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Comparative Analysis of Microbial Status of Raw and Frozen Freshwater Prawn (*Macrobrachium rosenbergii*)

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Abstract: The study was carried out to detect the health hazard microbial status in raw (head on, shell on) and frozen products of freshwater prawn (*Macrobrachium rosenbergii*) and to make comparison between them on the point of microbiological load at fish processing plant "ARK Sea Foods Ltd." in Chittagong, Bangladesh during June to November, 2011. The microbial quantity of total bacterial load, total coliforms, fecal coliforms, *Salmonella* spp. and *Vibrio cholerae* were determined with three different samples of raw and frozen products of freshwater prawn (*Macrobrachium rosenbergii*). In this study, total bacterial load (Aerobic Plate Count) of raw freshwater prawn (head on, shell on) was $4.37 \times 10^5 \pm 0.328 \times 10^5$ CFU/g whereas in frozen freshwater prawn was $1.42 \times 10^5 \pm 0.187 \times 10^5$ CFU/g, respectively. Total coliforms of raw and frozen freshwater prawn were 37.65 ± 13.32 MPN/g and 4.23 ± 0.95 MPN/g, respectively. Meanwhile, faecal coliform of raw freshwater prawn (head on, shell on) and frozen freshwater prawn were 3.23 ± 1.77 and 2.20 ± 1.11 MPN/g, respectively. Furthermore *Salmonella* spp. and *Vibrio cholerae* were totally absent in raw and frozen freshwater prawn. The above mentioned data were within the limit of international standards. The lots of each sample both raw and frozen which containedheavy microbial contamination were destroyed in processing plant. The results of the study revealed that the raw lots of freshwater prawn can be bought if processed well and the frozen prawns were excellent for exporting.

Key words: *Macrobrachium rosenbergii* · Coliforms · *Salmonella* · *Vibrio*

INTRODUCTION

Bangladesh is blessed with rich and extensive fishery resources with a wide variety of indigenous and exotic aquatic fauna. The soil, water and climate of Bangladesh are very favorable to inland fisheries, both open and closed water. Inland fisheries cover an area of 46,52,665 ha [1]. The country has sizeable area of land and water suitable for development of brackish water aquaculture. It has limited access to marine fish resources in the Bay of Bengal. The country has coast line of 710 km and marine water area covers an area of 41,040 nautical miles^2 [1]. The rapid expansion of prawn culture in the past fifteen years is one of the most remarkable developments in the country's fisheries sector.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 [2] and in 2009-2010 the earnings comes to BDT 3408.52 core [1]. There are 162 fish processing plants in Bangladesh of which 75 plants are in operation [1]. Annual production capacity of 75 plants is about 3396.28 MT [3]. Out of 75 plants European Commission has approved 74 plants [1]. Department of Fisheries has three inspection and quality control stations located at Khulna, Chittagong and Dhaka facilitated with testing laboratories [3].

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 [1]. Since the year 2000,

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global landings of all types of shrimp (*Penaeusindicus*) including freshwater prawn (*Macrobrachium rosenbergii*) rose by 66% to reach 7.1 million MT in 2007. Farmed shrimp alone made up almost 52% of this with the rest coming from wild catches [4]. Freshwater prawn constituted 4% of the world landings of shrimp (all types), up from 3.1% in 2006. The proportion may seem paltry, but freshwater prawn is gaining popularity in national and international markets. Almost 98% of the global freshwater prawn supply comes mainly from Asian countries with China, India, Thailand, Bangladesh, Taiwan and Vietnam being major producers [4]. Bangladesh is a big producer and consumer of fresh water prawn with a total production of about 23240 MT almost 60 to 80 percent of this are sold to processors while the remaining are channeled to local markets as head-on fresh products. The larger wild caught species are highly in demand as it has the best taste. Freshwater prawn is largely exported headless to the EU and the USA markets [4].

Shrimp or freshwater prawn is contaminated by water. The raw and exportable products may loss own quality due to netting, transportation, handling, processing and packaging. Thus the microbial assessment should be done for maintaining own quality and to gain satisfaction of buyers of European countries.

The present study was designed to assess the microbiological quality of raw (head on, shell on) and frozen freshwater prawn and to make a comparison about microbial load of raw and frozen products of freshwater prawn.

METERIALS AND METHODS

Collection of Samples: Frozen freshwater prawn (*Macrobrachium rosenbergii*) was examined for microbiological quality assessment. Fresh raw prawn were purchased from approved prawn producers and brought to the factory with ice in insulated trucks [5]. Receiving of sample was done in a protected platform. The samples were processed in three different processing techniques. Three different samples from three different lots each with four replicates were tested. The processing and preservation were done by the plants while the microbiological analysis of the samples was done in the Microbiology Laboratory of the same plants.

Processing Techniques:

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

Process 2: Raw IQF (Raw Individual Quick Frozen) – Freezing temperature -38 to -40°C.

