Sri Lanka J. Aquat. Sci. **10** (2005): 73-83

Microbiological quality of fresh and frozen cultured black tiger shrimp (*Penaeus monodon*) cultured in Sri Lanka

K.W.S. ARIYAWANSA*, K.S.HETTIARACHCHI AND S.P. JAYASOORIYA

Post Harvest Technology Division, National Aquatic Resources Research and Development Agency, Crow Island, Colombo 15, Sri Lanka.

*Corresponding author (E-mail: sariyawansa@hotmail.com)

Abstract

The prevalence of the pathogenic bacteria, *Salmonella* sp. *Vibrio cholerae, Vibrio parahaemolyticus*, total coliforms, faecal coliforms and *Escherichia coli* in cultured shrimp, pond sediments, water and the pelleted feed were analysed. Raw frozen shrimps were also analysed for total plate count, coliforms, faecal coliforms, *E. coli, Staphylococcus aureus, Salmonella, Vibrio parahaemolyticus* and *Vibrio cholerae*.

In shrimp, water and sediments the total coliform counts were <3-93 MPN/g, 0-45 MPN/100ml and 3.6-93 MPN/g, while the faecal coliform counts were <3-15 MPN/g, 0-11 MPN/100ml and <3-23 MPN/g respectively. *E. coli* were in the ranges of 0-8 MPN/100 ml (water), <3-9.1 MPN/g (sediment) and <3-7.3 MPN/g (shrimp). In one occasion, feed was highly contaminated with coliforms (>1100 MPN/g), faecal coliforms (>1100 MPN/g) and *E. coli* (460 MPN/g). *Salmonella* sp. were not recovered from samples of shrimp, pond water, sediments and feed. *V. parahaemolyticus* was detected in farm shrimp, sediment and water at a density of 10^1-10^2 cfu/g.

The total plate counts for frozen shrimp were 10^2-10^7 cfu/g. In 27% samples it ranged from 10^5 cfu/g to 10^6 cfu/g while in 59% it was 10^6-10^7cf/g. The *E. coli* count of frozen shrimp ranged from <3 to 10 MPN/g with 94% counts less than 3 MPN/g. *Vibrio cholerae, Vibrio parahaemolyticus, Salmonella* sp. and *Staphylococcus aureus* were not found in frozen shrimp samples.

Introduction

Frozen shrimp are one of the major export fisheries products from Southeast Asia. Failure of producers to meet the bacteriological standards of importing countries has labeled shrimp in this region as inferior quality products and has reduced their competitiveness in world markets (Reilly and Twiddy 1992). Today one of the major challenges faced by the shrimp industry, is the implementation of stringent microbiological standards for the shrimp exports set by authorities of importing countries (EU 1991). A study conducted in Sri Lanka in 1986 indicated the presence of *E. coli* in shrimp culture environment, which ranged from 3 to 11/g in shrimp and from 3/100 ml to 49/100 ml in pond water (Fonseka 1988). Another study conducted in Sri Lanka (Jayaweera and Subasinghe 1988) recorded that 58% of frozen shrimp samples tested had total bacterial counts in the 10^5-10^6/g range and 17% of the samples had total bacterial counts in the 10^6-10^7/g range. The microbial flora of fish and shellfish is a reflection of their environment (Shewan 1977). Therefore water and sediment samples were also tested to find out the micro flora associated with them. The objective of this study was to find out the microbial quality of both frozen and unprocessed cultured shrimp as well as their culture environment.

Materials and Methods

Samples of shrimp feed, cultured shrimp, water and sediments of the culture ponds were obtained from commercial semi-intensive shrimp farms in Puttalam District from January to August 2000. A total of 18 samples of farmed shrimp, 28 samples of pond water, 28 samples of sediments and 8 samples of feed were tested. Water was collected in sterile glass bottles while sediments and shrimp samples were collected in sterile polythene pouches. Pelleted feed samples were collected from feed bags and placed in sterile plastic bags. Samples were brought to the laboratory in ice for bacteriological analysis. Bacteriological analysis consisted of examination for *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, total coliforms, faecal coliforms and *E. coli*. Pond water samples were also tested for suspended solids, salinity and pH value.

