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ORIGINAL ARTICLE

Bacteriological quality of individually quick-frozen (IQF) raw and cooked ready-to-eat shrimp produced from farm raised black tiger shrimp (*Penaeus monodon*)

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*One thousand, two hundred and sixty four samples of individually quick-frozen (IQF) peeled and deveined raw and 914 samples of cooked ready to eat shrimp samples produced from farm raised black tiger (*Penaeus monodon*) obtained from a seafood unit working under HACCP concept were analysed for total aerobic plate count (APC), coliform count,* Escherichia coli*, coagulase positive Staphylococci and* Salmonella*. The overall bacteriological quality of the product was found to be good. Of the frozen raw shrimp, 96% of samples showed APC below 10^5 while 99% of the frozen cooked ready-to-eat samples showed APC less than 10^4. The APC ranged from $1{\cdot}0\times10^2$ to $4{\cdot}2\times10^6$ cfu/gm in frozen raw shrimp and from $1{\cdot}0\times10^2$ to $6{\cdot}4\times10^4$ cfu/gm in the frozen cooked shrimp. Prevalences of coliforms in raw shrimp and cooked shrimp samples were 14·4% and 2·9% respectively. The coliform count in raw products ranged from $1{\cdot}0\times10^1$ to $2{\cdot}5\times10^3$ cfu/gm and in the cooked products, from $1{\cdot}0\times10^1$ to $1{\cdot}8\times10^2$ cfu/gm. Although all the cooked shrimp samples were free of coagulase positive staphylococci,* E. coli *and* Salmonella*, 1·0, 2·0 and 0·1% of the frozen raw shrimp samples tested positive for coagulase positive Staphylococci,* E. coli *and* Salmonella *respectively. The* Salmonella *strain was identified as* Salmonella typhimurium*. The results of the present study highlight the importance of implementation of HACCP system in the seafood industry to ensure consistent quality of frozen seafood.*

Introduction

Increased consumption of frozen food in recent years has focused attention on establishing bacteriological standards for these products (Silverman et al. 1961). Microorganisms in frozen food remain viable to different extents over extended storage periods and the microbial population in these foods is altered by processes like rate of cooling or thawing and storage temperatures (Holcombe and Weiser 1959, Kereluk and Gunderson 1960). Frozen shrimp presents an additional problem in that it is sold cooked or raw, peeled or unpeeled and with or without breading (Silverman et al. 1961). Examination of fresh and frozen shrimp revealed that spoilage of this product is largely due to biochemical changes induced by microbial population and to a lesser degree by enzymes and chemical compounds in shrimp (Fieger 1950). In an effort to control the microbial

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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.

In recent years, India has witnessed a boom in aquaculture mainly in farm raised shrimp 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 the USA. Although there are few reports on the bacteriological quality of frozen raw and cooked shrimp in India (Varma et al. 1985, Iyer and Shrivastava 1988), there is no information on the bacteriological quality of IQF raw and cooked ready-to-eat shrimp produced from farm raised shrimp. The present investigation was undertaken to determine the bacteriological quality of IQF raw and cooked ready-to-eat shrimp produced from farm raised shrimp obtained from a plant working under HACCP guidelines.

Materials and methods

Selection of firm

A firm that exclusively produces individually quick-frozen (IQF) raw and cooked shrimp from farm raised black tiger shrimp (*Penaeus monodon*) was selected. The processing plant is functioning under the HACCP system incorporating all current good manufacturing practices (CGMPs). The shrimps were collected from the farm site, iced in individual plastic boxes and brought to the processing plant in temperature-controlled trucks. The head-on shell-on raw material was then subjected to filth washing using a pneumatic filth washing machine. Raw material after filth washing was stored in flake ice and subjected to pre-processing, which included peeling, grading and soaking. GMPs were strictly followed and the shrimp temperature was kept below 10°C at all stages using flake ice. The soaked raw shrimp then underwent freezing, glazing, packing and frozen storage in addition to, in the case of cooked ready-to-eat shrimps, cooking and cooling. Only properly attired, authorized persons were allowed to touch the product.

Bacteriological procedures

Samples for bacteriological analysis were collected in sterile polythene bags at the end of the processing line. The samples were collected on each production day, and the total number of samples collected during each month were pooled together as the number of samples collected during each month. The frozen samples were softened by overnight storage at 5°C in a refrigerator and processed for APC, coliforms *E. coli*, coagulase positive staphylococci and *Salmonella*. The methods used were those described in the bacteriological analytical manual of FDA (1992).

