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Microbiological Quality of Raw Shrimps Processed in Seafood Processing Plants of Tuticorin, Tamilnadu, India

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Abstract

The microbiological quality of raw shrimps collected from three seafood processing plants (viz. A, B and C) located in Tuticorin were tested. Total bacterial load of the shrimps were almost uniform with 10^4 to 10^5 cfu·g. Raw shrimps from plants 'A' and 'B' had lower counts of total bacteria and coliforms, followed by plant 'C'. The pathogens like *Vibrio cholerae, Salmonella* and *Listeria monocytogenes* were totally absent in raw shrimps. Significant difference ($p < 0.05$) was observed only in *E. coli* of the shrimps collected from different seafood processing plants at different months. The results suggested that seafood processing industries should exercise proper care in the collection of raw materials from the landing centres of the different regions in various seasons to produce uniformly good quality products.

Introduction

Shrimps are the most important items among the range of seafoods exported by India. Frozen shrimps, the highest foreign exchange earner among seafood items, account for more than 70% of the total earnings of Indian marine export products. It is generally accepted that the quality of a finished product depends largely on the quality of its raw material. Hence, preserving the freshness of a raw material becomes a very difficult task, more so when the time gap between harvesting and processing is very long. During this time gap shrimps, which are improperly handled, continue to deteriorate and further processing can never restore its freshness. Hence, spoilage of any food product is attributed to microbial growth due to improper handling and inadequate processing. Frozen shrimps are normally subjected to preshipment inspection based on physical and sensory characteristics followed by microbiological characteristics.

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Seafood processing plants usually receive their raw material supplies both from nearby and outside fishing centers. Rajadurai (1985) reported that the time interval between the landings of shrimps and their arrival at the processing plants is very important. Rao et al. (1986) studied the seasonal variations in the supply of raw material, with respect to shrimp processing plants located at Cochin, Veraval and Kakinada in India. It has been observed by Iyer et al. (1970) that bacterial content and handling of raw materials influence the bacteriological quality of frozen shrimps. Reilly et al. (1986) reported the microbiological changes that occur when shrimps are insufficiently iced and improperly stored at elevated temperatures. Zuberi and Qadri (1992) reported the important role of microorganisms in shrimp quality deterioration. Dalsgaard et al. (1995) examined shrimp samples for the prevalence of *Vibrio and Salmonella* while Jeyasekaran et al. (1996) reported the incidence of *Listeria* spp., particularly *L. monocytogenes* in seafoods landed in the Mangalore coast of India.

The recent introduction of Hazard Analysis Critical Control Point (HACCP) system and European Union (EU) hygienic regulations in seafood industries will pave the way for the production of safe and quality seafoods. The Tuticorin region on the east coast of India has about 19 seafood processing plants that export a substantial quantity of frozen shrimps. Losses due to the export of marginal quality seafoods or seafoods contaminated with pathogens will be heavy, and can affect the entire seafood industry located in that region. Since raw material quality determines the quality of the finished product, the present study was undertaken aimed at assessing the quality of raw shrimps used for processing in seafood industries located in Tuticorin, Tamil Nadu, India.

Materials and Methods

Raw shrimps belonging to the species *Penaeus indicus, P. monodon, P. semisulcatus, Metapenaeus dobsoni,* and *Parapenaeopsis stylifera* were obtained from three seafood processing plants (viz. A, B and C) located in and around Tuticorin, Tamil Nadu, India. The processing plants were selected for the present study based on their facilities, capacity, and status as representative plants for the region. Shrimps procured from these places were immediately placed in ice in insulated boxes (13.5 l capacity) with a drain valve and brought to the laboratory within half an hour for analysis.

Fresh shrimps (both headless and whole) from these seafood processing plants were collected in triplicates for a period of five months (September to January) at monthly intervals to determine their microbiological quality as per the sampling proforma, which included among others the name and location of the processing plant, pack type, date and time of sampling, temperature, sanitary status of raw material receiving hall, place and date of catch, distance between the place of catch and plant. The first batch of shrimp samples was taken in the month of September. The raw materials for plant 'A' were from the Kottaipattinam, Thondi, Ramanathapuram and Mandapam fishing centers; for plant 'B', the sources were Tuticorin, Ramanathapuram and Ovari and the sources for plant 'C' were Rameswaram, Kanyakumari and Tuticorin.

