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Microbiological Status in a Fully Export-Oriented Shrimp Processing Plant

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Abstract: The present study was carried out in the period from 1st April 2010 to 1st August 2010 to asses the microbial load of frozen shrimps processed for exporting to different countries of the world. For the microbial analyses, samples were collected from cooked IQF (Individual Quick Freezer) shrimp and raw block frozen shrimp. Total bacterial load of water, ice, contact surfaces at different stages were also investigated. The mean total coliforms observed in Cooked IQF shrimp was <3 ±0.00 MPN/g, while it was 23.50 ± 13.72 MPN/g in raw block frozen shrimp. Fecal coliforms for both raw block frozen and cooked IQF shrimp were <3 MPN/g. In the current investigation the SPC of normal water, UV radiated water, ice, receiving table, grading table and panning tray and worker's hand (average of 3 samples) were 8.34, 0, 5.14 44, 40, 31 and 23 cfu/cm^2 respectively, which were under limit of international standard. So the present findings indicate that the hygienic condition of the investigated fish processing plant was good and the quality of Cooked IQF shrimp was excellent for export.

Key words: Coliforms · IQF · Block Frozen Shrimp

INTRODUCTION

Fisheries items are the major protein contributing source of Bangladesh. Fisheries sector contributes 3.74% of our GDP and 22.23% of agricultural sector [1]. Bangladesh is a sea food exporting country and exports mainly frozen shrimps, fresh water fishes and marine water fishes to Japan, USA, Europe, Saudi Arabia, the UAE and Gulf States [2]. Export earnings from fisheries sector have increased from 1283 Taka core in 1995-1996 to Taka core 3025.93 in 2009-2010 [2].

By the year 1985, a good number of processing plants has developed without biological survey between capture and culture fishery. In the past time most of the factory led the emphasis on quantity rather than quality. Processed products quality depends on the quality of raw materials but it is too difficult to retain freshness of raw materials due to long period of time between harvesting and processing periods. Inadequate processing has resulted in microbial growth which deteriorates food products [3]. Now export market of Bangladesh is threatened for inadequate processed foods which may be contaminated by decomposition, high bacterial load, filth, unexpected foreign matters as well as pathogenic microbes (*E. coli, Salmonella, V. cholerae etc*). These activities occur occasionally due to improper implementation of HACCP system as well as poor established of GMPs (Good Manufacturing Practices), GHPs (Good Hygienic Practices) and inadequate sanitation procedures (Pers. Com.). On July 30, 1997, the EU (European Union) banned imports of fishery products from Bangladesh after the inspection of Bangladesh's seafood processing plants. Inspection found serious deficiencies in the infrastructure and hygiene in processing establishments and insufficient guarantees of quality control by Bangladeshi government inspectors. The ban was estimated to cost the Bangladesh shrimp-processing sector nearly US$15 million in lost revenues from August to December 1997 [4].

Bangladesh frozen shrimp exporters continue facing 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 did not follow the HACCP system and EU hygienic regulations. Recently, Bangladesh government has been taking serious action against the shrimp and fish processing plants which are not following importers conditions and not implementing HACCP system for ensuring quality products [4]. Therefore the present study was undertaken to asses' microbial load of frozen shrimps processed for exporting to different countries of the world.

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MATERIALS AND METHODS

Study Area: The investigation was carried out in the microbiology laboratory of the processing plant (ARK Sea Food Ltd. Chittagong Bangladesh) for 4 months during 1st April 2010 to 1st August 2010.

Sampling Procedure and Processing: For microbial analysis four samples were taken from different stages of processing e.g. from normal water, UV radiated water, ice, receiving table, grading table, panning tray and worker's hand. Shrimp samples were collected from different grades of processed shrimp in the plant e.g. Sample 1: shrimp IQF (Individual Quick Freezer) (tail-on), Sample 2: shrimp IQF (tail-off), Sample 3: Shrimp Peel Deveining Tail on and Sample 4: Shrimp Peel Deveining Tail off.

Representative samples of shrimps/ice/water were collected at specific steps of processing and were assessed for microbial analyses soon after sampling. The samples were carried to the laboratory in a sterile wide mouthed bottle within 5 minutes. Frozen shrimps were stored at -20°C and unfrozen perishable samples held at 0-4°C for not longer than 6 hrs.