Total Aerobic Plate Counts were obtained after 48 h of incubation at 35°C in tryptone glucose beef extract (TGBE) agar. Lauryl sulphate tryptose (LST) broth was used for the coliform count after 24 h incubation at 37°C. MacConkey agar and tergitol-7 agar were used as the selective media for *E. coli* and typical colonies were confirmed by **IMViC** test.

To detect Staphylococci, samples were plated for isolation on Baird Parker (BP) medium and incubated at 35°C for 48 h. Thereafter, coagulase positive isolates were detected using tube coagulase test (FDA, 1992).

To detect *Salmonella*, samples were pre-enriched in lactose broth at 37°C for 24 h, then 0·1 ml and 1 ml of the pre-enriched culture was transferred into Rappaport Vasilliadis (RV) broth and tetrathionate (TT) broth and incubated at 41°C and 37°C respectively, for selective enrichment. These cultures were then streaked onto bismuth sulphite agar (Oxoid, London, UK), xylose lysine deoxycholate (XLD) agar and hektoen enteric agar (HEA) and incubated at 37°C for 24–48 h. Typical colonies were stored on tryptic soy agar (TSA) slants. These isolates were subjected to standard biochemical tests for *Salmonella* (FDA, 1992). Isolates that yielded typical biochemical results were serologically confirmed as *Salmonella* using the poly-

valent antiserum (Wellcome Laboratories, UK) by the slide agglutination test. Complete confirmation and serotyping of isolates were kindly done at the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, India.

Results and discussion

A total of 1264 samples of individually quick-frozen, peeled and deveined raw shrimp and 914 samples of cooked ready-to-eat shrimp were analysed during the period January 1994 to December 1995. A summary of the results of bacteriological analysis of the samples is presented in Table 1. For the raw shrimp samples 96% showed an APC less than 10^5 cfu/gm, out of which 74% were less than 10^4 cfu/gm. APC values of cooked ready-to-eat shrimp were less than 10^4 in 99% of the samples. The total aerobic plate counts were much lower than those reported by Varma et al. (1985) who reported that 6·7% raw and 4·5% of cooked shrimps collected from processing plants at Cochin exceeded the limit of 10^6 and 10^5 cfu/gm respectively. This may probably be due to the strict adherence to CGMPs during processing, in the plant under study. In the plant under survey, shrimp temperature is kept below 10°C at all stages and bacteriological surveys of utensils, machinery and workers hands were taken on a daily basis to ensure proper sanitation. Wearing of sterile latex gloves and mouth piece is mandatory and the workers are asked to sanitize their hands in 50 ppm chlorine water at regular intervals.

The prevalence of coliforms in frozen raw shrimp was 15% but 3% in frozen cooked shrimp samples. No correlation was observed between aerobic plate count and incidence of coliforms. This agrees with the findings of Buchanan (1991) who reported that the total aerobic plate count (TAPC) increased even in adequately refrigerated shrimp whereas the level of coliforms and thermal tolerant coliforms increased only in the product that was temperature-abused. The prevalence of coliforms in raw and cooked shrimp samples from the plant under investigation were lower than those reported by Iyer and Shrivastava (1989b) in frozen raw and cooked shrimp.

While 1% of the raw shrimps contained coagulase positive staphylococci none of the cooked shrimps tested positive for this micro-organism. Since the prevalence of staphylococci is mainly from workers (Garret 1988), absence of *Staphylococcus* in cooked product is indicative of lack of post-process contamination Iyer and Shrivastava (1988) reported 38% incidence of coagulase-positive staphylococci in frozen cooked shrimp and considered it as a serious problem in frozen fishery products processed in this country.

Two percent of the raw shrimps tested positive for *E. coli* and 0·1% (1 sample) tested positive for *Salmonella typhimurium*. The incidence of *Salmonella* and *E. coli* is suspected to be from shrimp harvested from contaminated culture ponds (Reilly et al. 1992). All the shrimp samples analysed were produced from farm raised shrimps that were relatively free from pathogenic micro-organisms when compared to those harvested from contaminated coastal waters (Eyles 1986). However the isolation of pathogenic micro-organisms like *Salmonella* and *E. coli* in fish

Table 1. Summary of the bacteriological results of the individually quick-frozen raw and cooked ready-to-eat shrimp analysed during the period Jan 1994–Dec 1995

