

## Microbiological Quality of Processed Frozen Black Tiger Shrimps in Fish Processing Plant

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**Abstract:** The present study was conducted to evaluate the effectiveness of processing techniques of shrimps by microbiological quality assessment. The abundance of total aerobic bacteria, total coliform, faecal coliform, *Vibrio cholerae* and *Salmonella* were determined in Raw Block Frozen shrimp, Cooked IQF (Individual Quick Freezer) shrimp and Raw IQF shrimp. In each process, five different samples were examined with three replicates. The density of total aerobic bacteria detected in all the samples except sample 3 of Raw Block Frozen shrimp was significantly higher than that of others, while the density of the same bacteria detected in all the samples of Cooked IQF shrimp was significantly lower than that of others ( $p < 0.05$ ). While total coliform observed in Cooked IQF shrimp was  $< 3$  MPN g<sup>-1</sup>, it was  $21.00 \pm 0.25$  and  $4.20 \pm 1.20$  MPN g<sup>-1</sup> in Raw Block Frozen shrimp and Raw IQF shrimp respectively. Faecal coliform, *V. cholerae* and *Salmonella* was not detected in any of the samples. In conclusion, the findings of the present study suggest that investigated processed frozen shrimps of the fish processing plant were qualified enough for export and Cooked IQF shrimp was much better than other shrimps from the microbiological point of view.

**Key words:** Coliform % Block Frozen shrimp % Cooked IQF shrimp % Raw IQF Shrimp % HACCP

### INTRODUCTION

Fisheries sector plays an important role in the socio-economic development of Bangladesh, whereas it contributing 4.92% to the national GDP, 23% of the agriculture sector and supplying 63% of the domestic animal protein consumption. This is the second highest source of export earning and providing direct or indirect employment to 10% of total population of the country [1]. The quality of the processed products largely depends on the quality of raw materials and it is difficult to preserve the freshness of raw materials when there is a long period of time between the harvesting and processing periods. During this period, shrimps continue to be deteriorated [2]. The time interval between the landings of shrimps and their arrival at the processing plants is very important [3]. Improper handling and inadequate processing result in microbial growth which causes spoilage of food products.

Bangladesh exports mainly frozen shrimp and different types of fresh and marine water fishes. But, export market of Bangladesh is threatened for low quality processed foods which may be contaminated with different types of bacteria such as *Vibrio cholerae*, *Salmonella*, coliform, faecal coliform, streptococci and *Staphylococcus aureus*. Salmonellosis is one of the most

prevalent diseases that may be caused due to consumption of seafood contaminated with *Salmonella*. In the USA, salmonellosis accounts for about 60% of all bacterial diseases [4]. In Japan, whereas raw sea food is very much popular, about 70% food poisons occurs in summer is caused mainly due to bacterial pathogens resulting from fish products [5].

Handling of raw materials influences the bacteriological quality of frozen shrimps [6]. Insufficiently iced and improperly storage of shrimps at higher temperature enhances the growth of microorganisms responsible for microbiological changes [7]. The recent introduction of the Hazard Analysis Critical Control Point (HACCP) system and EU hygienic regulations in seafood industries will pave the way for the production of safe and high quality seafood. Bangladesh frozen shrimp exporters continue to have both real and perceived problems with buyers in the U.S., the EU and Japan, concerning the safety and quality of their products. Because many fish processing plants in Bangladesh do not follow the HACCP system and EU hygienic regulations for which many of them have been banned and the export of shrimps, fish and fish products have been regulated. Buyers in Japan also reported quality problems with previously imported shrimp from Bangladesh [8].

Microbiological quality assessment of frozen shrimp processed in different techniques in fish processing plants has not been done earlier in Bangladesh. Thus, the present study was designed to assess the microbiological quality of frozen shrimp for export and to determine the effectiveness of different processing techniques.

## MATERIALS AND METHODS

**Collection of Samples:** Frozen Black Tiger shrimp, *Penaeus monodon* was examined for microbiological quality assessment. Fresh raw shrimp were purchased from approved shrimp producers and brought to the factory with ice in insulated trucks [9]. Receiving of samples was done in a protected platform. Usage of any antibiotics or pesticides was prohibited strictly. After receiving, the samples were dipped in 50 ppm chlorinated water to remove adhered bacteria and other external materials. Deheading was done manually and the head waste was collected in disposal box and shifted to the waste disposal room through pocket door. The samples were processed in three different processing techniques. Five different samples from five different lots each with three replicates were tested. All the samples were taken from each processing techniques.

