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Microbial quality of shrimp products of export trade produced from aquacultured shrimp

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Abstract

Bacteriological quality of individually quick frozen (IQF) shrimp products produced from aquacultured tiger shrimp (*Penaeus monodon*) has been analysed in terms of aerobic plate count (APC), coliforms, *Escherichia coli*, coagulase-positive staphylococci, *Salmonella*, and *Listeria monocytogenes*. Eight hundred forty-six samples of raw, peeled, and deveined tail-on (RPTO), 928 samples of cooked, peeled, and deveined tail-on (CPTO), 295 samples of headless, undeveined shell-on (HLSO), and 141 samples of raw, peeled, and deveined tail-off (RPND) shrimps were analysed for the above bacteriological parameters. *Salmonella* was isolated in only one sample of raw, peeled tail-on. Serotyping of the strain revealed that it was *S. typhimurium*. While none of the cooked, peeled tail-on shrimp samples exceeded the aerobic plate count (APC) of 10^5 colony forming units per gram (cfu/g), 2.5% of raw, peeled, tail-on, 6.4% of raw, peeled tail-off, and 7.5% of headless shell-on shrimp samples exceeded that level. Coliforms were detected in all the products, though at a low level. Prevalence of coliforms was higher in headless shell-on (26%) shrimps followed by raw, peeled, and deveined tail-off (19%), raw, peeled tail-on (10%), and cooked, peeled tail-on (3.8%) shrimps. While none of the cooked, peeled tail-on shrimp samples were positive for coagulase-positive staphylococci and *E. coli*, 0.6–1.3% of the raw, peeled tail-on were positive for staphylococci and *E. coli*, respectively. Prevalence of staphylococci was highest in raw, peeled tail-off (5%) shrimps and the highest prevalence of *E. coli* (4.8%) was noticed in headless shell-on shrimps. *L. monocytogenes* was not detected in any of the cooked, peeled tail-on shrimps. Overall results revealed that the plant under investigation had exerted good process control in order to maintain superior bacteriological quality of their products.

Keywords: Bacteriological quality; Frozen shrimp; *Penaeus monodon*; *Escherichia coli*; *Salmonella*; *Staphylococcus*; *Listeria monocytogenes*; APC

1. Introduction

In recent years, India has witnessed a boom in aquaculture, mainly in farm-raised shrimps and fishes. Most of these farm-raised products are being processed as either block frozen or individually quick frozen (IQF) for export to various countries in Europe, Japan, and USA. Out of the total export of frozen seafood from India, shrimps contribute approximately 70%. The frozen shrimp exported from India has faced many problems in the past such as high bacterial count in the cooked and peeled frozen shrimps, as

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well as the presence of *Salmonella*. Central Institute of Fisheries Technology (CIFT) and Export Inspection Agency (EIA) have taken up these problems, and improved methods of handling and processing were suggested to the industry.

Increased import of seafood from developing countries by the developed economies also resulted in the adoption of international guidelines for food processing such as Hazard Analysis Critical Control Point (HACCP) and European Union (EU) guidelines by these countries. In an effort to control the microbial contamination of foods, National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1990) and International Commission on Microbiological Specification for Foods (ICMSF, 1988) have recommended a microbiological criteria as a means of assessing the effectiveness of HACCP programme. The present paper reports the findings of bacteriological quality of different individually quick frozen (IQF) raw and cooked shrimps produced from farm raised black tiger shrimp (*Penaeus indicus*) produced by a processor operating under HACCP.

2. Materials and methods

2.1. Sample collection

Samples were collected from a processor operating under HACCP guidelines. Farm raised tiger shrimps (*P. monodon*) were procured from local farmers by the processor and brought to the processing unit in insulated trucks with temperature control system.

2.2. Processing steps and critical control points

The samples were processed either cooked or raw as individually quick frozen. Different colour codes were given for the utensils used in different stages of processing. Current good manufacturing practices (CGMPs) were strictly followed during processing, and shrimp temperature was maintained below 10 °C at all stages of preprocessing using flake ice made from potable water. The processing environment, equipment, and utensils were cleaned using high-pressure water jets after each production shift of 8-h duration. After cleaning, the processing area floor, wall, equipment, and utensils were sterilized with steam. After steam sterilization, the conveyor belts of the equipment were wiped dry using clean, dry cotton towels. The process flow for raw and cooked shrimp is given in Fig. 1.