A total of 162 samples of frozen shrimp were collected in sterile polythene bags from the cold storage (below -20^oC) of processing plants. Among those 162 samples, 160 were in shell on headless form while 2 were in peeled un-deveined (PUD) form. Microbiological analysis consisted of examination for total bacterial counts (TBC), *Salmonella* spp. *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, total coliforms, faecal coliforms and *E.coli*. TBC were obtained as an index of proliferation of bacteria that had taken place along the processing line. Total Coliforms,

E. coli, *Salmonella* spp. and *S. aureus* were used as indicator organisms in this study.

Total Bacterial Counts

Ten grams of sample were macerated with 90 ml of sterile peptone water. Appropriate dilutions of shrimp samples were transferred onto Nutrient Agar (NA) and the plates were incubated at 30°C for 48-72 hours (SLS 1991).

Enumeration of total coliforms, faecal coliforms and *E. coli*

MPN-5 and MPN-3 tube technique was used with MacConkey broth to determine presumptive coliforms in water, shrimp, sediments and feed. Faecal coliforms were quantified by the 5/3-tube Most Probable Number (MPN) technique using Brilliant Green Bile Broth (incubated at 44.0°C± 0.5). Presence of *E. coli* in tryptone (incubated at 44.0°C± 0.5) was tested by adding Kovac's reagent and confirmed by streaking on Eosine Methylene Blue (EMB) (SLS 1982a, 1983).

Examination for *Salmonella*

The two water samples (100 ml) were filtered through a 45 μ-pore membrane and the membrane filters were pre-enriched for 24 hours at 37°C in 225 ml of buffered peptone. Twenty-five grams of shrimp samples were aseptically weighed into 225 ml buffered peptone and incubated for 24 hours at 37°C. Similarly, 25 g of sediment samples and 25 g of feed samples were also aseptically weighed separately into 225 ml of buffered peptone and each of these were incubated for 24 hours at 37°C. After that, 1 ml of from the buffered peptone was transferred to 10 ml of Tetrationate (improved by brilliant green dye) and Rappaport Vasilliadis (RV) and incubated at 37°C for 24 hours and at 42°C for 24 hours respectively. Enriched cultures were streaked on Xylose Lysine Desoxycholate (XLD) agar and Brilliant Green Agar (BGA) plates. Presumptive colonies were screened biochemically and confirmed serologically with polyvalent (O and H) antiserum (Murex Biotech Ltd, UK) by the slide agglutination test (SLS 1992a).

Examination for *Vibrio parahaemolyticus*

One hundred ml of water samples were filtered through 45μ pore membrane and enriched for 18-24 hours at 37°C in 225 ml of 3% alkaline peptone. Twenty-five grams of shrimp and sediment samples were aseptically weighed separately into 225 ml alkaline peptone solution and each of these were incubated for 18-24 hours at 37°C. Enriched cultures were streaked on thiosulphate citrate bile salt sucrose (TCBS) agar plates. Typical colonies of *V. parahaemolyticus* on TCBS were tested

biochemically (SLS 1982b). API 20E identification system purchased from BioMeriux was used for the confirmation of *V. parahaemolyticus.*

Examination for *Vibrio cholerae*

Alkaline peptone was used as the enrichment medium and suspected colonies on TCBS were tested biochemically for confirmation (SLS 1982b).

Examination for *Staphylococcus aureus*

Baird-Parker medium with egg yolk tellurite emulsion was used for this study. Two 0.1 ml portions of dilution (1/10) were spread plated on Baired parker agar and incubated at 37°C for 48 hours. Typical colonies on Baird parker agar were tested biochemically (SLS 1992b).

Results

The ranges of the counts of total coliforms, faecal coliforms, *E. coli* and *V. parahaemolyticus* are given in Table 1. The total coliform counts of shrimp varied between <3 and 93 MPN/g whereas in pond water and sediments these counts were 0-45 MPN/100 ml and 3.6-93 MPN/g, respectively. Faecal coliform counts in shrimp varied from <3 to 15 MPN/g while in pond water and sediment 0-11 MPN/100 ml and <3-23 MPN/g respectively. *E. coli* counts ranged from 0-8 MPN/100 ml in pond water <3-9.1 MPN/g in sediment and <3-7.3 MPN/g in shrimp.

