Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(6): 263-267

Received: 07-09-2016
Accepted: 08-10-2016

Md. Golam Sarwer
Fish Inspection and Quality Control (FIQC) Laboratory, Department of Fisheries (DoF), Chittagong, Bangladesh

Sayed Abdus Sadik Ahnaf Tahmid
Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali, Bangladesh

Md. Mahmudul Hasan Rony
Fish Inspection and Quality Control (FIQC) Laboratory, Department of Fisheries (DoF), Chittagong, Bangladesh

Correspondence
Md. Golam Sarwer
Fish Inspection and Quality Control (FIQC) Laboratory, Department of Fisheries (DoF), Chittagong, Bangladesh

Microbial quality assurance of finished, cooked and raw shrimp (*Penaeus monodon*) products of a marine fish processing industry of Bangladesh

Md. Golam Sarwer, Sayed Abdus Sadik Ahnaf Tahmid and Md. Mahmudul Hasan Rony

Abstract
The vision of this research was to examine the quality assurance of three different stages of frozen black tiger shrimp (raw head on & shell on, cooked and finished) products of a marine fish processing industry of Bangladesh. The microbial quality of those frozen black tiger shrimp (raw head on, shell on, cooked and finished) products were determined by the numerical value of APC (Aerobic Plate Count). In this study, total bacterial load (Aerobic Plate Count) of three raw (head on and shell on) samples was on average 1.6± 0.29× 105 CFU/g. In addition, three samples of finished frozen shrimp products was on average 1.09±0.102× 105 CFU/g and three samples of cooked frozen products was on average 0.23± 0.0116× 105 CFU/g. Here above mentioned all these data was under the limit of ICMSF recommended microbiological value, only because of maintaining possible critical control points (CCPs) and Hazard Analysis critical control point (HACCP) system.

Keywords: Finished, cooked and raw shrimp products, APC (aerobic plate count), hazard analysis and critical control point (HACCP), microbial quality assurance

Introduction
Shrimp is one of the leading exportable products in Bangladesh. Bangladesh is earning about 500 millions of foreign currency yearly by exporting shrimp and contributing 3.78% in GDP. To gauge the prospects of shrimp farming, the south-western region of Bangladesh has been considered as the core farming areas. Shrimps and Prawns are the main exportable items which earn a substantial amount as BDT 2744.12 core against 50368 ton of product with demandable variation in 2007-2008 [4] and in 2009-2010 the earnings comes to BDT 3408.52 core [4]. Production of shrimp by culture and capture fisheries increased to a great extent in the beginning of 1980's in Bangladesh. In 2007-2008, 217,877.05 ha have been brought under shrimp culture [4]. Although shrimp farming has had a significant impact on the economy of Bangladesh, it is generally agreed. Major export items from Bangladesh are raw shrimp block frozen, IQF Shrimp and prawn, consumer pack of raw frozen shrimp, dry, salted and dehydrated fish and a little quantity of value added shrimp products. 63% of frozen shrimp exported to the European countries and 34% to USA from Bangladesh [4]. However, that this rapid expansion has had considerable environmental cost as well. The area under shrimp culture tripled in 10 years, from the mid-1980s to the mid-1990s, covering 130,000 hectares by 1999 [10]. In the process, mangroves have been removed and replaced by coastal shrimp ponds. The ponds have increased the salinity of adjacent lands, jeopardizing its future productivity.

Bangladesh government has also taken necessary measures along and to promote export performance. Long supply chain of raw material collection, inadequate infrastructure facilities, poor level of maintaining cool chain and lack of adequate HACCP based training on hygiene and sanitation. Among the different people involved in field level are the main problems of quality loss of raw materials. Shortage of raw materials resulted poor capacity utilization of the processing plants. The bagda (*P. monodon*) hatchery sector has expanded rapidly over the last few years mostly concentrated in Cox's Bazar region is enough to meet the target production. However, there is a shortage of pelleted shrimp feed in Bangladesh. A large number of export processors are now producing increasing amounts of value-added products such as individually quick-frozen, peeled and deveined, and butterfly cut shrimp, as well as cooked products and the export earnings from value added products is about half of the total export value. About 95% of total fish products are exported to European countries, USA and Japan and remaining is exported to Southeast Asia and the Middle East. Most of EU approved shrimp processing industries have been upgraded with laboratory facilities and provided HACCP training to the workers. As of now, HACCP is applied on the processing plants, but to ensure the quality of raw materials and to reduce risks, shrimp farms are also required to adopt HACCP plan. There is increased pressure from importing countries for fish processors time to time to establish effective quality assurance systems in processing plants.