Processing of Samples: 20g of each sample was addedto 180 ml of sterile 0.1% peptone water in a sterile polythene bag thatwas placed in a sterile stomacher and blended for 2 minutes. This homogenate sample resulted dilution at this stage was 10^{-1}. Again 1 ml of the 10^{-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 10^{-2}. In this way 10^{-3}, 10^{-4} and 10^{-5} dilutions were made [6].

Microbial Analysis: Microbial analysis was performed according to the standard procedures for the enumeration and identification of microorganisms [7].

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 [8].

Total Coliforms: For enumeration of coliform organisms by using liquid media, 1 ml portion of each decimal dilution was inoculated into 3 separate tubes of lauryl tryptosebroth (LTB) containing inverted Durham's tube (10^{-1}, 10^{-2} and 10^{-3}) and incubated at 37°C for 48 hours. The tubes showing growth turbidity and gas production were selected [8].

Fecal Coliforms: The tubes of LTB positive for gas production were selected and a loopful of broth from each positive culture was inoculated 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 24 hours in a circulation water bath [8]. After 48 hours of incubation, tryptone waters were tasted with Kovac's reagent to determine the presence of indole [9]. Sufficient gas production in tubes of BGLB and corresponding indole positive test of tryptone water were recorded[10]. For both total and fecal coliforms, the results were computed using MPN chart [11].

Detection of Salmonella spp.: 25g sample was taken in 225 ml sterile buffered peptone water [pH 7.5] aseptically and incubated for 24 hours at 37°C for pre-enrichment [12]. One ml of pre-enrichment medium was then pipetted to selective enrichment medium namely selenite Cystenebroth and incubated for 24 hours at 37°C. One

loopful of culture 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. Suspected colonies were identified by characteristic appearance, Gram staining and biochemical tests [13]. All cultures giving positive biochemical reactions were confirmed by agglutination test with *Salmonella* polyvalent (O) somatic antisera [14].

Detection of *V. Cholerae*: 25g sample was taken in 225 ml sterile alkaline peptone water aseptically and incubated at 37° C for 24 hours [12]. After incubation a loopful of culture was streaked on the thiosulphatecitrate bile salt sucrose (TCBC) agar plate and incubated at 37°C for 24 hours. Then the suspected colonies were confirmed biochemically [15]. Finally the strains were confirmed serologically by agglutination test using polyvalent *Vibrio cholerae* (O) antiserum [14].

RESULTS

Total Aerobic Bacteria: The density of total aerobic bacteria detected in all frozen samples of freshwater prawn was significantly lower than raw samples of freshwater prawn (Fig. 1). Aerobic plate counts(APCs) of raw freshwater prawn among three samples were 5.0×10^5, $4.25 \times 10^5$and 3.89×10^5 CFU/g of samples 1, 2 and 3, respectively (Table 1). Furthermore, APCs of frozen products of freshwater prawn of three samples were 1.75×10^5, 1.40×10^5 and 1.1×10^5 CFU/g of samples 1, 2 and 3, respectively (Table 2).

Total and Fecal Coliforms: Total coliforms of raw freshwater prawn among three samples were 64, 28 and 21 MPN/g of samples 1, 2 and 3, respectively (Table 1). Total coliforms in frozen product of freshwater prawn among three samples were 6.1, 3.6 and 3.0 MPN/g of samples 1, 2 and 3, respectively (Table 2). Fecal coliforms of raw (head on, shell on) freshwater prawn of three samples were 6.1, 3.6 and <3 MPN/g in samples 1, 2 and 3, respectively (Table 1). Whereas, fecal coliform countsof frozen freshwater prawn were 3.6, 3 and <3MPN/g in samples 1, 2 and 3, respectively (Table 2). In this study MPN count (Mean ± SEM) of total coliforms observed in different samples of raw (head on, shell on) freshwater prawn was 37.65 ± 13.32 MPN/gm (Table 1), while in frozen prawn product, the MPN count of total coliform was 4.23 ± 0.95 MPN/gm (Table 2). In raw samples of freshwater prawn, the MPN count of total coliform ranged from 21 to 64, the values varied too much (Fig. 2). In the study, the mean MPN count of fecal coliforms in raw (head on, shell on) freshwater prawn and frozen prawn products were 3.23±1.77 MPN/gm (Table 1) and 2.20 ± 1.11 MPN/gm (Table 2), respectively.

DISCUSSION

As prawns are bottom dwelling animals, the livelihood of their becoming contaminated with bacteria from the muddy substrate have always possibility.

In this work, mean APC, total and fecal coliform counts of frozen (1.42×10^5±$0.187 \times 10^5$4 CFU/g, 23±0.95 and 2.20±1.11 MPN/g, respectively) andraw (4.37×10^5 ± 0.328×10^5 CFU/g, 37.65±13.32 and3.23±1.77 MPN/g, respectively) prawn samples were determined. *Salmonella* spp. and *Vibrio cholerae* were not detected in all raw and frozen freshwater prawn samples. The acceptable upper limits of total bacterial load, total and fecal coliformsare 10^6 CFU/g, 100 and <3 MPN/g, respectively, while *Salmonella* spp. and/or *Vibrio cholerae*should not be present [16].