In one occasion feed was found to be highly contaminated with coliforms (>1100 MPN/g), faecal coliforms (>1100 MPN/g) and *E. coli* (460 MPN/g) (Table 1). When this sample was excluded, the total coliform, faecal coliform and *E. coli* counts in feed samples ranged from <3 MPN/g to 3 MPN/g. In the present study *Salmonella* was not recovered from any sample of shrimp, feed, pond water or pond sediment. *V. parahaemolyticus* was detected in farmed shrimp, pond sediments and pond water at low levels of 11-900 cfu/g or 100 ml.

The physicochemical parameters monitored in pond water were as follows: pH 7.9-9.6; salinity 15-28 mg/l and suspended solids 43-74 mg/l (Table 2). Majority of total coliforms, faecal coliforms and *E. coli* were isolated from waters at salinities ranging from 18 to 27 mg/l, pH ranging from 7.9 to 9.6 and suspended solids levels ranging from 57 to 73 mg/l.

Bacteriological analysis of frozen shrimp

The TBC, total coliforms, faecal coliforms and *E.coli* of frozen shrimp are presented in Tables 3 and 4. The TBC for frozen shrimp ranged from 10^2 cfu/g to 10^7 cfu/g (Table 3) with majority being around 10^5 cfu/g (30%) and 10^6cfu/g (58%). The *E. coli* count of frozen shrimp ranged from <3 MPN/g to 10 MPN/g with 94% of the counts being <3 MPN/g (Table 4).

No correlation was observed between total plate count and incidence of coliforms. *Vibrio cholerae* (N=54), *Vibrio parahaemolyticus* (N=32), *Staphylococcus aureus* (N=47) and *Salmonella* (N=60) were not found in the frozen shrimp samples analysed.

Table 1. Ranges of the total coliforms, faecal coliforms, *E. coli* and *V. parahaemolyticus* counts in unprocessed shrimp, pond water, pond sediment and shrimp feed.

Type of sample	Total coliforms	Faecal coliforms	*E. coli*	*V. paraahemolyticus*
Feed	<3->1100	<3->1100	<3-460	-
Shrimp	<3-93	<3-15	<3-7.3	0-280
Pond water	0-45	0-11	0-8	11-70
Pond sediment	3.6-93	<3-23	<3-9.1	60-900

Total coliform, faecal coliform and E coli counts are given as MPN/g for feed, shrimp and pond sediments and as MPN/100 ml for pond water while *V. parahaemolyticus* counts are given as cfu/g for feed, shrimp and pond sediments and as cfu/100 ml for pond water

Table 2. Some physico-chemical parameters of pond water.

Parameter	Mean ± SD	Range
pH	8.8 ± 0.47	7.9 – 9.6
Salinity (ppt)	23.0 ± 4.48	15 – 28
Suspended solids (mg/l)	62.3 ± 10.83	43 – 74

Table 3. The number of frozen shrimp samples in different ranges of TBC (cfu/g).

	$<10^2$	10^2	10^3	10^4	10^5	10^6	10^7
Headless, shell-on (Number of samples)	1	-	5	10	48	93	3
Peeled, un-deveined	-	-	-	-	-	2	-

Table 4. The number of frozen shrimp samples in different ranges of coliform, faecal coliform and *E. coli* counts (MPN/g).

	<3	3-10	11-100	101-500
Headless, shell on				
Total coliform	22	6	12	6
Faecal coliform	36	7	1	2
E. coli	44	3	-	-
Peeled un-deveined				
Total coliform	1	-	-	-
Faecal coliform	1	1	-	-
E. coli	2	-	-	-

Discussion

The results of the present study indicate that the total coliform count, faecal coliform count and *E. coli* count in pond sediments were higher than those of the pond water and cultured shrimp. However, in Indonesia, total coliform count in cultured shrimp was found to be higher than that of pond sediments and pond water (Putro et al. 1988).