There are 162 fish processing plants in Bangladesh of which 75 plants are in operation [4]. Annual production capacity of 75 plants is about 3396.28 MT. out of 75 plants European Commission has approved 74 plants [4]. FIQC of the Department of Fisheries has moderate laboratory facilities equipped with chemical, bio-chemical and microbiological testing facilities and qualified technical personnel, but so far there is no facility available for testing of antibiotics. FIQC mainly supervise quality aspects of the processing plants and has little or no control on raw material supply chain from farm to processing plants. Bangladesh export consignments sometimes face rejection due to poor quality of the products.

Most developing countries lack the resources to put up food microbiology laboratories adequately to international standards. The globalization of food trade and increasing problems worldwide with emerging and re-emerging food born diseases have increased the risk of cross border transmission of infectious agents. In this regard, developing countries are required to ensure that their sanitary and phytosanitary measures are based on an assessment, as appropriate to the circumstances, of the risks to human, animal or plant life or health, taking into account the risk assessment techniques developed by the relevant international organizations. The raw and frozen product of shrimp is demandable to the Buyers of European countries. but if the microbial loads of frozen shrimp products exit there limit, then the acceptance of frozen shrimp product will be drastically low which has seen last few years of export activities of foreign countries. That is why the microbial assessments are important to the processing plants of Bangladesh. So the main objective of the current investigation was to have better understanding about the quality assurances procure of fish and shrimp products.

Materials and Methods

Study area and time

The present research work was a study on microbial quality assurance of finished, cooked and raw shrimp (*Penaeus monodon*) products of a marine fish processing industry of Bangladesh started on March 2015 and it was conducted in the fish processing plant located in the ARK Sea Foods Ltd of Chittagong, Bangladesh from April 2015 to July 2015.

Observation of Shrimp Processing Method

The present method of shrimp processing in the investigated plant was as follows:

1. At first, baskets were unloaded and shrimp were dumped on receiving table just after arriving.
2. Shrimp were then inspected, sorted, graded as supplier's grade and finally weighed.
3. After receiving, the received shrimp were rinsed with water and were stored in the chill room as buffer store.
4. As production line permitted, the head on shrimps were brought in the processing hall for preparing different types of finish products as per buyer's specification, such as HLSO, HLSOTO, BTHLSO-IQF, P & D, PUD etc.
5. The processed shrimps were then washed and graded according to the export standard and finally washed with spray washing water. Few minutes were allowed to drain excess water.
6. After draining, the weighing is performed as per buyer's demand. Weighed shrimps were arranged in a pan lying with polyethylene.
7. Chilled water as glaze added to pan, were waited for freezing in contract (3 hours) or blast freezer (8 hours) at -40 °C.
8. After completion of freezing the pans were unloaded and the blocks were de-panned. Then the blocks were passed through the metal detector plant to detect metal or any metallic substance inside the block. Having assurance of metal free, the blocks of frozen shrimp were then packed individually in a wax coated paper inner carton with proper labeling. Six such cartons were again packed in double corrugated master cartoon. The master cartons were then kept in cold storage at about -18 °C until they were ready for shipment or export.