Sreenivassam [17] found that 1.8×10^6 CFU/gbacteria per gram in freshly caught whole shrimp [17]. Alam *et al.* [18] found the bacterial levels in the muscleof shrimp in processing plant decrease over time in five suppliers and varied between 8×10^5 and 6×10^5 CFU/g. Furthermore, Ali *et al.* [19] observed that the mean total coliform countof 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. The results of present study indicated that mean APC of the raw (head on, shell on) fresh water prawn is $4.37 \times 10^5 \pm 0.328 \times 10^5$ CFU/g (Table 1).

Salmonella spp. 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 [20]. *Salmonella* has been isolated from fresh, frozen, canned and sun dried marine fish products [21].

In the present study, *Salmonella* spp. and *Vibrio cholera* were not detected in any of examined samples. The present study shows the absence of *Salmonella* spp. and *Vibriocholerae* in frozen prawn because of proper handling, freezing and storing at -18°C which be properly ready for exportfrom Bangladesh following the guidelines of international standards.

Table 1: Density (CFU/g) of Total Aerobic Bacteria, MPN Count of Total Coliforms and Fecal Coliforms and Presence of *Salmonella* spp. and *Vibrio cholerae*in the examined samples of raw (head on, shell on) freshwater prawn

Sample No.	APC (CFU/g)	Total Coliforms (MPN/g)	Fecal Coliform (MPN/g)	*Salmonella* spp.	*Vibrio cholerae*
Sample 1	5.0×10^5	64	6.1	Absent	Absent
Sample 2	4.25×10^5	28	3.6	Absent	Absent
Sample 3	3.89×10^5	21	<3	Absent	Absent
Mean ± SEM	$4.37 \times 10^5 \pm 0.328 \times 10^5$	37.65±13.32	3.23±1.77		

Table 2: Density (CFU/g) of Total Aerobic Bacteria, MPN Count of Total Coliforms and Fecal Coliforms and Presence of *Salmonella* spp. and*Vibrio cholerae* in the examined samples of frozen freshwater prawn

Sample No.	APC	Total Coliform	Faecal Coliform	*Salmonella* spp.	*Vibrio cholerae*
Sample 1	1.75×10^5	6.1	3.6	Absent	Absent
Sample 2	1.40×10^5	3.6	3	Absent	Absent
Sample 3	1.10×10^5	3	<3	Absent	Absent
Mean ± SEM	$1.42 \times 10^5 \pm 0.187 \times 10^5$	4.23±0.95	2.20±1.11		

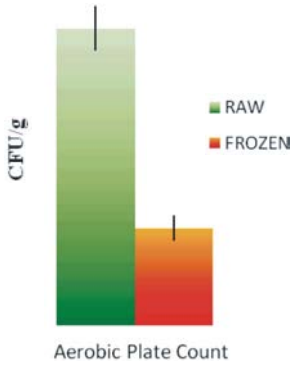

Fig. 1: Aerobic Plate Count (Mean ± SEM) of raw and frozen freshwater prawn samples

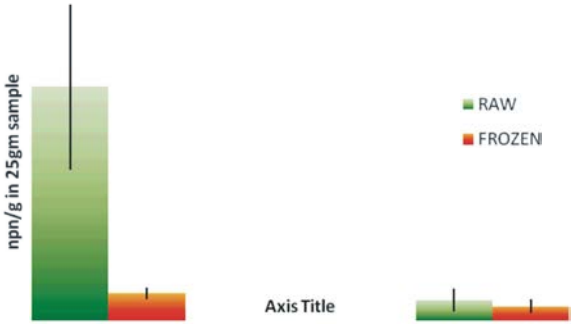

Fig. 2: Total and fecal coliform MPN counts (Mean± SEM)of raw and frozen freshwater prawn samples

Hossain *et al.* [22] reportedthat total coliform count of cooked IQF shrimp is<3 MPN, while it is 21.00±0.25 and 4.20±1.20 MPN in Raw Block Frozen Shrimp and Raw IQF shrimp, respectively. Fecal coliforms, *Salmonella* spp.and *Vibriocholerae* were not detected in any of the samples. During the preservation processes, the sample of cooked IQF shrimp showed the lowest total coliforms. In cooked IQF shrimp, elimination of bacteria occurred in two steps first during cooking and then freezing [23]. On the other hand, in the present study, raw (head on, shell on) freshwater prawn contained more MPN counts because not eliminated head and shell contain maximum microbiological load of total body and in frozen prawn the elimination of microorganisms occurs only during freezing. The processing method, species variation and also the aquaculture practices in fish farm might be also responsible for these reasons of variation.

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