The critical control points common during the processing of both raw and cooked shrimps were raw material receiving, peeling, freezing, glazing, and packing. The processing steps such as cooking and cooling are critical and specific to cooked product.

The microbial hazard identified are high bacterial load associated with the raw material, cross-contamination of the meat from the raw material, as well as from the workers hand during peeling, microbial growth of possible psychrotrophs due to improper freezing, possibility of high microbial load in the

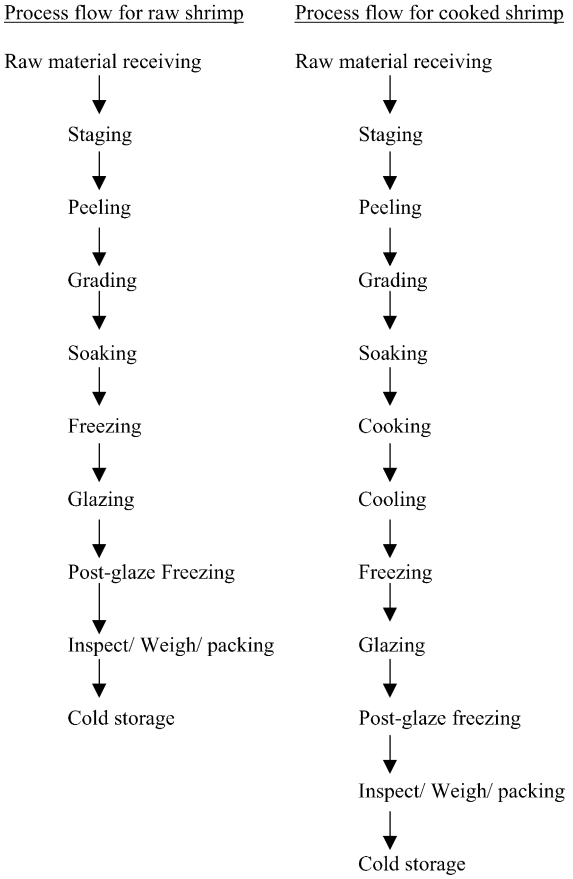

Fig. 1. Process flow for the raw and cooked shrimp.

recirculated glaze water and resultant contamination of the frozen product, and possible cross-contamination of the finished product at the packing region. The microbial hazards specific to cooked product are improper cooking and resultant inactivation of microbial load and possibility of high bacterial load in the recirculated cooler water and resultant cross-contamination of the cooked product during cooling.

The high microbial load associated with the raw material is neutralized to great extent by allowing the raw material to go through a preliminary wash in a 50-ppm chlorine water as soon as the raw material reaches the processing plant. The raw material then undergoes a wash through pneumatic filth washing machine in order to remove microfilth attached to the shrimp. This also helps the removal of bacteria associated with microfilth. The cross-contamination during peeling, as well as all other steps are kept under check by wearing of sterile surgical gloves and periodical hand dip in chlorinated water maintained at 50 ppm. Wearing of nosepiece is also mandatory. The peeling knife and utensils in all section are also rinsed periodically in chlorinated water maintained at different sections of the processing plant. The peeled-off waste material is removed regularly to a large polythene bag kept outside the plant through a chute. The cooker and freezer temperature are constantly monitored and maintained at 100–102 °C for cooker and kept at −35 to −40 °C for freezer in order to avoid problems due to improper cooking and freezing. This is done by the engineering personnel and also verified and recorded by Q.C. personnel in-charge of the respective area.

Since the cooler water and glaze water has to be maintained at a very low temperature (less than 3 °C for cooler water and less than 1 °C for glaze water) they are recirculated to a great extent and the possibility of a higher load of microorganisms in these waters are considered as possible microbial hazards. However, this is kept under control by maintaining proper chlorination and passage of water through UV filters. The microbial content of cooler water and glaze water was also monitored on a daily basis, and the limit for total heterotrophic bacterial content of this water is kept at 100 colony forming units per milliliters (cfu/ml). In the packing region, only authorized personnel are allowed to enter and all steps to avoid cross-contamination such as wearing of sterile gloves, nosepiece, and chlorine hand dip are strictly followed. Separate colour codes are maintained for all utensils used for raw and cooked product.