The processing and preservation were done by the plants while the microbiological analysis of the samples was conducted in the present study. The study was done in the Microbiology Laboratory of the same plants.

### Shrimp Samples

**Sample 1:** SW.BT. PD. T/ON (26/30) - Salt Water Black Tiger Shrimp Peel Deveining Tail on, size of shrimps per Kg.

**Sample 2:** SW.BT. PD. T/ON (26/30) - Sea Water Black Tiger Shrimp Peel Deveining Tail on, size of shrimps per Kg.

**Sample 3:** SW.BT. PD. T/OFF (16/20) - Sea Water Black Tiger Shrimp Peel Deveining Tail off, size of shrimps per Kg.

**Sample 4:** SW.BT. PD. T/OFF (26/30) - Sea Water Black Tiger Shrimp Peel Deveining Tail off, size of shrimps per Kg.

**Sample 5:** SW.BT. PD. T/OFF (21/25) - Sea Water Black Tiger Shrimp Peel Deveining Tail off, size of shrimps per Kg.

### Processing Techniques

**Process 1:** Raw Block Frozen – Freezing temperature -38 to -40°C for 2.5 to 3.0 hours by contact freezing.

**Process 2:** Cooked Individual Quick Frozen (Cooked IQF) – Cooking temperature 95-98°C, core temperature 70-72°C, cooking time 140 sec for 16/20, 130 sec for 20/25 and 120 sec for 26/30.

**Process 3:** Raw Individual Quick Frozen (Raw IQF) - Freezing temperature -38 to -40°C for 35 to 40 min by spiral freezing.

**Processing of Samples:** A sample of 20 g was taken in a sterile polythene bag and poured in 180 ml of sterile 0.1% peptone water in that bag and placed in a sterile stomacher and blended for 2 minutes. This homogenate sample resulted dilution at this stage was  $10G^1$ . Again 1 ml of the  $10G^1$  dilution was transferred to a screw cap vial containing 9 ml of sterile 0.1% peptone water. The vial was shaken thoroughly and it gave a dilution of  $10G^2$ . In this way  $10G^3$ ,  $10G^4$  and  $10G^5$  dilutions were made.

**Microbiological Analysis:** Microbiological analysis was performed according to the standard procedure for the enumeration and identification of microorganisms [10].

**Total Aerobic Bacteria:** Each dilution of the samples was pour- plated on nutrient agar (Becton Dickinson, France). The colonies were counted after incubation at 37°C for 24 hours [11].

**Total Coliform:** For enumeration of coliform organism by using liquid media, 1ml portion of each decimal dilution is inoculated into 3 separate tubes of Lauryl Tryptose Broth (LTB) containing inverted Durham's tube ( $10G^1$ ,  $10G^2$  and  $10G^3$ ) and incubated at 37°C for 48 hrs. Gas positive was deserved and selected the last three gas positive dilutions [11].

**Faecal Coliform:** The tubes of Lauryl Tryptose Borth (LTB) positive for gas production were selected and a loopful of broth from each positive culture were inoculated by a sterilize wire loop into Brilliant Green Lactose Bile Broth (BGLB) tube containing inverted Durham's tube and a tube of Tryptone Water. Then the tubes were incubated at 44.4°C for 48 hrs in a circulation water bath [11]. After 48 hrs of incubation, the tryptone waters were tested with Kovac's reagent to determine the presence of indole [12]. Sufficient gas production in tubes

of BGLB and corresponding indole positive test of Tryptone water were recorded. The methods are followed as described by Health & Welfare of Japan [13]. For both total coliform and faecal coliform, the results were computed using MNP chart [14].

**Detection of Salmonella sp.:** 25 g sample was taken in 225 ml sterile Buffered Peptone Water [P<sup>H</sup> 7.5] aseptically and incubated for 48 hours at 37°C for pre-enrichment [2]. 1 ml of pre-enrichment medium was then pipetted to selective enrichment medium namely Selenite Cystene Broth and incubated for 24 hours at 37°C. One loopful of medium was streaked onto pre-dried selective plating medium, viz. Brilliant Green Agar and Xylose Lysine Desoxycholate Agar and incubated for 24 hours at 37°C.

