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Microbiological evaluation of ready to eat product of Horina (*Metapenaeus Monoceros*) shrimp at a sea food industry of Bangladesh

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Abstract
Microbiological quality assessment is a crucial part of sea food processing industries. The food processing companies must safeguard the quality and safety of their products for the consumers. The study was carried out to assess the microbial load (total bacterial load- total coliforms, fecal coliforms, *Salmonella spp*, and *Vibrio cholera*) of ready-to-eat exportable Horina shrimp (*Metapenaeus Monoceros)* products processed in a sea food processing industry of Bangladesh. For the microbial analyses, samples were collected from cooked IQF (Individual Quick Freezer) shrimp and BF (Block Frozen) shrimp. Microbial analysis was conducted using the standard protocol for counting and identifying microorganisms. Total bacterial load in cooked four samples of Horina shrimp (Br. CPBF, Br. Cooked & peeled BF, Hr. PUD cooked IQF, Br. CP&D BF) were 0.059×10^5cfu/g, $0,054\times10^5$cfu/g, 0.069×10^5cfu/g, 0.059×10^5cfu/g, respectively. Total coliform and fecal coliform of cooked shrimps was < 3 MPN/g. *Escherichia coli*, *Salmonella* and *V. Cholera* were totally absent in cooked shrimp products. The data demonstrated that all the values were under the limit of ICMSF recommended microbiological standard might be because of maintaining possible critical control (CCPs) and critical control point (HACCP) system. The result of the present study implies that the Horina shrimp ready-to-eat product in sea food industry from Bangladesh was of excellent quality for export, and posed no risk to human health.

Keywords: Microbiological evaluation, ready-to- eat, cooked and peeled, block frozen

1. Introduction
Shrimp is one of the top exportable products from Bangladesh. The fishing industry was responsible for over one-fourth (26.50%) of the agricultural GDP and roughly 3.57% of the overall national GDP. Bangladesh exported fish, shrimp, and other fisheries products, accounting for 1.24% of all export revenue and these exports have brought in a substantial amount of foreign currency for Bangladesh (DoF, 2022) [2]. The main exports of fishery products are raw shrimp block frozen, IQF shrimp and white fish, PUD and P&D shrimp block frozen, consumer packs of raw frozen shrimp, chilled & frozen Hilsa, dry, salted and dehydrated fish, live fish, eel fish, crab, and a small amount of value-added fish and shrimp products (Islam *et al*., 2015) [8]. Around 98% of all fish exports worldwide are sent to the USA (30.06%), European nations (48.51%), and Japan (9.32%) (Hossain, 2003) [6]. The bulk of sickness outbreaks (80%) that result in fatalities are caused by bacterial infections like *Salmonella* spp. and *Vibrio* spp., according to numerous research (Hamdan *et al*., 2008) [4]. The processing of raw materials has an impact on the bacteriological quality of frozen shrimp (Iyer *et al*., 1970) [9]. Inadequately iced and improperly stored shrimp at higher temperatures promote the growth of the bacteria that cause microbiological changes (Reilly *et al*., 1986) [12]. Due to poor attention given to product quality and sanitation standards between 1975 and 1978, rejection rates for shrimp goods significantly decreased (Limpus, 1978) [10]. As a result, the United States Food and Drug Administration (USFDA) placed the country under automatic detention. Bangladesh was added to the USFDA's "Black List" along with other countries as a result of the contamination of frozen marine seafood with Vibrio, *Salmonella*, dirt, flies, cockroaches, and other insects.

Due to foreign buyers' refusal and the relatively low price they were ready to pay for Bangladeshi fish products, it sustained significant losses. Consequently, conducting a microbiological investigation is essential to maintaining product quality and satisfying European customers. The isolation and identification of microbial food pollutants aids in the comprehension on the development of methods to prevent or decrease consumer exposure to contagious pathogens. The microbiological quality of processed frozen black tiger shrimps was tested at fish processing plant (Hossain *et al*., 2010) [5]. Due to containing high bacterial load, decomposition, filth, unexpected foreign objects, as well as harmful microorganisms (*E coli*, *Salmonella*, *V cholerae*, etc.) the processed shrimp and fish are rejected by the importing countries.