Another microbial hazard identified is cross-contamination from the processing environment, machinery, and utensils. Random samples from these are regularly included for the sanitary survey. The THB limit set for various samples are 100 cfu/25 cm^2. Worker's hands were also included in the survey and the limit for it is kept at 200 cfu/area equal to 25 cm^2 and with no coliforms. The workers showing higher counts were called in the quality assurance laboratory and educated about the importance of personal hygiene on product quality.

2.3. Bacteriological procedures

Samples for bacteriological analysis were collected aseptically at the end of the processing line by the Q.C. personnel designated in that area and submitted to the laboratory in a sterile polythene bags. Samples were collected at random on each day of production. Since the major product of the processor was raw, peeled tail-on and cooked, peeled tail-on shrimps, more number of samples were collected for those two products. The frozen samples were softened before analysis by overnight storage at 5 °C in a refrigerator and then processed for aerobic plate count (APC), coliform load, *Escherichia coli*, coagulase-positive staphylococci, and *Salmonella*. Cooked, peeled tail-on shrimps were also tested for *Listeria monocytogenes*. The methods used were those described in the bacteriological analytical manual of Food and Drug Administration (FDA; 1992).

Tryptone glucose beef extract (TGBE) (Himedia, Bombay) agar was used for the estimation of APC after incubation at 35 °C for 48 h. EC broth was used for the preliminary screening of *E. coli* followed by selective streaking on Tergitol-7 agar and Macconkey agar. Typical *E. coli*-like colonies were confirmed by IMViC test.

Samples for staphylococci were spread plated on Baird parker agar (Himedia) and incubated at 35 °C for 48 h. Typical colonies were confirmed using tube coagulase test (FDA, 1992).

For *Salmonella* detection, Rappaport–Vasilliadis (RV) broth and tetrathionate broth (TTB) were used for selective enrichment after preenrichment in lactose

broth. Selective media used were xylose lysine deoxycholate (XLD) agar, bismuth sulphite agar (BSA), and hektoen enteric agar (HEA). Cultures screened after preliminary and secondary biochemical tests (FDA, 1992) were confirmed by slide agglutination test using polyvalent O serum (Wellcome Laboratories, UK). Confirmed cultures were then serotyped at National *Salmonella* and *Escherichia* Centre, Kasauli, Himachal Pradesh, India.

The cooked, peeled tail-on shrimp samples for *L. monocytogens* detection were enriched in *Listeria* enrichment broth, and *Listeria* selective agar (Oxoid, UK) was used as selective plating medium. No typical *Listeria*-like colonies were encountered and further screening tests such as Gram staining, motility, and biochemical tests were not carried out.

3. Results and discussion

Eight hundred forty-six samples of raw, peeled, and deveined tail-on (RPTO), 928 samples of cooked, peeled, and deveined tail-on (CPTO), 295 samples of headless tiger (HLSO), and 141 samples of raw, peeled, and deveined tail-off (RPND) were analysed for bacteriological parameters such as APC, coliform load, presence of *E. coli*, coagulase-positive staphylococci, and *Salmonella* in order to assess the effectiveness of HACCP programme on the bacteriological quality of these products.

The bacteriological quality of the raw, peeled tail-on samples analysed during this period is given in Table 1. It is found that only 2.5% of the samples were found to be having APC values higher than 10^5 cfu/g,

Table 1
Bacteriological quality of individually quick frozen (IQF) raw, peeled, and deveined tail-on (RPTO) tiger shrimp