Presence of high number of coliforms in feed could be due to several factors such as inadequate measures taken during handling and storage to protect the finished feed from contamination at all stages of the processing process and inadequate heat treatment. The manure and the feeds are presumed to be the major sources of *E. coli* in the pond. The frequent and consistent isolation of total coliforms, faecal coliforms and *E. coli* from the sediments and water samples of the shrimp farms may be due to the fact that these pathogens survive better in pond sediments (Burton et al. 1987) and pond water (Townsend 1992). The survival rate of these pathogens increase due to the nutrients present (Venkateshwaran et al. 1985) as a result of organic loading in the form of manure and the feed, and due to the favourable interaction of various biological and physical factors (Rhodes and Kator 1988).

Non-recovery of *Salmonella* spp. from farms suggests that there would be a very low level of incidence of this organism in shrimp immediately after harvest unless there is post-process contamination. In a study conducted in Pakistan (Zuberi and Qadri 1981) it has been reported that *Salmonella* contamination of fish occurred due to washing in polluted waters. Leangphibul et al. (1998) also found a low (0.5%) prevalence of Salmonella in 283 water and sediment samples obtained from shrimp farms in Bangkok, Thailand. Further, there are reports on the absence of *Salmonella* from all samples of water, sediment, shrimp and chicken manure collected from cultured shrimp producing areas of southern Thailand (Dalsgaard 1998). A previous study has shown that there is a

strong correlation between the prevalence of *Salmonella* in estuarine water and the levels of total coliforms and faecal coliforms (Kaper et al. 1979). Although not observed in the present study, *Salmonella* was found in 16% of cultured shrimp samples and in 22% of mud/water samples obtained from shrimp farms in Thailand (Reilly and Twiddy 1992). The results of the present study are in agreement with the observations of Liston (1980), Gilbert (1982), Hobbes and Hodgkiss (1982) and Hobbes (1983) that traditional faecal indicator bacteria are of little value as indicators of the possible presence of pathogenic bacteria.

Non-recovery of *Salmonella* in the feed suggests there is no serious contamination of pathogenic bacteria. However, it should be noted that the number of samples used for the determination of *Salmonella* is not sufficient to conclude that the feed is completely free from it. The high prevalence of *Salmonella* was suspected to be associated with the use of large amounts of fresh chicken manure as organic fertilizer and supplemental feeding with small fish and crushed shellfish (Reilly and Twiddy 1992). In Sri Lanka, chicken manure is rarely applied to shrimp ponds.

The organic enrichment (in the form of manure, clam meat and artificial feed), temperature and salinity provide a complex situation in the shrimp culture environment and each of these has been proved to aid in the development and /or survival of *Vibrio* spp. (Singleton et al. 1982). Fonseka (1988) found low counts of *V. parahaemolyticus* in samples of cultured shrimp in Sri Lanka and showed that it would not be a major threat as subsequent washing, processing, freezing and cold storage or cooking within a short period of harvesting would ensure elimination of these microorganisms and thereby prevent any chance of food poisoning. However, according to the Dalsgaard (1998), the occurrence of *Vibrio* spp. does not correlate with traditionally used bacterial indicator organisms of faecal pollution, and their presence in aquaculture products is to be expected and cannot be controlled by water quality measures such as waste water treatment. Reilly et al. (1984) in their work on brackish water shrimp have reported that *V. parahaemolyticus* was present commonly as part of the natural flora, but have not been isolated after processing. *V. parahaemolyticus* can grow at a salinity level of 1-8% NaCl, with best growth occurring at 2-4% range (Sakazaki 1979) and over the pH range 4.8-11.0, with 7.6-8.6 being optimum (Beuchat 1973).

Aklani et al. (1988) reported *V. parahaemolyticus* counts to be 10^2-10^3 cfu/g in farm shrimp in Philippines. In this study, water in the shrimp farms as well as in the sources had optimum salinity and pH ranges for the growth of *V. parahaemolyticus*.

