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RESEARCH ARTICLE

MICROBIOLOGICAL QUALITY ASSURANCE OF PROCESSED SHRIMP PRODUCT AND SANITATION STATUS OF A SEA FOOD INDUSTRY, BANGLADESH

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ABSTRACT

Food quality, including safety, is a major concern facing the food industry today. A number of surveys have shown that consumer awareness about quality of their food is increasing. The extensive of microorganism (total coliform, *Vibrio cholerae* and *Salmonella sp.*), growth promoters, residues of pesticide and dioxin, antibiotics etc. make fear and unease to consumer about what they eat. Moreover, Bangladesh export fish to developed countries and they are very conscious to their food quality rather than price. Therefore, the current study was undertaken to evaluate effectiveness of Hazard Analysis and Critical Control Point (HACCP) in the investigated processing plant. For this purpose, microbial status of Cooked IQF (Individual Quick Freezer) shrimp was assessed. In addition to SWAB tests of water, ice and different contact surface of shrimp were also conducted to assess the magnitude of contamination during processing. The abundance of total aerobic bacteria, total coliform, *Vibrio cholerae* and *Salmonella sp.* were determined in Cooked IQF (Individual Quick Freezer) process. In Cooked IQF Shrimp, while the MPN count of total coliform of the sample was <3, *V. cholerae* and *Salmonella* were not detected. A large amount of water is used in the fish processing plant, which is a superior source of microbial contamination of finish products. For good quality finish product, hygienic water management is now a prime concern. In the current investigation, the SPC of normal water, UV radiated water, ice, receiving table, grading table and panning tray, worker's hand were 8.66 ± 0.45 cfu cm^{-2}, 0 cfu ml^{-1}, 5 ± 0.36 cfu ml^{-1}, 45 ± 0.63 cfu cm^{-2}, 91.33 ± 0.81 cfu cm^{-2}, 79.33 ± 1.09 cfu cm^{-2}, 42.33 ± 0.54 cfu cm^{-2} respectively, which were under the limit of international standard. In conclusion, the result of the present study implies that the hygienic condition of the investigated fish processing plant was good and the quality of Cooked IQF shrimp was excellent for export.

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INTRODUCTION

The geographical location of Bangladesh makes it suitable for both marine and freshwater fish production. Due to high demand for shrimps, prawn and other crustaceans in the world market some entrepreneurs stepped into a new sophisticated industrial processing to earn foreign currency utilizing our available resources of shrimps and frog legs. The evaluation of the shrimps processing industries in Bangladesh dates bake to 1959 when the first processing and freezing plant was installed in Chittagong. During pre-liberation period from 1960-70 due to political crisis, less emphasis was given oil the industrial development. Even then due to initiative of some private entrepreneurs, a few fish processing plant were established (Uddin and Das, 1994).

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By 1971, there were nine such processing plants in the country with a total production capacity of 58.5 metric tons per day. During 1972-76, only four processing plants were established. The trend of installation of freezing plants speeded up since 1977 and reached its climax during 1986-89 when 39 plants were commissioned in three years (Hussain, 1993). Due to insufficient attention toward standard of hygiene and quality of the product, reasonable numbers of seafood products have gone out of business. As a result, the export of shrimp products has suffered considerable losses in rejection from 1975 to 1978 and the country was placed under automatic detention by United State Food and Drug Authority USFDA (Limpus, 1978). In October 1979, Bangladesh was black listed along with other countries by USFDA for having the evidence of Salmonella, filth, flies, cockroach and other insects in frozen shrimps and frog legs. It faced heavy loss in the form of rejection and of relatively low price offered by the foreign buyers for fish products from Bangladesh. So, Government then felt necessity

of establishing Fish inspection and quality control (FIQC) service in the country to regulate the sanitation status of the factory and the quality of fish and shrimps meant to export. Under this scheme, two testing laboratories were established in Chittagong and Khulna. In the year 1994, the existing processing of Bangladesh was filtered as per fish inspection and quality control (FIQC) act and EU (European Union) regulations. On July 30, 1997, the EU imposed ban on imports of fishery products from Bangladesh as a result of EU inspections of Bangladesh's seafood processing plants. Inspections 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 revenue loss from August to December 1997.