Month	No. of samples analysed	Samples exceeding APC[a] $>10^5$ cfu/g	No. of samples showing:			
			Coliform	*E. coli*	*Staphylococcus*[b]	*Salmonella*
1998						
January	25	0	0	0	0	0
February	46	0	4	0	0	0
March	52	0	2	0	1	0
April	47	0	12	1	1	0
May	38	0	17	3	1	0
June	22	0	7	0	0	0
July	28	2	5	1	0	0
August	49	5	0	0	0	0
September	15	0	0	0	0	0
October	46	3	4	0	0	0
November	24	1	0	0	0	0
December	55	0	2	1	1	0
1999						
January	45	0	1	0	0	0
February	51	0	0	0	0	0
March	39	0	0	0	0	0
April	14	1	0	0	0	0
May	27	0	4	2	0	0
June	12	0	0	0	0	0
July	17	0	0	0	0	0
August	14	1	1	0	0	1
September	39	2	7	0	1	0
October	48	2	2	2	0	0
November	54	3	9	1	0	0
December	39	1	8	0	0	0
Total	846	21 (2.5)	85 (10)	11 (1.3)	5 (0.6)	1 (0.1)

Numbers in the parenthesis indicate percentage value.
[a] Aerobic plate count.
[b] Coagulase-positive staphylococci.

which is set as the highest permissible level in this product by the processor. As the shrimps are procured exclusively from farms, the chances of natural contamination expected in case of samples from polluted coastal waters (Eyles, 1986) was not there. However, the microbial load present in the raw material can go up during transportation and storage. Excellent temperature control of the raw material (less than 10 °C) during preprocessing might have controlled the multiplication of mesophilic microflora present in the raw material. Results are indicative of considerable improvement from the results reported by Varma et al. (1985) who has observed 10^6 cfu/g in more than 7% of the raw samples collected from seafood processing units at Cochin.

Coliforms are detected in 10% of the raw, peeled tail-on shrimp samples and 1.3% of the samples were found to be contaminated with *E. coli*. There was no correlation between high APC and coliforms. This agrees with the finding of Buchanan (1991) who reported that thermo-tolerant coliforms could be used as a better indicator of process integrity than the APC. This indicates the scope for improvement in process integrity. *E. coli*, though at permissible level were detected in 1.3% samples. The coliform and *E. coli* levels were less than those reported by Iyer and Shrivastava (1989) in frozen raw shrimps. Staphylococci were detected in less than 1% of the samples. As humans are the major source of this organism in the processing environment (Garret,

Table 2
Bacteriological quality of individually quick frozen (IQF) raw, peeled, and deveined tail-off (RPND) tiger shrimp

Month	No. of samples analysed	Samples exceeding APC[a] $>10^5$ cfu/g	No. of samples showing:			
			Coliform	*E. coli*	*Staphylococcus*[b]	*Salmonella*
1998						
January	9	0	0	0	0	0
February	4	0	0	0	0	0
March	10	1	5	1	2	0
April	13	0	4	1	0	0
May	4	0	1	0	1	0
June	8	0	5	1	0	0
July	18	0	8	1	1	0
August	9	1	0	0	0	0
September	8	1	0	0	0	0
October	3	0	3	0	0	0
November	6	1	0	0	2	0
December	6	2	0	0	0	0
1999						
January	8	0	0	0	0	0
February	4	0	0	0	0	0
March	7	0	0	0	0	0
April	–	0	0	0	0	0
May	1	0	0	0	0	0
June	–	0	0	0	0	0
July	–	0	0	0	0	0
August	–	0	0	0	0	0
September	–	0	0	0	0	0
October	2	1	0	0	0	0
November	3	0	0	0	0	0
December	18	2	1	1	0	0
Total	141	9 (6.4)	27 (19)	5 (3.6)	7 (5)	0 (0)

Numbers in the parenthesis indicate percentage value.
[a] Aerobic plate count.
[b] Coagulase-positive staphylococci.

1988), a good personal hygiene and adherence to GMPs are a must to control this organism. Wearing of nosepiece and periodical dipping of worker's hand in chlorinated water are strictly followed in the processing unit under investigation. There was only one sample tested positive for *Salmonella* and it was isolated from raw, peeled tail-on shrimp. The serotype encountered was *S. typhimurium*. It was suspected that contamination was from raw material. Though aquaculture ponds are relatively uncontaminated, periodical visits by aquatic birds may contaminate the system with their droppings. Incidence of pathogenic microorganisms such as *Salmonella* and *E. coli* has been reported from aquaculture ponds of Thailand (Reilly et al., 1992) and India (Sivakami et al., 1996).