Raw shrimps were analyzed for total bacterial load (TPC); indicator organisms like total coliforms, fecal coliforms, *E. coli* and *Staphylococcus aureus* and human pathogens such as *Salmonella, Vibrio cholerae, V. parahaemolyticus* and *L. monocytogenes.* The shrimps, brought in insulated boxes were aseptically removed, peeled and weighed separately in sterile containers. To one part of shrimp, nine parts of diluent (physiological saline) were added and homogenized in a sterile homogenizer. Dilutions were made from shrimp homogenate and appropriate dilutions were selected for enumeration. The isolation and identification of bacteria were done as per APHA (1976) methods. Identification of the isolates was done as per the methods described by EIC (1995a) and Jeyasekaran (1996).

Appropriate dilutions of shrimp homogenate were spread plated onto Plate Count Agar (PCA) and incubated at 37°C for 24 to 48 h and the colonies were counted and reported as TPC. The Most Probable Number (MPN) technique was used to determine the level of total coliforms, fecal coliforms and *E.coli* in shrimp samples. Shrimp homogenate was transferred to Lauryl sulphate tryptone broth (LSTB) tubes and incubated at 37°C for 24 h for total coliform estimation. Samples from positive LSTB tubes were further transferred to *E. coli* (EC) broth tubes and incubated at 44.4 ± 0.5°C for 18 to 24 h for estimating fecal coliforms. Samples from positive EC broth tubes were streaked onto Eosine Methylene Blue (EMB) agar plates and incubated at 37°C for 24 to 48 h for estimating *E. coli.* Typical colonies were subjected to biochemical tests for final confirmation.

Shrimp homogenate was spread plated onto Baird parkar agar (BPA) and incubated at 37°C for 24 to 48 h for the estimation of *S. aureus*. Typical colonies were counted, purified and further subjected to biochemical tests for final confirmation. Shrimp homogenate was spread plated onto Thiosulphate citrate bilesalt sucrose (TCBS) agar plates and incubated at 37°C for 24 to 48 h and colonies were counted for the determination of *V. parahaemolyticus*. Typical colonies were subjected to biochemical tests for confirmation, 25g of shrimp sample was aseptically taken and homogenized with 225 ml of alkaline peptone water (APW) and incubated at 37°C for 6 h for enrichment. A loopful of enriched sample was streaked onto TCBS agar plates and incubated at 37°C for 24 to 48 h to test the presence of *V. cholerae.* After incubation, the plates were checked for colony morphology. Typical colonies were confirmed by subjecting them to different biochemical tests.

Shrimp (25 g) was taken aseptically and homogenized with 225 ml of lactose broth and incubated at 37°C for 24 h for pre-enrichment. One ml of pre-enriched sample was transferred to 10 ml of selenite cystine broth and tetrathionate broth and incubated at 37°C for 24 h for selective enrichment. A loopful of enriched sample was streaked onto Bismuth Sulphite agar (BSA) and Xylose lysine deoxycholate (XLD) agar plates and incubated at 37°C for 24 to 48 h for the examination of *Salmonella*. After incubation, the plates were observed for typical colonies. Suspected colonies were later subjected to various biochemical tests for confirmation. Shrimp sample (25 g) was aseptically taken and homogenized in 225 ml of Listeria pre-enrichment broth and incubated at 37°C for 24 h for pre-enrichement. Ten ml of pre-enriched sample was transferred to 90 ml of University of Vermont I (UVM I) broth and incubated at 37°C for 24 h for primary enrichment; 0.1 ml of enriched sample from UVM I broth was transferred to 10 ml of UVM II broth and incubated at 37°C for 24 h for secondary enrichment. A loopful of secondary enriched sample from UVM II broth was streaked onto PALCAM (Polymyxin Aesculin Lithium chloride Ceftazidime Acriflavin Mannitol) agar and Modified McBride's Listeria agar (MMLA) plates and incubated at 37°C for 24 to 48 h to test the presence of *L. monocytogenes*. After incubation, the plates were checked for typical colonies and suspected colonies were further subjected to biochemical tests for confirmation.