Despite the fact that a lot of our nation's frozen and ready-to-eat goods are exported, the Export Promotion Bureau (EPB) has a ton of data showing that when competing with other nations, the exported fisheries goods lost money (Ahmed, 1999) [17]. Without a doubt, as a result, our country has experienced a significant economic loss. These activities occasionally occur as a result of poor GMP (Good Manufacturing Practices) compliance, faulty HACCP system implementation, and ineligible sanitation procedures. GMPs is a prerequisite before starting HACCP because GMPs act as a foundation of any fish processing plant. On a strong foundation of GMP compliance and acceptable sanitation methods, HACCP systems must be built. Recently, strict action was taken by the Bangladeshi government against fish and shrimp processing plants that don't follow importers' demands and don't employ the HACCP system to guarantee high-quality products (Cato, & Subasinge, 2003) [1]. Because of this, microbiological evaluations are highly valued in Bangladeshi processing facilities. Therefore, the present study was investigated the microbiological status of finished or ready to eat shrimp products for export to various countries of the world.

2. Materials and Methods

2.1 Sample collection, preparation and microbial analysis

A modern shrimp processing plant (ARK seafood Ltd) was chosen for microbiological assessment of its readiness to cook shrimp, due to its modern cooking facilities and approved HACCP plan. The processing plant had been constantly evaluated and verified by the relevant authorized organization. The present study conducted for microbial quality assurance of ready to eat Horina (*Metapenaeus Monoceros*) shrimp products at seafood industry of Bangladesh and from December 2015 to June 2016. Four samples of ready-to-eat products mainly cooked and peeled with Block frozen and Individual Quick freezing according to buyer's specification Br. CPBF (Brown Cooked Peeled Block Frozen), Br. Cooked &Peeled BF (Brown Cooked & Peeled Block Frozen), Hr. PUD cooked IQF (Horina Peeled and un-deveined cooked Individual Quick Freezing) as well as Br. CP&D BF (Brown Cooked Peeled and Deveined Block Frozen) of Brown/ Horina shrimp (*Metapenaeus monoceros)* were randomly selected from four different lots in the receiving hall at the onset of processing. Counting and identifying microorganisms for microbial analysis by used standard protocol.

2.2 Total bacterial load (APC) detection

20 grams of the sample was blended for one minute with 180 milliliters of sterile 0.1% peptone in an automatic blender, resulting in a dilution of 10^{-1}. The progressive dilution used with repeated method to prepare dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. In the Durham's tube (10^{-1}), 1ml of 10^{-1} solution was introduced together with 9ml of 0.1% peptone water and 1ml of LTB (lauryl tryptose broth). Then, the 10^{-2} solution was changed by adding 0.1% peptone solution to the 10^{-3}, 10^{-4}, and 10^{-5} solutions (Haq *et al*., 2009) [16]. The sterile Petri dish received 1ml of each test tube's solution. Around 15 ml melted and heated to 45 °C agar was put into the plates. For an even distribution of the media, plates were manually rotated five times in each of the following directions: clockwise, counterclockwise, and multiple times crosswise. The time between creating the dilution and pouring the agar was less than 15 minutes. After the medium had solidified, the plates incubate at 37 °C for 18 hours in an incubator (USFDA, 1984) [15]. Following 48 hours, a colony counting machine was used to accurately count the number of colonies that had grown in the Petri dishes. By dividing the average number of colonies on Petri dishes by the appropriate dilution factor, one can calculate the total number of bacteria per gram of material. Aerobic Plate Count (APC) was detected by averaging the total number of bacteria discovered in each petri dish for each dilution.