Selection of Sample for Microbial Analysis

- **Raw shrimp:** Some sample (250 g of each) of shrimp (BTHLSO: Black Tiger Head Less Shell on 250gm**)** from different lots were collected randomly in receiving hall at the beginning of processing.
- **Finished Shrimp product:** Cooked product of shrimp (BTHLSO: Black Tiger Head Less Shell on 250gm**)** was selected from three different lots were collected randomly in packaging hall at the end of processing for the microbial analysis.
- **Processed (frozen) shrimp product:** Some samples of shrimp (BTHLSO: Black Tiger Head Less Shell on 250gm**)** of processed frozen shrimp were collected from the same lots of raw freshwater prawn which kept in cold storage at about -18 ^{0}C.

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

Swab Test

a) Bacteria from a known area of a surface were removed by passing a sterile cotton wool which was moistened with sterile peptone water.
b) 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.

a) Powder was weighted properly and then dissolved required amount of distilled water.
b) The mixture then sterilize in autoclave for 15-30 minutes at 121 °C under 15 lbs pressure.
c) 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 raw shrimp sample was blended for 1 min with 180 ml of sterile dilute 0.1% peptone in an automatic blender. 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}. The same procedure was followed for processed (frozen) shrimp product & finished (cooked) shrimp product samples which were kept in refrigerator for microbial analysis.

Standard Plate Count (Pour Plate Method)

General procedure for the detection of SPC:

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}).
2. Then 10^{-2} solution was converted to 10^{-3}, 10^{-4}, 10^{-5} solution with the 0.1% peptone solution.
3. Poured 1ml. of solution from each test tube in the sterile Petri dish.
4. Approximately 15 ml agar which has been melted and brought to 45 °C was poured into the plates.
5. 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.
6. 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 [3].

Computing Standard Plate Count

1. After 48 hours, the number of colonies which were developed in the Petri dishes was properly counted by colony counter machine.
2. 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.
3. The total number of bacteria found from each Petri dish for each dilution was averaged to find a reliable Standard Plate Count (SPC) or APC (Aerobic plate count).

Results

Microbial Quality Assessment

The comparison of bacterial colony among raw (head on, shell on) shrimp product, cooked shrimp product and frozen finished shrimp product are mentioned below:

Total Aerobic Bacteria: APC (Aerobic Plate Count) of raw shrimp products were 1.45×10^5 CFU/g, 1.19×10^5 CFU/g, 2.15×10^5 CFU/g for sample 1, 2 and 3 respectively (Table 3.1). Furthermore, APC of frozen finished shrimp products for three samples were 1.3×10^5 CFU/g, $.98 \times 10^5$ CFU/g, 1.01×10^5 CFU/g respectively (Table 3.2). Finally APC of cooked shrimp products were $.23\times 10^5$ CFU/g, $.21\times 10^5$CFU/g, $.25 \times 10^5$ CFU/g for sample 1, 2 and 3, respectively (Table 3.3).

Table 3.1 Density (CFU/g) of total aerobic bacteria detected in three different samples of raw (head on, shell on) shrimp products.

Sample No.	APC (CFU/g)	Mean	Mean ± SEM
Sample 1	1.45×10^5	1.6×10^5	$1.6\pm 0.29\times 10^5$
Sample 2	1.19×10^5		
Sample 3	2.15×10^5		

Table 3.2 Density (CFU/g) of total aerobic bacteria detected in three different finished samples of frozen shrimp products.

Sample No.	APC (CFU/g)	Mean	Mean ± SEM
Sample 1	1.3×10^5	1.09×10^5	$1.09\pm0.102\times 10^5$
Sample 2	0.98×10^5		
Sample 3	1.01×10^5		

Table 3.3 Density (CFU/g) of total aerobic bacteria detected in three different cooked samples of frozen shrimp products.