Swab Test: A solution of peptone (1 g/L) and sodium chloride (8.5 g/L) was made up. The solution was distributed in bottles and was sterilized for 15 min at 121°C. Bacteria from a known area of a surface were removed by passing a sterile cotton wool moistened with sterile peptone water, from which bacterial counts were made as the procedure adopted for standard plate count (colony/cm^2) [5].

Microbial Analysis:

Enumeration of Total Bacterial Load (SPC): Twenty gm of the sample were blended for 1 min with 180 ml of sterile dilute (0.1% peptone) in an automatic blender (dilution of 10^{-1}); 1 ml of the 10^{-1} dilution was transferred to a screw cap vial containing 10 ml of sterile dilute to give a dilution of 10^{-2}. The containing screw cap vial was shaken gently. This process was repeated, using the progressively increasing dilution to prepare dilutions of 10^{-3}, 10^{-4} and 10^{-5} [6]. Each dilution was plated by pipette 1 ml into a sterile plate containing 15 ml agar which was melted and brought to 45°C and poured into the plates. Fewer than 15 minutes were elapsed between making the dilution and pouring the agar. After solidification of the media, the plates were inverted and incubated at 37°C for 18-24 hrs [7]. The total number of bacteria per gm of sample was obtained by multiplying the average number of colonies on paradises by the respective dilution factor. The total numbers of bacteria found from each Petridis for each dilution were averaged to find a reliable standard plate count (SPC).

Enumeration of Total Coliforms: Samples were prepared as described in section serial dilution. Dilution blanks remaining from the determination of the plate count were used. One ml of each of the decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}) was poured into each of the three separate tubes of lauryl tryptose broth (LTB) containing Durhams Tube. The tubes were incubated at 37°C for 48 hrs. The formation of gas after 48 hrs was considered a sufficient evidence of the presence of coliforms. The positive gas production was recorded and the result was computed using MPN chart [7].

Test of Fecal Coliforms: Tubes of lauryl tryptose broth that was positive for gas production were selected and a loopful of Broth from each positive culture were inoculated into a tube of Brilliant Green Bile (2%) Broth and a tube of tryptone water tube were tasted with Kovac's reagent to determine the presence of indole [5]. A positive indole reaction in a broth that has produced gas at 44°C indicates the presence of *E. coli*. The positive gas production tubes were recorded and the results were computed using MPN chart [7].

Detection of Salmonella: The presence of *Salmonella* was detected by homogenizing a 25 gm portion of the composite sample in 225 ml (pH 7.5) sterile buffered peptone water aseptically and incubating for 24-48 hrs at 37°C in an incubator. After incubation 1 ml sample was transferred to duplicate tubes of tetrathionate (9 ml) and selenite cysteine broth (9 ml), incubated for 24 hrs at 37°C and sub-cultured into xylose lysine deoxycholate (XLD) and brilliant green agar (BGA) [8]. After incubation for 24-48 hrs at 37°C the characteristics colonies (on XLD; black centered, convex entirely glossy colonies and on BGA-pink, red, convex, entirely glossy colonies surrounded by brilliant red zones in the agar) were streaked with sterile platinum wise loop and incubated at 37°C for 6 hrs. If black color and H_2S gas were found, that indicated the presence of *Salmonella* [7].

Detection of V. Cholerae: Twenty-five gm portion of the composite sample was added in 225 ml sterile alkaline peptone water aseptically and incubated at 37°C for 24 hrs. After incubation a loopful from the alkaline peptone water was streaked on the surface of separate plates of thiosulfate citrate bile salts (TCBS) agar in such a manner

to obtain individual colony and incubated at 37°C for 24 hrs. The colonies of *V. cholera* were plain, yellow in color and of very big size (generally 2-3 mm). From TCBS the selected colony was transferred to triple sugar iron (TSI) agar slant with streaking. Then TSI agar tubes were incubated at 37°C for 24-48 hrs. The presence of gas and black color in TSI slants, indicated that *V. cholerae* was absent. If the media became yellow in color then *V. cholerae* is present [7].

RESULTS AND DISCUSSION

Total Bacterial Load/Standard Plate Count (SPC): The SPC of normal water, UV radiated water, ice, receiving table, grading table and panning tray, worker's hand (average of 3 samples) were 8.34, 0, & 5.14 cfu/ml, 44, 40, 31 and 23 cfu/cm^2, respectively (Fig. 1& 2).