2.3 Enumeration of total coliform

In an automated blender, 20g of the material was blended for 1 minute with 180 ml of sterile dilute 0.1% peptone. This resulted in a 10-1 dilution. The Durham's tube (10-1) was filled with 1ml of 10^{-1} solution, 9ml of 0.1% peptone water (10^{-2}), and 1ml of LTB (Lauryl Tryptose Broth). Then, using the 0.1% peptone solution, the 10^{-2} solution was changed into the 10^{-3}, 10^{-4}, and 10^{-5} solutions. Durham's tubes were incubated with a solution of 10^{-1}, 10^{-2}, and 10^{-3} for 48 hours at 37°C. It was deemed sufficient proof of coliform presence when gas formed after 48 hours. Noted with gas formation, MPN chart was used for outcome calculation (USFDA, 1984) [15].

2.4 Detection of faecal coliform

The tubes of lauryl tryptose broth that produced gas were chosen, and a loopful of the broth from each positive culture was added to a tube of Brilliant Green Bile (2%) to produce gas. To detect the presence of indole, Kovac's reagent was used to taste broth and a tube of tryptone water. If a red ring appeared on the surface after 48 hours, that was seen as proof that E. coli was present at 44 °C. A MPN chart was used to calculate the results after the positive gas production tubes were recorded (USFDA, 1984) [15].

2.5. Detection of *Salmonella spp*.

Salmonella spp. were found after a piece of the composite sample weighing 25 g was homogenized in 225 ml of sterile buffered peptone water (pH 7.5) and incubated for 24 to 48 hours at 37 °C (figure 3.20). 1 ml of the material was added to duplicate tubes of Tetrathionate and Selenite Cysteine Broth, incubated for 24 hours at 37 °C, and then sub-cultured into Xylose Lysine Deoxycholate (XLD) and Brilliant Green Agar (BGA). Characteristic colonies (on XLD- black centered, convex, glossy entrances and on BGA- pink, red, convex, glossy colonies surrounded by brilliant red zones in the agar) were streaked with sterile platinum wise loop and incubated at 37 °C for 6 hours. Changes in features following incubation may aid in determining if *Salmonella* is present or absent. *Salmonella* was not present in the sample, as H_2S gas could

not be identified (USFDA, 1984) [15].

2.6 Detection of *Vibrio cholera*

A 25g portion of the composite sample was aseptically dissolved in 225 ml of sterile alkaline peptone water and incubated at 37 °C for 24 hours. In order to obtain individual colonies, loopful alkaline peptone water was streaked on the surface of different plates of Thiosulfate Citrate Bile Salts (TCBS) agar. The plates were then incubated at 37°c for 24 hours. *V. cholerae* colony was examined 24 hours later. The *V. cholerae* colony was simple, yellow in appearance, and relatively large (typically 2-3 mm). The chosen colony was moved from TCBS to the butt of a Triple Sugar Iron Agar (TSIA) Slant with streaking. After that, TSIA tubes are incubated for 24 hours at 37 °C. According to black color gas found in TSIA, *V. Cholerae* was not present (USFDA, 1984) [15].

3. Results and Discussion

3.1 Total bacterial load (APC)

Aerobic plate count of ready to eat (Br. CPBF, Br. Cooked &peeled BF, Hr. PUD cooked IQFA and Br. CP &D BF) Horina shrimp among four samples were 0.059×10^5cfu/g, 0.054×10^5cfu/g, 0.069×10^5cfu/g, 0.059×10^5 cfu/g respectively (Table 1 and Figure 1). According to ICMSF (1982), *Salmonella* and *V. cholerae* shouldn't be present; the permitted upper limits for total bacterial load, total coliform, and faecal coliform are 10^6 cfu/g, 100 MPN/g, and <3 MPN/g, respectively. In the study, APC, total coliform and faecal coliform in the ready-to-eat shrimp were, 0.06×10^5 CFU/g, <3MPN/g, and <3 MPN/g, respectively, which is below the ICSMF limit. Total bacterial load (Aerobic plate count) of three cooked frozen shrimp products was on average $0.23\pm 0.0116\times 10^5$ CFU/g (Sarwar *et al*., 2016) [13].