The TBCs of cultured shrimp reported from tropical waters in South East Asia are normally within the ranges reported in studies by Llobrerra et al. (1988), Putro et al. (1988) and Fonseka (1988). Jayaweera

and Subasinghe (1988) reported that the 58.3% frozen shrimp samples in Sri Lanka had TBCs in the range of 10^5-10^6 cfu/g and only 16.6% were in the range of 10^6-10^7 cfu/g. The total plate counts in 98% of the samples of frozen shrimp analysed in the present study fall within the limits of Sri Lankan standards. PUD shrimp undergo a further processing step compared to shell-on product, which is the removal of exoskeleton. PUD products are subjected to more handling and this may be the reason for their higher bacterial load compared to shell-on shrimp. Though the cultured shrimp showed higher levels of *E. coli* in the raw material, a progressive reduction was noted during processing procedure. This may be due to freezing as coliforms are susceptible to freezing (Hobbes 1983).

Absence of *V. parahaemolyticus* in the final frozen product may be also due to their susceptibility to chilling and freezing. Washing at each stage of processing may also have contributed to this. Baross and Liston (1970) found *V. parahaemolyticus* to be more readily inactivated by freezing than other food poisoning organisms. The sensitivity of *V. parahaemolyticus* to cold has been reported by many investigators (Lamprecht and Ferry 1980; Baross and Liston 1970).

Since the contamination of *Staphylococci* occurs mainly from workers (Garret 1988), absence of *Staphylococci* in frozen shrimp is indicative of maintenance of good hygienic conditions during processing.

It is known that the products with good bacteriological quality could be consistently produced by application of simple hygienic precautions during processing. Strict adherence to Good Manufacturing Practices (GMP) and HACCP plan based processing can greatly enhance the product quality.

Conclusions

The overall bacteriological quality of frozen cultured shrimp that were ready to be marketed was satisfactory. The results also highlight the importance of implementation of HACCP system in the shrimp industry to ensure consistent quality of frozen shrimp. A poor correlation was observed between the pathogens and population of indicator organisms such as total coliforms, faecal coliforms and *E. coli*.

Acknowledgements

The financial support provided by the National Aquatic Resources Research and Development Agency, Sri Lanka is gratefully acknowledged.

References

Aklani, R. G. C., M.S. Leonor & D. Evelyn. 1988.
Microbiology of prawn processing. In: Seventh Session of the Indo-Pacific Fishery Commission Working Party of Fish Technology and Marketing. Bangkok, Thailand, pp. 86-98. FAO Fisheries Report No. 401, FAO, Rome.

Barros, J. & J. Liston 1970.
Occurrence of *Vibrio parahaemolyticus* and related haemolytic Vibrios in marine environments of Washington state. Applied Microbiology 20: 179-186.

Beuchat, R. 1973.
Interacting effects of pH temperature and salt concentration on growth and survival of *Vibrio parahaemolyticus*. Applied Microbiology 25: 844-846.

Burton, G. A., D. Gunnison & G. R. Lanza, 1987.
Survival of pathogenic bacteria in various fresh water sediments. Applied Environmental Microbiology 53: 633 -638.

Dalsgaard, A. 1998.
The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. International Journal of Food Science and Technology 38: 127-138.

EU 1991.
European Union Directive, 1991. Council Directive 91/493/EEC.

Fonseka, T.S.G. 1988.
Microbial flora of pond cultured prawn (*Penaeus monodon*). Seventh Session of the Indo-Pacific Fishery Commission Working Party of Fish Technology and Marketing. Bangkok, Thailand, pp. 25. FAO Fisheries Report No. 401, FAO, Rome.

Garret, E.S. 1988.
Microbiological standards, guidelines, specifications and inspection of seafood products. Food Technology 42: 90.

Gilbert, R. J. 1982.
Control of the microbial contamination of foods and feeds in international trade: In: Microbial Standards and Specifications (H. Kurata and C. W. Hesseltine eds), pp. 105. Sailcon publishing Company Limited, Tokyo.

Hobbes, G. & W. Hodgkiss 1982.
Development of food microbiology. In: The Bacteriology of Fish Handling and Processing (R. Davis ed.), pp. 71-117. Applied Science Publisher, London.

Hobbes, G. 1983.
Marker organisms in fresh fish in relation to spoilage and public health, Torry Research Station, Aberdeen. Scotland.