Sample No.	APC (CFU/g)	Mean	Mean ± SEM
Sample 1	0.23×10^5	0.23×10^5	$0.23\pm 0.0116\times 10^5$
Sample 2	0.21×10^5		
Sample 3	0.25×10^5		

Comparison of three different Stages of (*Penaeus monodon)* products - Raw (Head on, shell on), Finished and Cooked

The average aerobic plate count of raw frozen shrimp sample is ($1.6\pm 0.29\times 10^5$). Its aerobic plate count are higher than finished ($1.09\pm0.102\times 10^5$) and cooked ($0.23\pm 0.0116\times10^5$) frozen sample (Table 3.4). Reduction amount and reduction rate of microorganisms between raw & finished frozen product are 0.51×10^5(cfu/g) and 31.88% (Table 3.4). Its value between raw & cooked frozen product are 1.37×10^5(cfu/g) and 85.62% (Table 3.4). But reduction amount and reduction rate of microorganisms between cooked & finished frozen product are 0.86×10^5 (cfu/g) and 78.90% (Table 3.4).

Table 3.4 MPN/g (Mean ±SEM) count, reduction and relative reduction rate of Aerobic plate count (CFU: Colony Forming Unit, MPN: Most Probable Number)

Sample name	APC, mean ± sem (cfu/g)	Reduction of microorganism between raw & finished frozen product (cfu/g)	Reducing rate (%)	Reduction of microorganism between raw & cooked frozen product (cfu/g)	Reducing rate (%)	Reduction of microorganism between cooked &finished frozen product (cfu/g)	Reducing rate (%)
Raw sample	$1.6\pm 0.29\times 10^5$	0.51×10^5	31.88	1.37×10^5	85.62	0.86×10^5	78.90
Finished frozen sample	$1.09\pm0.102\times 10^5$						
Cooked frozen sample	$0.23\pm 0.0116\times10^5$						

Discussion

The study worked on three different products of shrimp (*Penaeus monodon*). For raw shrimp product, three samples took randomly from the receiving room and found the average number of APC is 1.6 ± 0.29× 10^5 (CFU/g). The value is comparatively less than ICMSF [7] (International Commission on Microbiological Specification for Foods) recommended microbiological limits for sea food. After taking raw shrimp product, three of finished shrimp product and three of cooked shrimp product are also taken, but the average number of APC are 1.09±0.102×10^5(CFU/g) for finished shrimp product and 0.23± 0.0116× 10^5 (CFU/g) for cooked shrimp product. The values are also remaining within the acceptable limit of ICMSF standard chart. From above three different samples, it is seen that the microbial load in raw product is higher than finished product and cooked product, due to the contamination of supply channel, labor hygienic condition, suitable temperature for microbial load etc.

Table 4.1: ICMSF [7] recommended microbiological limits for sea foods.

ICMSF Recommended Microbiological Limits for Sea foods					
Product	Microorganism/test	n	c	Limit per g or cm 2 m M	
Fresh and frozen fish	Aerobic plate count (APC)	5	3	5×10^5	10^7
Frozen Shrimp (Raw)	Aerobic plate count (APC)	5	3	10^6	10^7
Frozen Cooked shrimp	Aerobic plate count (APC)	5	2	5×10^5	10^7
n = number of representative sample units; c = maximum allowable number of sample units which exceed microbial level m; m = microbial level which separates good quality from marginally acceptable quality; M = hazardous or un acceptable microbial level.					

But the reduction rate of microorganisms between raw & finished frozen product is 31.88%, between cooked & finished frozen product is 78.9% and between raw & cooked frozen product is 85.62%. So it can be clearly seen that the reduction rate of microorganism in cooked product is comparatively higher than other two products due to standard cooking temperature 65-72 °C. In Cooked IQF shrimp, elimination of bacteria occurs in two steps first during cooking and then freezing [9]. In this study, it can be clearly seen that temperature is a key factor to reduce microbial load of frozen food. Shrimp is a bottom dwelling organism, so there is a huge chance to be contaminated with bacteria from the muddy substrate. Sreenivassam [8] worked on freshly caught whole shrimp. He found that 1.8 × 10^6 CFU/g bacteria per gram in freshly caught whole shrimp. Alam *et al.* [1] performed a study on the prevalence of bacteria in the muscle of shrimp in processing plant. They found that the bacteria levels in the muscle of five supplied shrimp were decreased over time and varied between 8 × 10^5 CFU/g and 6 × 10^5 CFU/g. But sometimes external contaminations are responsible for incidence of this bacterium in fish, shrimp [6]. This bacterial growth may occur due to survival of pathogen for time and temperature abuse. If proper cooking time and temperature is not maintained then the pathogens can survive in the cooking product and caused hazard to the consumers. The standard cooking time and temperature for cooked shrimp product are 65-72 °C at 3 minute. The investigated plant maintained the standard cooking time and temperature for cooked shrimp product. If the beheading is done in that case, shrimp has leaded to reduce 75% of bacterial counts. This effect of reduction of microbial load of prawns has been documented by Green [5] and Williams [11].