In order to lift the ban, the Bangladesh shrimp processing industry had invested US$17.6 million in plant upgrades, the government had invested US$382,000 in laboratory and personnel upgrades and outside partners had invested US$72,000 in training programs in Bangladesh in 1997 (Cato and Subasinge, 2003). After successful upgradation by July 1998, a total of 11 plants were approved for export to the EU. Now out of 65 plants licensed for export by the government, 62 plants had EU approval. EU advised the Bangladesh government to implement HACCP system in shrimp industries for hygienically safe production of frozen food. For implementation of the system by the shrimp industry, the government along with the BFFEA obtained experts' services from the USFDA, EU, FAO/ WHO, SGS and other sources. The government promulgated Fish and Fish Products (Inspection and Quality Control) Rules in December 1997 to implement the programme. The EU experts subsequently visited Bangladesh to inspect the arrangement made by government. The experts were satisfied by the government/BFFEA efforts in this respect and the EU ban on import of frozen fish from Bangladesh was lifted in 1998.

MATERIALS AND METHODS

Research Material

Processed product (Shrimp) and the equipments involved in the processing techniques of a fish processing plant named "Meenhar Fisheries Ltd." located at BSCIC Industrial Area. Samples were collected from the suitable stages of processing for completing idea about the commercial method of processing of shrimp.

Selection of Sample for Microbial Analysis

Shrimp: Cooked product of shrimp (BTHLSO: Black Tiger Head Less Shell on) was selected for the microbial analysis.
Water: Samples were collected from Over head tank and water used for shrimp washing.

Ice: Flack Ice.
For SWAB Test: Samples were collected from the contact surface of receiving table, panning tray, grading table and worker's hand.

Sampling

Shrimps, Ice and Water

- Representative samples of shrimps, ice and water were collected at specific steps of processing and were assessed for microbial analyses.
- The water samples were carried to the laboratory in a sterile wide mouthed bottle within 5 minuets.
- The shrimp sample was carried to the laboratory in a sterile polythene bag within five minutes.
- Frozen shrimp was stored in the freeze.

SWAB Test

- Bacteria from a known area of a surface were removed by passing a sterile cotton wool which was moistened with sterile peptone water.
- This inoculums of bacteria were shaken from the swab into a known volume (9.5ml) of sterile peptone water from which bacterial counts were made as the procedure adopted for standard plate count (colony cm^{-2})

Microbiological Media

Media was prepared as per instruction given by the company. The company was follow ICMSF (International Commission on Microbiological Specification for Foods) standard. The recommended quantities of powder are written on packet of the synthetic powdered media (plate 1C, 1D & 2A).

- Powder was weighted properly and then dissolved required amount of distilled water.
- The mixture then sterilize in autoclave for 15-30 minutes at 121°C under 15 lbs pressure.
- Sterilization media were taken out of autoclave and cooled as per necessary.

Microbial Analysis

Enumeration of Total Bacterial Load (SPC)

Serial Dilution

20 g of the sample was blended for 1 min with 180 ml of sterile dilute 0.1% peptone in an automatic blender (Plate 1B). That provided a dilution of 10-1. 1 ml of the 10-1 dilution was transferred to a screw cap vial containing 9 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 dilution of 10-3, 10-4, and 10-5 (Seeley, *et al*. 1970) (Plate, 2D).

Standard Plate Count (Pour Plate Method)

General procedure for the detection of SPC:

- 1ml 10-1 solution was added with 9ml.0.1% peptone water (10-2) and 1ml. with 9ml LTB (Lauryl Tryptose Broth) in the Durham's tube (10-1).

- Then 10-2 solution was converted to 10-3, 10-4, 10-5 solution with the 0.1% peptone solution.
- Poured 1ml. of solution from each test tube in the sterile Petri dish (Plate 3A).
- Approximately 15 ml agar which has been melted and brought to 45°C was poured into the plates.
- Plates were rotated by hand 5 times in the clockwise direction, 5 times in the counter clockwise direction and several times crosswise for equal distribution of the media.
- Fewer than 15 minutes were elapsed between making the dilution and pouring the agar. After solidification of the media, the plates were inverted and placed in incubator to incubate at 37°C for 18 hrs. (Carpenter, P. L. 1980) (Plate 3B).