Bacteriological quality of raw, peeled, and deveined shrimps is given in Table 2. Around 7% of these samples had APC levels higher than 10^5 cfu/g and 19% of the samples were having coliform contamination. As this product is generally produced from soft-shelled shrimps and from shrimps with damaged tail, it is noticeable that their muscles are more prone to invasion by bacteria. It is also noticeable that in the processing unit, which is under investigation, there is lack of attention by the workers and resultant temperature abuse for this product. There is a suspicion among the workers that this product is coming from second quality shrimps. Around 4–5% of these samples were found to be contaminated with *E. coli* and coagulase-positive *Staphylococcus*, respectively. However, the present results have considerable improvement from

Table 3
Bacteriological quality of individually quick frozen (IQF) headless shell-on (HLSO) tiger shrimp

Month	No. of samples analysed	Samples exceeding APC[a] $>10^5$ cfu/g	No. of samples showing:			
			Coliform	*E. coli*	*Staphylococcus*[b]	*Salmonella*
1998						
January	10	0	0	0	0	0
February	19	0	5	0	0	0
March	12	0	1	0	1	0
April	11	1	3	0	1	0
May	34	0	23	4	0	0
June	11	0	10	2	0	0
July	16	0	7	0	0	0
August	17	0	0	0	0	0
September	23	4	2	1	0	0
October	36	9	10	0	0	0
November	28	5	5	2	0	0
December	12	1	0	0	0	0
1999						
January	6	0	0	0	0	0
February	6	0	3	1	0	0
March	3	0	0	0	0	0
April	4	0	1	0	0	0
May	4	0	0	0	0	0
June	1	0	1	0	0	0
July	6	0	0	0	0	0
August	13	0	3	2	0	0
September	5	0	0	0	0	0
October	11	1	0	0	0	0
November	6	2	2	2	0	0
December	1	0	0	0	0	0
Total	295	22 (7.5)	76 (26)	14 (4.8)	2 (0.7)	0 (0)

Numbers in the parenthesis indicate percentage value.
[a] Aerobic plate count.
[b] Coagulase-positive staphylococci.

those reported by Iyer and Shrivastava (1988), who observed that 38% of the frozen shrimps were contaminated with coagulase-positive *Staphylococcus*.

Table 3 represents the bacteriological parameters recorded in the headless shell-on shrimp samples. This sample only undergoes beheading during processing. This product is generally produced during heavy raw material inflow in order to convert the raw material into a product. Around 8% of the samples had APC levels higher than 10^5 cfu/g and 26% of the samples had coliform bacteria. Though *E. coli* contamination was recorded in approximately 5% of the samples, staphylococcal contamination was relatively less. The low staphylococcal contamination of the product may be due to the reduced handling of this product, as the chief source of contamination is the workers' hand. Higher coliform and *E. coli* levels of this product may be due to the semiprocessed nature of this organism. Even though the entire raw material received by the processor undergoes washing through pneumatic filth washing machine, fine sediment particles from the bottom of the pond may adhere to the pleopods of the shrimp, which can act as a possible source of coliform and *E. coli* for this product.

The only ready-to-eat product (cooked, peeled tail-on shrimp) produced by the processor showed good bacteriological quality (Table 4). None of the samples exceeded APC levels higher than 10^5 cfu/g, and there was no sample contaminated with *E. coli*, *Staphylococcus*, *Salmonella*, and *L. monocytogenes*. Temper-

Table 4
Bacteriological quality of individually quick frozen (IQF) cooked, peeled, and deveined tail-on (CPTO) tiger shrimp