Pink, red, convex, entire glossy colonies surrounded by brilliant red zones in the Brilliant Green Agar and black centered, convex, entire glossy colonies Xylose Lysine Desoxycholate Agar were suspected for *Salmonella spp.* 2 or 3 typical (or suspected) colonies were selected from each selective agar. Then several biochemical tests such as Lysine, Dulcitol, Malonate, Indole, Lactose, Sucrose, MR-VP, Simmon's citrate etc. were conducted for the further confirmation of *Salmonella spp.* according to the procedure of American Society of Microbiology [15] with some modifications. All cultures giving biochemical reactions were confirmed by agglutination test with *Salmonella* polyvalent (O) somatic antisera [16].

**Detection of V. Cholerae:** 25 g sample was taken in 225 ml sterile Alkaline Peptone Water aseptically and incubated at 37°C for 24 hours [2]. After incubation a loopfull of medium was streaked on the Thiosulphate Citrate Bile

Salt Sucrose Agar plate and incubated at 37°C for 24 hours. The characteristics colonies, yellow, 2-3 mm diameter, slightly flatten with adequate center and translucent peripheries were suspected for *V. cholerae*. Then the suspected colonies were confirmed biochemically [17] with some modifications. Biochemical tests included TSI, KIA, Salt tolerance (0%, 6% 8%) oxidase Grain reaction, motility, fermentation of carbohydrates (Glucose, Sucrose, Arabinose, Mannose, Mannitol and Inositol) Decarboxylase (Lysine, Arginine and Ornithine). Finally the strains were confirmed serologically by agglutination test using polyvalent *Vibrio cholerae* (O) antiserum [16].

## RESULTS AND DISCUSSION

**Total Aerobic Bacteria:** The density of total aerobic bacteria detected in all the samples of Raw Block Frozen Shrimp (except sample 3) was significantly higher than that of cooked IQF shrimp and Raw IQF shrimp (Figure 1, p<0.05). Bacterial bloom observed in Sample 3 of Raw Block Frozen Shrimp was similar to Raw IQF Shrimp, but was higher than that of Cooked IQF Shrimp (p<0.05). Bacterial concentration detected in all the samples of Cooked IQF Shrimp was significantly lower than that of others (p<0.05). In Raw IQF Shrimp the bacterial bloom was significantly higher than that of Cooked IQF Shrimp, but lower than that of Raw Block IQF Shrimp (P< 0.05). The highest bacterial load ( $8.13 \pm 0.47 \times 10^4$  CFUg<sup>-1</sup>) was detected in Sample 4 of Raw Block Frozen Shrimp, while the lowest ( $1.30 \pm 0.29 \times 10^3$  CFUg<sup>-1</sup>) was in the same sample of Cooked IQF Shrimp (Figure 1).

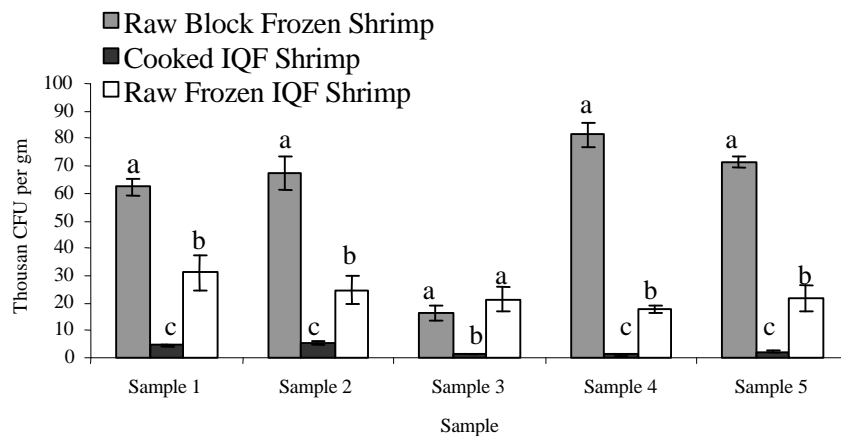


Fig. 1: Density (cfug<sup>-1</sup>) of total aerobic bacteria detected in different samples of tiger shrimp preserved in different process in fish processing plant. Bars (Mean ± SEM) with same colors different letters are significantly different (P < 0.05).