Analysis of variance (ANOVA) technique was used (Snedecor and Cochran 1962) to find out whether any significant difference exists between the samples of shrimps collected in different months and seafood processing plants in relation to overall microbiological quality.

Results

The shrimps from the processing plants had the TPC within the range of 10^4 and 10^5 cfu·g (Table 1). Shrimps collected in the month of November were found to have a higher bacterial load of 10^5 cfu·g, while those collected in the months of September and October had a lower bacterial load of 10^4 cfu·g. Total coliforms were detectable in shrimps collected from all the plants and their MPN counts ranged from 12 to 1600·g (Table 2). Shrimps from plant A recorded a lower level while the shrimps from plant C were found to have the highest total coliforms. Fecal coliforms were not detected in the shrimps collected during one month of sampling i.e. October for plant A and September for plant C. Shrimps from plant A recorded a low level of fecal coliforms, followed

Table 1. Total bacterial load (cfu·g) in raw shrimps collected from different seafood processing plants

Month of sampling	Plant A	Plant B	Plant C
September	$7.60 \pm 0.50 \times 10^4$	$3.35 \pm 1.05 \times 10^4$	$4.00 \pm 0.20 \times 10^4$
October	$1.40 \pm 0.10 \times 10^4$	$5.40 \pm 0.60 \times 10^4$	$1.75 \pm 0.05 \times 10^4$
November	$1.61 \pm 0.10 \times 10^5$	$1.95 \pm 0.35 \times 10^5$	$1.70 \pm 0.09 \times 10^5$
December	$4.30 \pm 0.20 \times 10^4$	$4.50 \pm 0.10 \times 10^4$	$2.05 \pm 0.10 \times 10^5$
January	$1.83 \pm 0.09 \times 10^5$	$8.95 \pm 0.25 \times 10^4$	$5.20 \pm 0.40 \times 10^4$

Table 2. Bacterial indicator organisms (MPN counts·g) in raw shrimps collected from different seafood processing plants

Month of sampling	Plant A			Plant B			Plant C		
	Total coliforms	Fecal coliforms	*E. coli*	Total coliforms	Fecal coliforms	*E. coli*	Total coliforms	Fecal coliforms	*E. coli*
September	33	33	ND*	13	13	ND	34	ND	ND
October	12	ND	ND	1600	1600	48	1600	1600	53
November	920	240	17	542	348	ND	1600	1600	345
December	1600	920	26	920	348	130	1600	920	542
January	1600	345	278	1600	1600	221	1600	348	138

ND* - Not detected

by plants B and C. *E. coli* was not detected in shrimps from plant A (in the months of September and October), plant B (in the months of September and November) and plant C (in the month of September). Shrimps from plants A and B had lower *E. coli* counts compared to those from plant C, which recorded a higher count of 542·g in the month of December (Table 2).

Shrimps collected from plant A had higher *S. aureus* count of 10^4 cfu·g, while the shrimps from plant B had low counts (10^3 cfu·g) except in the month of September (Table 3). Shrimps from plant C had the lowest count in the month of October, but had higher counts during the other months. *V. parahaemolyticus* was not detected in the month of September in shrimps from plants A and“”. It was found to be in high in shrimps collected from plant C, with 10^4 cfu·g in the months of November and December, while the shrimps from plants A and B recorded lower counts (10^3 cfu·g), with the exception in the shrimps from plant B collected in the month of October, which had the lowest count (Table 4). All the shrimps examined from the three processing plants were found to be free from *V. cholerae*, *Salmonella* and *L. monocytogenes* (Table 4).