Jayaweera, V. & S. Subasinghe 1988.
Microbiological changes in prawn (*Penaeus* sp) during processing in Sri Lanka. Seventh Session of the Indo-Pacific Fishery Commission Working Party of Fish Technology and Marketing. Bangkok, Thailand, pp. 57-67. FAO Fisheries Report No. 401, FAO, Rome.

Kaper, J., H. Lockman, R.R. Colwell & S.W. Joseph 1979.
Ecology, serology and enterotoxin production of *Vibrio cholerae* in Chesapeake bay. Applied Environmental Microbiology 37: 91-103.

Lamprecht, E. & K. Ferry 1980.
South African fishing industry research institute, Annual report.

Leangphibul, P., C. Nilakul, C. Sornchai, S.Tantimavanich & K. Kasemsukakul 1998.
Investigation of pathogenic bacteria from shrimp farms. Kasetsart Journal 20: 333-337.

Liston, J. 1980.
Advances in fish science and technology. In: Microbiology in Fishery Science (J.J. Connell eds), pp. 138-157. Torry Jubiliee Conference.

Llobrerra, A.T., M.L.Balalacao & A. Tan 1988.
Effect of farming phase and in plant processing on the microbiological quality of prawns (*Penaeus monodon*). Seventh Session of the Indo-Pacific Fishery Commission Working Party of Fish Technology and Marketing. Bangkok, Thailand, pp. 1-6, FAO Fisheries Report No. 401, FAO, Rome.

Putro, S., A.M. Anggawati, Y. N. Fawzya & F. Ariyani 1988.
Studies on microbiology of farmed shrimp. Seventh Session of the Indo- pacific Fishery Commission Working party on Fish Technology and Marketing. Bangkok, Thailand, pp. 6–17, FAO Fisheries Report No. 401, FAO, Rome.

Reilly, A. M.A. Bernate & E. Dangla 1984.
Storage stability of brackish water prawns during processing for export. Food Technology 35: 77-88.

Reilly, P.J.A. & D.R. Twiddy 1992.
Salmonella and *Vibrio cholerae* in brackish water cultured tropical prawns. International Journal of Food Microbiology 16(4): 293-301.

Rhodes, M.W. & H Kator 1988.
Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. Applied Environmental Microbiology 54: 2902-2907.

Sakazaki, R. 1979.
Food-borne infection and intoxication. In: Vibrio Infection (H Riemann and F.L. Bryan eds), pp. 189 –209, Academic Press, New York..

Shewan, J.M. 1977.
The bacteriology of fresh and spoiling fish and biochemical changes induced by bacterial action. In: Handling, Processing and Marketing of Tropical Fish. pp. 51-66. Tropical Product Institute, London.

Singleton, F. L., R.W. Attwell, M.S. Jangi & R.R. Colwell 1982.
Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. Applied Environmental Microbiology 43(5): 1080-1085.

SLS 1982a.
Sri Lanka Standards 516: Part 3: 1982, Bureau of Standards, Colombo.

SLS 1982b.
Sri Lanka Standards 516: Part 7: 1982, Bureau of Standards, Colombo.

SLS 1983.
Sri Lanka Standards 614: Part 2: 1983, Bureau of Standards, Colombo.

SLS 1991.
Sri Lanka Standards 516: Part 1: 1991, Bureau of Standards, Colombo.

SLS 1992a.
Sri Lanka Standards 516: Part 5: 1992, Bureau of Standards, Colombo.

SLS 1992b.
Sri Lanka Standards 516: Part 6: 1992, Bureau of Standards, Colombo.

Townsend, S.A. 1992.
The relationship between *Salmonella* and fecal indicator organisms in two Australian wet/dry tropics. Journal of Applied Bacteriology 73: 182-188.

Venkateshwaran, K., S. Manavalan & R. Natarajan 1985.
Salmonella contamination in freshly caught prawns. In: Harvest and post harvest technology of fish. (K. Ravinran, N. Unnikrishnan Nair, P. A. Perigreen, P. Madhavan, A.G. Gopalkrishna Pillai, P.A. Panicker & M. Thomas eds), pp. 482-484. Cochin.

Zuberi, R. & R.B. Qadri 1981.
Organisms of public health significance in fish and shrimp from Karachi coastal waters-a survey. Pakistan Journal of Scientific and Industrial Research 24: 77-81.