Computing Standard Plate Count

- After 48 hours, the number of colonies which were developed in the Petri dishes was properly counted by colony counter machine.
- The total number of bacteria per gram of sample was obtained by multiplying the average number of colonies on Petri dishes by the respective dilution factor.
- The total number of bacteria found from each Petri dish for each dilution was averaged to find a reliable Standard Plate Count (SPC).

Enumeration of Coliform

General procedures for the detection of total coliform:
20 g of the sample was blended for 1 min with 180 ml of sterile dilute 0.1% peptone in an automatic blender (Plate 1B). That provided a dilution of 10-1.

- 1ml 10-1 solution was added with 9ml.0.1% peptone water (10-2) and 1ml. with 9ml LTB (Lauryl Tryptose Broth) in the Durham's tube (10-1).
- Then 10-2 solution was converted to 10-3, 10-4, 10-5 solution with the 0.1% peptone solution.
- Solution of 10-1, 10-2, 10-3 were incubated durham's tubes at 37°c for 48 hours.
- The formation of gas after 48 hours was considered sufficient evidence of the presence of coliform.
- No gas formation was recorded and the result was computed by using MPN chart.

Detection of Salmonella sp

- The presence of Salmonella sp. was detected by homogenizing a 25 g portion of the composite sample in 225 ml (pH 7.5) sterile buffered peptone water aseptically and incubating for 24-48 hr. 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 hours at 37°c and sub-cultured into Xylose Lysine Deoxycholate (XLD) and Brilliant Green Agar (BGA) (Seelay *et al*. 1970).
- After incubation for 24 hr at 37°c, characteristics colonies (on XLD- black centered, colony, convex, entries, glossy and on BGA-pink, red, convex, entree glossy colonies surrounded by brilliant red zones in the agar) were streaked with sterile platinum wise loop incubated at 37°c for 6 hrs.
- After incubation characteristics change may help about presence or absence of Salmonella. H2S gas was not found which indicate that Salmonella was absent in the collected sample.

Detection of V. cholera

- 25g portion of the composite sample was added in 225 ml. sterile alkaline peptone water aseptically and incubated at 37°c for 24 hrs.
- Loopful 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.
- After 24 hours, V. cholerae colony was tested. The colony of V. cholerae was plain, yellow color and very big size (generally 2-3 mm).
- From TCBS the selected colony was transferred to the butt of Triple Sugar Iron Agar (TSIA) Slant with streaking.
- Then, TSIA tubes are incubated at 37°C for 24 hrs. Black color gas was observed in TSIA indicated that V. cholerae was absent.

Data Analysis

All data were analyzed with Microsoft Excel 2003. Data were presented as mean ± SEM.

RESULTS

Microbiological Quality Assessment

Total bacterial load, total coliform, Solmonella sp., Vibrio cholerae etc. were investigated throughout the study period. Microbiological test of normal water, UV radiated water, ice, receiving table, grading table, panning tray, worker's hand and cooked product (Black Tiger Head Less Shell on) were also investigated.

Total Bacterial Load/ Standard Plate Count (SPC)

SPC of normal water (Table 1), UV radiated water (Table 2) and ice (Table 3) were 8.66 ± 0.45 cfu ml-1, 0 cfu ml-1, 5 ± 0.36 cfu ml-1 respectively. SPC of receiving table, grading table and panning tray, worker's hand were 45 ± 0.63 cfu cm-2, 91.33 ± 0.81 cfu cm-2, 79.33 ± 1.09 cfu cm-2, 42.33 ± 0.54 cfu cm-2 respectively (Table 4). Furthermore, SPC of cooked product of Black Tiger Head Less Shell on (BTHLSO) was 0.7×105 g-1 (Table 5).

Total Coliform, *Salmonella sp.* and *Vibrio cholera*

Total coliform was found nil from normal water (Table 1) and ice (Table 2). Total coliform was also found nil from receiving

table, grading table and panning tray and worker's hand (Table 4). Total coliform of Black Tiger Head Less Shell on (BTHLSO) cooked product was <3 (Table 5). Furthermore *salmonella* and *V. cholerae* were totally absent from Black Tiger Head Less Shell on (BTHLSO) cooked product (Table 5).