Discussion

The TPC of the shrimps collected from the processing plants varied from 10^4 to 10^5 cfu·g, with about 66% having 10^4 cfu·g (Table 1). It was found that TPC was lower in plant B, with about 80% of the shrimps having the average counts of 10^4 cfu·g, whereas in plants A and C, only 60 % of the samples had a similar bacterial load. Most of the earlier reports indicated that bacterial load in freshly landed tropical shrimps ranged from 10^3 to 10^5 cfu·g (Vanderzant et al. 1970; Zuberi et al. 1987; Thampuran and Gopakumar 1990; Zuberi and Quadri 1992; Karunasagar et al.1992 and Iyer and Joseph 1995). The total bacterial load of the shrimps tested was lower than the standard limits (5.00 x 10^5 cfu·g) prescribed by EIC (1995b).

The occurrence of total and fecal coliforms was very low in the samples collected in the month of September (Table 2). Total coliforms were relatively high in the month of January in all the plants. However, the shrimps from plant C collected from October till January recorded a higher level of total coliforms. In respect of fecal coliforms, the counts were lower in the month of September in plants A and B, while it was not detected in plant C. On the contrary, in the month of October, fecal coliforms were relatively very high in plants B and C. The total and fecal coliform counts were less than 100·g in

Table 3. Levels of *Staphylococcus aureus* (cfu/g) in raw shrimps collected from different seafood processing plants

Month of sampling	Plant “A”	Plant “B”	Plant “C”
September	1.20 x 10^4	2.50 x 10^4	3.00 x 10^3
October	1.02 x 10^4	5.85 x 10^3	4.00 x 10^2
November	2.70 x 10^4	4.45 x 10^3	1.51 x 10^4
December	1.05 x 10^4	3.65 x 10^3	3.20 x 10^4
January	7.50 x 10^4	4.25 x 10^3	1.05 x 10^4

Table 4. Bacterial pathogens in raw shrimps collected from different seafood processing plants

Month of sampling	*Vibrio parahaemolyticus* (cfu/g)			*V. cholerae*			*Salmonella*			*Listeria monocytogenes*		
	Plant "A"	Plant "B"	Plant "C"	Plant "A"	Plant "B"	Plant "C"	Plant "A"	Plant "B"	Plant "C"	Plant "A"	Plant "B"	Plant "C"
September	ND*	ND	5.00×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
October	4.50×10^3	4.55×10^2	1.35×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
November	1.87×10^3	3.00×10^3	2.35×10^4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
December	1.55×10^3	4.85×10^3	1.25×10^4	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
January	3.50×10^3	3.65×10^3	9.50×10^3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

ND* – Not detected

only 40% of shrimps collected from plant A. Iyer and Joseph (1995) reported that the incidence of total coliforms in cultured *P. indicus* was 230·g, while Jeyasekaran et al. (1990) reported an MPN total coliform count of >240·g in tropical shrimps. Jeyasekaran et al. (1990) and Karunasagar et al. (1992) have reported a MPN faecal coliform counts of 11 to 240·g and 2 to >2400·g, respectively in freshly caught penaeid shrimps. In the present study also, the MPN total and fecal coliform counts were found in the range of 12 to 1600·g and 0 to 1600·g, respectively. Fecal coliform contents in shrimps vary depending on the sanitary and hygienic condition of the landing centers.

Wide variation in *E. coli* counts was observed among the shrimps and it ranged from 0 to 542·g (Table 2). Significant difference ($p<0.05$) in *E. coli* counts of shrimps collected in different months was noticed. Jeyasekaran et al. (1990) reported that *E. coli* was present in shrimps in the range of 0.6 to 240·g. About 40% of the samples from plants A and B had lesser *E. coli* count than the limit (20·g) prescribed by EIC (1995b). It has been observed that the occurrence of *E. coli,* fecal and total coliforms in the shrimps collected in the month of September from the processing plants were low, which increased in the subsequent months due to seasonal variation. In the Tuticorin region, rainy season generally starts at the end of October, which results in heavy runoff and carries away domestic sewage from the land to sea. Iyer et al. (1970) have also stated that season plays a role in controlling the bacterial quality of fresh shrimps and observed that the bacterial counts were higher in certain specific seasons. They have recorded a high incidence of *E. coli* in raw shrimps during rainy season, which is probably due to the high degree of fecal pollution of water during that period. Among the different microbial quality characteristics tested, only *E. coli* counts were found to differ considerably among the different seafood processing plants ($p<0.05$). This shows that by the estimation of *E. coli* counts in raw shrimps, the minimal quality differences among the shrimps can be found out.