Table 1: MPNgG<sup>1</sup>(mean ± SEM) count of total coliform detected in different samples of different process

Sample Name	Raw Block Frozen Shrimp	Cooked IQF Shrimp	Raw IQF Shrimp
Sample 1	9	<3	3
Sample 2	24	<3	9
Sample 3	9	<3	3
Sample 4	20	<3	3
Sample 5	43	<3	3
Mean ± SEM	21.00 ± 6.25	<3 ± 0.00	4.20 ± 1.20

*V. cholerae* and *Salmonella* spp.

After biochemical and serological tests it was confirmed that no *V. cholerae* and *Salmonella* was detected in any of the sample.

Table 2: MPNgG<sup>1</sup> (mean ± SEM) count of faecal coliform per gm sample observed in different samples of different process

Sample Name	Raw Block Frozen Shrimp	Cooked IQF Shrimp	Raw IQF Shrimp
Sample 1	<3	<3	<3
Sample 2	<3	<3	<3
Sample 3	<3	<3	<3
Sample 4	<3	<3	<3
Sample 5	<3	<3	<3

**Total Coliform and Faecal Coliform:** Coliform bacteria are the indicator organisms whose presence in food in large quantity indicates the probability of having pathogenic bacteria. Faecal coliform are considered to be present, especially in the gut and feces of warm-blooded animals. Because the origins of faecal coliforms are more specific than the origins of the total coliform group, faecal coliforms are considered a more accurate indication of animal or human waste than the total coliforms [18]. The presence of faecal coliform is not permitted in the shrimp samples in Japan, USA and other European countries [19].

MPN count of total coliform per gram of sample observed in different samples of Raw Block Frozen Shrimp was between 9 and 43, while in Cooked IQF Shrimp, the MPN count of all the samples was <3 (Table 1). The mean MPN count per gram of Raw Block Frozen Shrimp, Cooked IQF Shrimp and Raw IQF Shrimp was 21.00 ± 6.25, <3 ± 0.00 and 4.20 ± 1.20, respectively. MPN count per gram of total coliform observed in sample 3, 4 and 5 of Raw IQF Shrimp was <3, while in sample 1 and 2, it was 3 and 9, respectively. Furthermore, the MPN count of faecal coliform per gram of sample observed in all the samples was <3 (Table 2).

*Salmonella* in aquaculture shrimp products mainly originates from the environment rather than from poor standards of hygiene and sanitation. But sometimes, incidence of this bacterium in fish, shrimp or similar foods of aquatic habitats may be happened due to external contamination. Most shrimps are cooked prior to consumption. These products, therefore, cause negligible health risks to the consumers except for cross contamination in the kitchens [20]. *Salmonella* has been

isolated from fresh, frozen, canned and sun dried marine fish products [21]. This bacterium is highly pathogenic and has been isolated from different frozen shrimp and shrimp products. *Salmonella* was detected in 8 of 20 samples of shrimp from departmental shop and local fish market in Dhaka city, Bangladesh [11].

According to ICMSF [22] the acceptable upper limit of total bacterial load, total coliform and faecal coliform is 10<sup>6</sup>cfu/g, 100 MPN/g and <3 MPN/g, respectively while *Salmonella* and/or *V. cholerae* should not present. In the present study the total aerobic bacteria, TC and FC in all the samples were under the limit of ICMSF [22]. Besides, *Salmonella* and *V. cholerae* were not detected in any of the sample. Thus, all the samples of all the process were under the acceptable limit according to ICMSF and FDA guidelines [22, 23].

During the preservation processes, all the samples of Cooked IQF Shrimp showed the lowest TC, FC and total aerobic bacterial count while Raw frozen shrimp showed the highest. In Cooked IQF shrimp elimination of bacteria occurs in two steps, first during cooking and then freezing. In the contrary, in raw block frozen and raw IQF shrimp, elimination occurs only during freezing. The lowest count in Cooked IQF shrimp might be because of this reason. This result is supported by elsewhere [24].

From the current study, it is revealed that Cooked IQF shrimps are highly qualified for export purpose. Furthermore, TC, FC and total bacterial count found in Raw Block shrimp were significantly higher than those of Raw IQF shrimp. This indicates that IQF shrimp is better than Block Frozen shrimp from the microbiological point of view.

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