Observation of Standard Operating Procedures (SOPs)

Water Quality Observation

The observed water of the investigated processing plant was free from microbes & viruses, harmful chemicals, heavy metals and filths. Observed water quality was as follows-

Table 1. Total bacterial load of water

Sample No.	SPC cfu/ml (Mean ± SEM)	Overall Mean (± SEM)	Total coliform
1	10 ± 0.54		
2	7 ± 0.54	8.66 ± 0.45	Absent
3	9 ± 0.27		

Table 2. Total bacterial load of water just after UV radiation

Sample No.	SPC cfu/ml	Average	Total coliform
1	0		
2	0	0	Absent
3	0		

Table 3. Total bacterial load of ice

Sample No.	SPC cfu/ml (Mean ± SEM)	Overall Mean (± SEM)	Total coliform
1	6 ± 0.27		
2	4 ± 0.54	5 ± 0.36	Absent
3	5 ± 0.27		

Time Observation

All the raw materials were processed within 72 hours from the time of receiving, for reducing bacterial activity (Table 8). The time for all operating steps was found as follows:

DISCUSSION

The present investigation was carried out some microbiological tests in the selected plant laboratory. During investigation a bacterial survey of water and ice were conducted. The results from the table 1 indicate that water used for washing the shrimp was found to contain SPC 8.66 ± 0.45 cfu ml^{-1} which added with the shrimp ice used for lowering temperature during processing and ice contained SPC 5 ± 0.36 cfu ml^{-1} Water just after UV radiation contained SPC 0 cfu ml^{-1}. Total coliform was absent in both ice and water. Among the five different sources of sample, the highest SPC 7 cfu ml^{-1} was counted in overhead tank and lowest SPC 3 cfu ml^{-1} in panning tape water (Haq, *et al*. 2009). There is evidence to support the fact that surface water carries less bacteria compared to bottom mud about 50ft below the surface (Williams *et al*., 1952). As shrimp are bottom dwelling animals, the livelihood of their becoming contaminated with bacteria from the muddy substrate is always possibility. Beheading of shrimps has lead to reduction of bacterial counts by 75% and the effect of through washing on the reduction of microbial load of prawns has been documented by (Green, 1949) and (Willams *et al*., 1952). These results demonstrate that a wash with clean water and proper handling techniques will reduce high SPC. The acceptable limit of SPC of water is 10 cfu ml^{-1} (ICMSF, 1988). All the values that were found at each sampling in the processing plant showed better quality as well as within the acceptable limit.

Table 4. Total bacterial load of contract surface at different stages

Stages	Sample No.	SPC cfu/cm^2 (Mean ± SEM)	Overall Mean (± SEM)	Total coliform
Receiving table	1	45 ± 1.09	45 ± 0.63	Absent
	2	47 ± 0.54		
	3	43 ± 0.27		
Grading table	1	90 ± 0.81	91.33 ± 0.81	Absent
	2	95 ± 1.09		
	3	89 ± 0.54		
Panning Table	1	80 ± 1.09	79.33 ± 1.09	Absent
	2	75 ± 1.36		
	3	83 ± 0.81		
Worker's hand	1	40 ± 0.54	42.33 ± 0.54	Absent
	2	45 ± 0.81		
	3	42 ± 0.27		

Table 5. Total Bacterial Load, Total Coliform, *Vibrio cholerae* and *Salmonella sp.* of Black Tiger Head Less Shell on Cooked product

Product	SPC/g	Total coliform/g	*Vibrio cholerae*	*Salmonella sp.*
BTHLSO cooked	0.7×10^5	<3	Absent	Absent

Temperature Observation

A sufficient room air cooler was installed in the studied processing plant to maintain the standard temperature (Table 7). For the proper maintenance of cold chain in the all processing steps, the following temperature was observed at all operating steps:

The SPC of shrimp contact surface such as, receiving table, grading table and panning tray, worker's hand were 45 ± 0.63 cfu cm^{-2}, 91.33 ± 0.81 cfu cm^{-2}, 79.33 ± 1.09 cfu cm^{-2}, 42.33 ± 0.54 cfu cm^{-2} respectively. SWAB samples were collected from worker hand, where mean SPC before and after working, were 20 and 30 per cm^2 respectively (Haq *et al.* 2009). After working the SPC was increased due to handling of shrimp.