S. aureus counts were found to be in the range of 10^3 to 10^4 cfu·g in all the shrimps collected in the months of October to January, except in the month of September (Table 3). It was found that 80% of the shrimps collected from plant B had a *S. aureus* count of about 10^3 cfu·g, whereas plant C had only 60% of shrimps with that load. On the other hand, all the shrimps from plant A had a load of above 10^3 cfu·g. However, the differences were not statistically significant ($p>0.05$). Krishnamurthy and Karunasagar (1986) also reported that S*taphylococcus* was present in significant numbers in raw shrimps. Higher counts of *S. aureus* observed in raw shrimps collected from plant A shows that personnel hygiene was not given much importance during handling and transportation of raw shrimps. The lower occurrence of *S. aureus* in plant B might have been due to proper care taken by the plant workers in handling the material on arrival. However, the *S. aureus* counts in shrimps were higher than the limit (100·g) prescribed by EIC (1995b). During sensory evaluation, it was observed by the panelists that strong chlorine odor was perceived in the shrimps collected in the month of October from plant C, which had *S. aureus* count of about 10^2 cfu·g. This might be due to excess chlorination.

V. parahaemolyticus was not detected in the shrimps collected in the month of September from plants A and B (Table 4). About 40% of the shrimps collected from plant B had *V. parahaemolyticus* counts below 10^3·g, while it was only 20% for plant A. With regard to plant C, none of the shrimps had counts less than 10^3 cfu·g. Bandekar et al. (1982) reported that all the shrimps tested had *V parahaemolyticus*. Karunasagar et al. (1985) have reported that freshly caught prawns from Mangalore coast were contaminated with *V. parahaemolyticus* in the range of 10^2 to 10^4 cfu·g, which is almost similar to the results of the present study. Venkateshwaran et al. (1996) reported the incidence of *V. parahaemolyticus* in headless and whole shrimps at a level of 1.60´10^2 and 3.60´10^2·g, respectively. The lower levels of *V. parahaemolyticus* in shrimps collected from plant B, therefore, show that the raw material was received from the fishing area, wherein the population of *V. parahaemolyticus* might be low. *V. cholerae* was absent in all the raw shrimps collected from the three processing plants (Table 4). But, Varma et al. (1989) reported that *V. cholerae* 01 was found to be present in only one raw shrimp out of 7238 shrimps tested.

Salmonella was not reported from any of the shrimps collected from the three processing plants (Table 4). Chen et al. (1990) analyzed the bacteriological quality of *P. monodon* and observed similar results. Dalsgaard et al. (1995) also reported that *Salmonella* was not recovered from shrimps tested in Thailand. However, there were very few reports on the incidence of *Salmonella* in fish and fishery products (Varma et al. 1985). Bhaskar et al. (1995) also reported the incidence of *Salmonella* in cultured shrimps. The results of the present study is supported by the findings of Hood et al. (1983), who observed that the low coliform levels indicate the absence of *Salmonella*, but high levels of fecal coliforms are somewhat limited in predicting the presence of *Salmonella*. *L. monocytogenes* was not found in the raw shrimps collected from the three processing plants (Table 4), which is well supported by earlier studies of Manoj et al. (1991) and Kamat and Nair (1994). However, Jeyasekaran et al. (1996) reported the incidence of *L. monocytogenes* in raw shrimps.

Conclusion

There is a difference between the shrimps collected in different months from the three seafood-processing plants with regard to their microbiological quality. It can be concluded that the quality of final products (i.e. frozen products) differs with the raw material received in different months and processed in the seafood processing plants. Hence, the present findings suggest that seafood processing plants should take proper care when collecting raw material from fish landing centers of different regions in various seasons to produce uniformly high quality product.

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