Month	No. of samples analyzed	Samples exceeding APC[a] $>10^5$ cfu/g	No. of samples showing:				
			Coliform	*E. coli*	*Staphylococcus*[b]	*Salmonella*	Listeria
1998							
January	13	0	0	0	0	0	NT[c]
February	22	0	0	0	0	0	NT
March	29	0	0	0	0	0	NT
April	41	0	0	0	0	0	NT
May	46	0	7	0	0	0	NT
June	15	0	9	0	0	0	0
July	34	0	7	0	0	0	0
August	53	0	0	0	0	0	0
September	65	0	1	0	0	0	0
October	54	0	0	0	0	0	0
November	44	0	0	0	0	0	0
December	62	0	1	0	0	0	0
1999							
January	21	0	0	0	0	0	0
February	4	0	0	0	0	0	0
March	–	0	0	0	0	0	0
April	21	0	0	0	0	0	0
May	28	0	2	0	0	0	0
June	34	0	1	0	0	0	0
July	31	0	0	0	0	0	0
August	20	0	0	0	0	0	0
September	58	0	1	0	0	0	0
October	78	0	3	0	0	0	0
November	86	0	1	0	0	0	0
December	69	0	2	0	0	0	0
Total	928	0	35 (3.8)	0	0	0	0

Number in the parenthesis indicate percentage value.

[a] Aerobic plate count.

[b] Coagulase-positive staphylococci.

[c] Not tested.

Table 5
Summary of the bacteriological quality of the shrimp products analysed in the present investigation

Item[a]	No. of samples analysed	Samples exceeding APC[b] $>10^5$ cfu/g	No. of samples showing:				
			Coliform	*E. coli*	*Staphylococcus*[c]	*Salmonella*	Listeria
RPTO	846	21 (2.5)	85 (10)	11 (1.3)	5 (0.7)	1 (0.1)	NT[d]
RPND	141	9 (6.4)	27 (19.1)	5 (3.5)	7 (5)	0	NT[d]
HLSO	295	22 (7.5)	76 (25.8)	14 (4.8)	2 (0.7)	0	NT[d]
CPTO	928	0	35 (3.8)	0	0	0	0

Number in the parenthesis indicate percentage value.
[a] For explanation of abbreviations, see previous tables.
[b] Aerobic plate count.
[c] Coagulase-positive staphylococci.
[d] Not tested.

ature control while cooking and cooling operations were closely monitored in the processing unit under study and only authorized persons were allowed to touch the cooked product. The communications between raw and cooking sections were made wireless in order to prevent any possible chance of cross-contamination through movement of supervisors from raw to cooking sections. Presence of staphylococci are considered as excellent indicators of postthermal contamination by food handlers in a variety of products including cooked, ready-to-eat shrimp and crabmeat (NAS/NRC, 1985; ICMSF, 1978, 1980, 1988). Furthermore, because this microorganism does not grow at refrigerated temperatures if a product has been maintained under proper refrigeration there should be no increase in the number of *Staphylococcus aureus* over the course of food production–distribution–retail chain. This is significant as a higher level of this organism is a well-known cause of food intoxication through its production of heat stable enterotoxin. The absence of this organism in cooked, ready-to-eat shrimp samples collected from the plant under investigation reflects the excellent personal hygiene maintained by the workers in the processing environment. However, 3.8% of the samples had coliform contamination. This warrants a review particularly with regard to adequacy of sanitation procedures, as the source of coliform after thermal processing appears to be in the processing environment.

The present bacteriological survey was carried out over a period of 2 years, as there was no systematic study on the effectiveness of the process control on the bacteriological parameters except for the reports from Varma et al. (1985) and Iyer and Shrivastava (1988) about the product quality from the seafood processing units at Cochin. The results of the present study have been presented in a month-wise manner in order to study the correlation between the months of high production and the bacteriological quality of the product. A small let up was noticed in temperature control during the months of intense production as evidenced by the high levels of APC. However, no correlation was noticed between higher levels of APC and coliforms. This is well represented by the CPTO samples where none of the samples exceeded 10^5 cfu/g APC, even though 3.8% of the samples showed presence of coliforms.

The summary of the bacteriological findings of the present investigation is represented in Table 5. Comparisons of the present investigation with the earlier reports (Zuberi et al., 1983; Varma et al., 1985; Iyer and Shrivastava, 1988) revealed that there is considerable improvement in the bacteriological quality of the samples produced in the unit under investigation, which is functioning under HACCP guidelines. Though data from a single investigation may not be considered as satisfactory basis for the formulation of the control or regulatory bacteriological standards, it may be noted that HACCP based food-processing systems may be able to produce products with consistently good bacteriological quality.

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