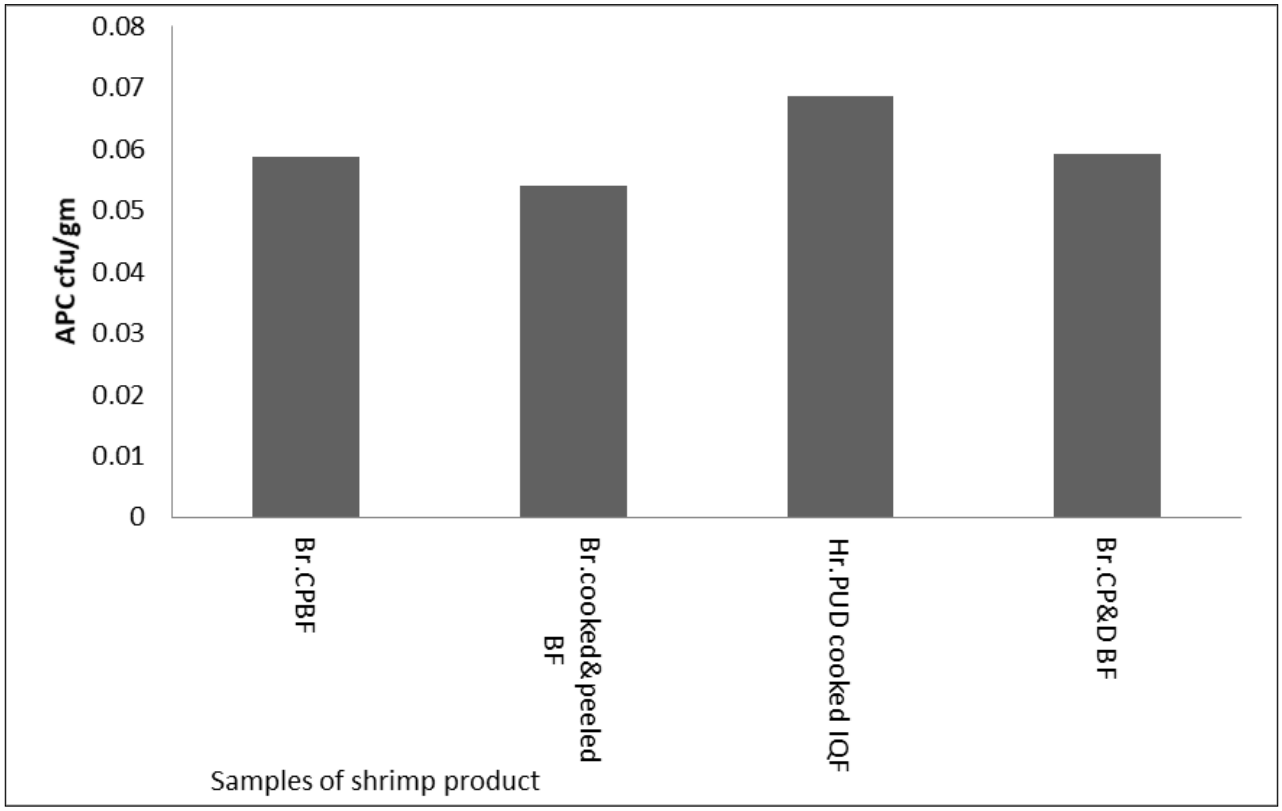

Fig 1: Aerobic plate count of four sample of ready to eat shrimp product. Br. CPBF =Brown Cooked Peeled Block Frozen, Br. Cooked &Peeled BF =Brown Cooked & Peeled Block Frozen, Hr. PUD cooked IQF =Horina Peeled and un-deveined cooked Individual Quick Freezing, Br. CP&D BF =Brown Cooked Peeled and Deveined Block Frozen

3.2 Total coliform and faecal coliform

Total coliform and fecal coliform in ready-to-eat product of Horina shrimp among four sample were 0.30 MPN/g, 0.30 MPN/g, 0.30 MPN/g and 0.30 MPN/gBr CPBF, Br. Cooked &peeled BF, Hr. PUD cooked IQF as well as Br. CP &D BF respectively. *E. coli* was totally absent in four types of Horina shrimp products (Table1). Total coliform was < 3.0 MPN in cooked IQF shrimp, 21.00±0.25 MPN in raw block frozen shrimp, and 4.20 ±1.20 MPN in raw IQF shrimp, respectively (Hossain *et al*., 2010) [5]. The chosen samples were devoid of fecal coliform, *Salmonella* spp., and Vibrio cholerae. Total and faecal coliform were also found in shrimp ready-to-eat products in the present study with MPN values of < 3 respectively.

