10^3. When the concentration of NaCl increased into 2% then the *Staphylococci* count decreased to 2.34×10^3 CFU/g.

After that 1% acetic acid was used, then the *Staphylococci* count found 1.32×10^3 CFU/g. After the concentration of acetic acid increase into 5% no *Staphylococci* count was obtained (Table 5).

Concussion

From the present investigation, it has been found that microbial load in local market were high in comparison with super shop markets as they showed higher counts in most of microbial parameters. The investigation that was conducted also indicates that the fish of local market were in high risk to transmit various types of pathogens to the consumers. From the result, it can be concluded that the fish are sold in the local retail markets are not standard to consume since the microbial levels were always higher than the recommended levels because they don't maintain the hygienic condition during preservation and lower cold storage facilities. To overcome this situation, proper hygienic condition should be maintained at every step of catching, landing and transportation, processing and marketing following HACCP steps for good quality of fish and fishery products. This study also reveals that, pathogenic microorganism from fish can be removed by washing with Acetic acid.

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