During handling the SPC come to direct contact with the workers hands. For this reason after working the SPC of SWAB test results increased. The standard limit of SPC incase of SWAB is 50 cfu cm^{-2} (ICMSF, 1988).

Table 6. Observed water quality of investigated plant

Parameters	Observed Quality	Standard Quality
Standard Plate Count	<10 cfu/ml	<10 cfu/ml
Total Coliform	Nil/100ml	Nil/100ml
E. coli/Fecal Coliform	Nil/100ml	Nil/100ml
P^H	6.7-7.9	6.5-8.5

Table 7. Observed temperature at all operating steps

Parameter	Observed Limit (° C)	Operational Limit (° C)
Receive Room temperature	18 - 21	<21
Raw material (iced) temperature	4 - 5	<5
Raw material(Freshly caught)temperature	Ambient	Ambient
Fish at all processing step temperature	8 - 10	<10
Raw material washing water temperature	9 - 11	<10
Chill storage temperature	3 - 4	<4
Process hall temperature	18 - 22	<21
Panning water temperature	2	<2
Freezer temperature loading	9 - 11	<10
Freezer temperature unloading	-34 to -36	<-35
Glazing water temperature	2	<2
Core temperature of block	-18	<-18
Anteroom temperature	13 - 15	<15
Frozen storage temperature	-18 to -20	<-20
Hot Water	75-78	>75

Table 8. Observed time for all operating steps

Parameter	Observed Limit (Minute)	Operational Limit (Minute)
Receive	26 - 29	<30
Dip & Wash	4 - 5	< 5
Grading	19 - 21	< 20
Pressure Wash	5	< 5
Weighing	2	< 2
Individual Wash	4 - 5	< 5
Panning	3 - 4	< 3
Stand by	28 - 31	<30
Freezing	118 - 120	<120
De-panning	4 - 5	< 5
Glazing	2	< 2
Packing	14 - 16	<15

Furthermore, SPC of Black Tiger Head Less Shell on (BTHLSO) cooked product was 0.7×10^5. Total coliform of Black Tiger Head Less Shell on (BTHLSO) cooked product was <3. *Salmonella* and *V. cholerae* were totally absent from Black Tiger Head Less Shell on (BTHLSO) cooked product. 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 sample was <3. (Hossain, *et al.* 2010) According to ICMSF, the acceptable upper limit of total bacterial load, total coliform and faecal coliform is 106 cfu/g, 100 MPN/g and <3 MPN/g, respectively, while *salmonella* and *V. cholera* should not present ICMSF, 1982). In the present study, the total coliform was under the limit of ICSMF. Besides, *salmonella* and *V. cholera* were not detected in the Cooked IQF product. Thus, the sample of Black Tiger Head Less Shell on (BTHLSO) cooked IQF product was under the acceptable limit according of ICMSF and FDA guidelines (ICMSF, 1988 and FDA, 2001). During the preservation processes, the sample of Cooked IQF Shrimp showed the lowest total coliform. 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 and raw IQF shrimp, elimination occurs only during freezing. The lowest count in Cooked IQF shrimp might be because of this reason. From the present study, it is reveled that Cooked IQF shrimps are highly qualified for export purpose.

Bangladesh is exported the frozen sea foods mainly in the EU, USA and other developed countries of the world. After 17 December, 1997 it was mandatory to prepare the all seafood products under the HACCP regulations (USFDA, I997). But the implementation of HACCP in a processing plant is not only narrative task. Good Manufacturing Practices (GMPs) and sanitation are the perquisite for the implementation of HACCP system. The plant or factory layout, facilities, Standard Operation Procedure (SOPs) and Sanitation Standard Operating Procedures (SSOPs) are the principal points of GMPs and sanitation procedures which are obviously needed to implement HACCP system. The current study showed that the GMPs and sanitation procedures of the selected fish processing plant was excellent, hence their product performance (quality) earned a better place in the foreign markets. The company arranged all facilities as well as SOPs and SSOPs which were prerequisite for GMPs then for the implementation to HACCP program. Moreover, in around the processing area sanitation practices were adopted. The entrance to the plant had a foot dip area which contained chlorinated solution to sanitize worker's foot while walking. Access to the plant was limited and the ambient area was clean and free from pollution which prevent the cross contamination.

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