Table 1: Density (CFU/g) of total aerobic bacteria, MPN count of total coliform, fecal coliform and presence of *Salmonella sp*, *Vibrio cholera* per g of sample detected in four sample of ready to eat Horina shrimp product.

Sample name	APC CFU/g	Total coliform (MPN/g)	Fecal coliform (MPN/g)	*Salmonella* sp.	Vibrio cholera
Br. CPBF	0.059×10^5	0.30	0.30	Absent	Absent
Br. Cooked & peeled BF	0.054×10^5	0.30	0.30	Absent	Absent
Hr. PUD cooked IQF	0.069×10^5	0.30	0.30	Absent	Absent
Br. CP &D BF	0.059×10^5	0.30	0.30	Absent	Absent
Mean	0.06×10^5	0.30	0.30		

CFU: Colony Forming Unit; MPN: Most Probable Number and APC: Aerobic Plate Count

3.3 *Salmonella sp*. and *Vibrio cholera*

Salmonella sp. and *vibrio cholera* both were absent in ready to eat product of Horina shrimp in sample Br. CPBF, Br. cooked & peeled BF, Hr. PUD cooked IQF, Br. CP & D BF respectively (Table 1). In addition, the chosen samples lacked Vibrio cholerae and *Salmonella* spp. In accordance with ICMSF and FDA rules (ICMSF, 1982; FDA, 2001) [7, 3], the sample of Brown/ Horina shrimp ready-to-eat product was therefore below the permitted level. Shewan (1970) [14] investigated the prevalence of the diseases Vibrio parahaemolyticus, *Salmonella*, and Clostridium botulinum in fish and fishery products. He provided bacteriological data on several fishery products and suggested bacteriological criteria for fish and fishery goods. According to Natarajan *et al*. (1985) [11], *Salmonella* has been found in goods made from fresh, frozen, canned, and sun-dried marine fish. The absence of *Salmonella spp*. and *Vibrio cholerae* in the ready-to-eat shrimp product revealed that sanitation condition of this industry was good. After December 17, 1997, all seafood products had to be prepared in accordance with HACCP guidelines. However, there are other factors to consider while implementing HACCP in a processing plant. Sanitation and Good Manufacturing Practices (GMPs) are required for the HACCP system to be implemented. The layout of the factory or plant, the facilities, the Standard Operating processes (SOPs), and the Sanitation Standard Operating Procedures (SSOPs) are the main components of GMPs and sanitation processes, both of which are obviously necessary to apply the HACCP system. According to the results of this study, this shrimp products that are ready to eat are highly qualified for export purpose.

4. Conclusion

Microbiological quality of ready to eat shrimp product depends on processing method, application of good manufacture and hygienic practices during processing. In conclusion, it may be pointed out that the product is ready for consumption and export because each step from initial production to packaging process was operated under good sanitation condition which made sure there was a reduced amount of microbial load. Shrimps from Bangladesh that are already cooked and ready to eat might be regarded as hygienic and safe for ingestion. In fact, following the incident in 1997, Bangladeshi seafood exporters made all the necessary preparations to their establishments and facilities. Most importantly, they updated their HACCP activities and provided top-notch training opportunities for their staff in order to satisfy the demands of the European Commission and other international food regulating bodies. Strict connection to GMPs and HACCP plan-based processing can greatly improve the product quality. The chosen fish processing facility's GMPs and sanitation practices were good, and as a result, its product performance (Quality) gained a superior position in international markets.

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