The microbial range in the shrimp is seasonally varied. So, fish processing plants are careful about the export quality of fish and shell fish product all the year round with special effort on sources of raw fishes and crustaceans. Shrimp and prawn culture faces a range of challenges including disease, compliance with quality standards in importing markets and in equitable terms of exchange among value chain actors [2]. Council of the European Communities (EEC) recently established new regulation for export of cooked crustaceans. The standard of ICMSF has also a worldwide acceptance beside this. In Bangladesh, very few data are available on microbial enumeration of shrimps from neighboring countries like India, and Pakistan. In most of their investigations, the microbial counts of raw and cook shrimps in comparison with international standard were overlooked. So the present investigation might meet this gap.

Conclusion

It is necessary to give more attention to the quality and safety aspects of shrimp products related to the harvesting, handling, processing and packaging. Now-a-days, the shrimp is creating a good market as a new product and test to the foreigner. This exportable product has a great chance to earn a lot from international market. Brackish water shrimp (*Penaeus monodon*) prefers for its taste, size and color. Lack of proper knowledge, negligence about sanitation and quality related factors at different stages of handling, transportation and processing results low graded frozen shrimp and huge qualitative and quantitative losses. So, we should concern about the quality and safety aspects of shrimp. Low quality shrimp products are not only a great concern of food security and public health but also of serious national economic loss. If the defects and hazards of shrimp are controlled then export of Bangladesh would be increased.

Acknowledgement

The facilities provided to us in the microbiological laboratory of ARK Sea Foods Ltd, Chittagong, Bangladesh to carry out this study gratefully acknowledged.

References

1. Alam SMN, Mustafa G, Bhuiyan MDH. Prevalence of bacteria in the muscle of shrimp in processing plant. Internet Journal of Food Safety. 2003; 5:21-23.
2. Belton DJ, Pearce RA, Sheridan JJ, Blair IS, McDowell DA, Harrington D. Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. Journal of Applied Microbiology. 2011; 92:893-902.
3. Carpenter PL. WB Saunders Company, London. Microbiology 3rd Ed: 1980, 433-446.
4. DoF (Department of Fisheries). Jatiyo Matshya soptaho. Ministry of Fisheries and Livestock, The Government of Peoples republic of Bangladesh, Dhaka, 2012.
5. Green M. Bacteriology of shrimp, qualitative studies of freshly caught and iced shrimp. Food Research. 1949; 14:372-383.
6. Huss HH. Assurance of seafood quality. 1994; FAO Fisheries Technical Paper No.334.
7. ICMSF. Microorganisms in foods, Sampling for

microbiological analysis: Principles and specific applications. Buffalo, NY. 1986; 2(2):55.

8. Sreenivassam A. A note in the bacteriological of shrimp and their preservation by freezing. J Sci. Ind. Res. 1958; 2(1):119.
9. Suwanrangsi S. Experiences in the application of HACCP for export and local markets: The case of Thai fisheries, Food safety management in developing countries. Proceedings of the international workshop, CIRAD-FAO, Montpellier, France, 2000.
10. USFDA. Bacteriological Analytical Manual Association of Official Analytical Chemist. 6^{th} edition, Arlington, 1984.
11. Williams OB, Ress HB, Campbell LL. The bacteriology of Gulfcoast shrimp, 1 and 2, Experimental procedures and quantitative results. Texas Journal Sci. 1952; 4:49-52.