Total and Faecal Coliforms: Total and fecal coliforms were nil from normal water, ice, receiving table, grading table and panning tray and worker's hand (Fig. 2). Total coliforms of cooked IQF <3 MPN/g and in raw block frozen 23.50 MPN/g. But faecal coliform is <3 MPN/g both of cooked IQF and raw block frozen (Table 1&2).

Salmonella and V. Cholera: *Salmonella* and *V. cholerae* were totally absent from all sorts of sample viz. normal water, UV radiated water, ice, receiving table, grading table, panning tray, worker's hand as well cooked IQF and raw block frozen shrimp.

According to ICMSF the acceptable upper limit of total bacterial load, total and fecal coliforms is 106 cfu/g, 100 and <3 MPN/g, respectively, while *Salmonella* and/ or *V. cholera* should not present. In the present study the total coliforms was under the limit of ICSMF [9] and presence of coliform is not permitted in the food products in importing countries [10]. Besides, *Salmonella* and *V. cholera* were not detected in the Raw Block Frozen and cooked IQF shrimp. Thus the samples of Raw Block Frozen and cooked IQF shrimp were under the acceptable limit according to ICMSF and FDA guidelines [9, 3]. Hossain, *et al.* [11] carried out an experiment on microbiological quality of processed frozen black tiger shrimps in fish processing plant. The study was conducted to evaluate the effectiveness of processing techniques of shrimps by microbiological quality assessment. The abundance of total aerobic bacteria, total coliforms, fecal coliforms, *Vibrio cholerae* and *Salmonella* were determined in Raw Block Frozen shrimp, Cooked IQF (Individual Quick Freezer) shrimp and Raw

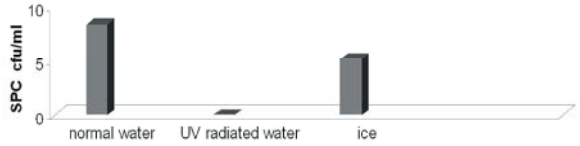

Fig. 1: Standard plate count in water.

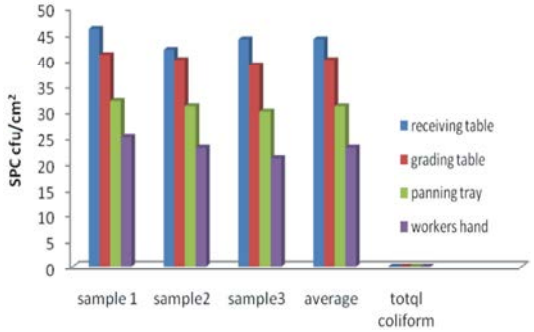

Fig. 2: Standard plate count in SWAB test at different stages.

Table 1: MPN/g (mean ± SD) counts of total coliform detected in different samples of different processes

Sample name	Raw block frozen shrimp	Cooked IQF shrimp
Sample 1	9	<3
Sample 2	23	<3
Sample 3	20	<3
Sample 4	42	<3
Mean	23.50 ± 13.72	<3±0.00

Table 2: MPN/g counts of fecal coliform observed in different samples of different processes

Sample name	Raw block frozen shrimp	Cooked IQF shrimp
Sample 1	<3	<3
Sample 2	<3	<3
Sample 3	<3	<3
Sample 4	<3	<3

IQF shrimp. They observed total coliforms in Cooked IQF shrimp <3 MPN, while it was 21.00 ± 0.25 and 4.20 ± 1.20 MPN in Raw Block Frozen shrimp and Raw IQF shrimp respectably. Fecal coliforms, *V. cholerae* and *Salmonella* were not detected in any of the samples. Huq *et al.* [6] worked on quality aspect of frozen shrimp product in processing industry: a case study in Khulna Bangladesh. They reported that the highest SPC was 7 CFU/cm^2 in the overhead tank and lowest was 3 CFU/cm^2 in the panning tape water. SWAB samples were collected from worker hand, where mean SPC before and after working, were 20 and 30 per cm^2 respectively. During the processing technique, the samples of Cooked IQF Shrimp showed the lowest total coliforms. In Cooked IQF shrimp elimination of bacteria occurs in two steps first during cooking and

then freezing. On the other hand, in raw block frozen, abolition occurs only during freezing. The lowest count in Cooked IQF shrimp might be because of this reason. From the present study, it is unveiled that Cooked IQF shrimps are highly qualified for export purpose.

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