Sample	No. of Samples analysed	Percent of samples with indicated count levels/gm						*Staphylococcus*[a]	*E. coli*	*Salmonella*
		APC				Coliforms				
		10^2	10^3	10^4	10^5	10^1	10^2			
Raw shrimp	1264	35	39	22	4	12	3	1	2	0·1[b]
Cooked shrimp	914	79	20	1	0·0	3	0·1	0·0	0·0	0·0

[a]Coagulase positive staphylococci
[b]serotype *Salmonella typhimurium*

and shrimp harvested from aquaculture ponds in India have been reported (Sivakami et al. 1996). Ogbondeminu (1993) also reported incidence of enteric bacteria like *Salmonella* and *E. coli* in fish and water from tropical aquaculture ponds. Iyer and Shrivastava (1989b) did a comprehensive study on the pattern of *Salmonella* serotypes in fishery products, frog legs and processing environments and reported the incidence of various *Salmonella* serotypes in these samples. He reported approximately 2% incidence of *Salmonella* in frozen shrimps collected from the processing environments of Cochin and Bombay, which is much higher than this research observed (0·1%) in the samples collected from the processing plant under investigation.

The number of frozen raw shrimp samples collected during each month and results of the bacteriological analysis are presented in Table 2. None of the sample collected during the months of January, February, March, May, June and July in 1994 and January, February, May and June in 1995 exceeded the aerobic plate count of 10^5. Nearly 10% of the samples collected during the months of September through December during 1994 and 1995 exceeded APC value of 10^5. These are months of intense production and a small let up in the adherence to GMPs was noticed, which may well have influenced the bacterial counts. The prevalence of coliforms in samples did not show any correlation between high APC. In fact, a high prevalence of coliforms (>50%) were noticed in the samples collected during the months of May and June 1994 during which none of the samples exceeded APC levels of 10^5. The use of coliforms as an index of sanitary quality has been questioned by many, who have found this group susceptible to freezing and frozen storage (Hobbs 1983).

Incidence of coagulase positive staphyloc-

Table 2. Monthwise data of the bacteriological analysis of individually quick-frozen raw shrimp analysed during the period Jan 1994–Dec 1995

Year & month	Samples analysed	Percent of samples with indicated count levels/gm						*Staphylococcus*[a]	*E. coli*	*Salmonella*
		APC				Coliforms				
		10^2	10^3	10^4	10^5	10^1	10^2			
1994 Jan	42	43	43	14	0·0	0·0	0·0	13	0·0	0·0
Feb	69	41	49	10	0·0	0·0	0·0	5	1	0·0
Mar	73	59	27	12	1	10	1	1	3	0·0
Apr	70	29	37	33	1	27	0·0	3	9	0·0
May	75	39	43	18	0·0	55	0·0	0·0	7	0·0
Jun	41	22	37	41	0·0	54	0·0	2	3	0·0
Jul	62	26	32	39	3	32	0·0	0·0	0·0	0·0
Aug	73	37	34	21	8	0·0	0·0	0·0	0·0	0·0
Sep	45	38	33	18	11	7	0·0	0·0	0·0	0·0
Oct	86	15	49	19	22	15	1	0·0	1	0·0
Nov	56	20	34	34	12	9	0·0	1	1	0·0
Dec	72	29	42	15	4	3	0·0	3	0·0	0·0
1995 Jan	59	71	22	7	0·0	2	0·0	0·0	2	0·0
Feb	61	51	41	8	0·0	5	0·0	0·0	0·0	0·0
Mar	47	49	40	11	0·0	0·0	0·0	0·0	0·0	2
Apr	18	22	50	22	6	0·0	0·0	0·0	6	0·0
May	33	58	39	3	0·0	12	0·0	0·0	0·0	0·0
Jun	13	31	54	15	0·0	8	0·0	0·0	0·0	0·0
Jul	16	50	38	12	0·0	0·0	0·0	0·0	0·0	0·0
Aug	27	41	37	19	0·0	0·0	0·0	0·0	0·0	0·0
Sep	57	25	39	33	3	7	0·0	1	0·0	0·0
Oct	50	14	36	42	8	4	0·0	0·0	0·0	0·0
Nov	61	10	49	33	8	13	3	0·0	0·0	0·0
Dec	58	28	50	17	5	16	0·0	0·0	2	0·0

[a]Coagulase positive staphylococci

occi were greater in samples collected during 1994 than in 1995. This may be due to the awareness among the workers about the role of personal hygiene in maintaining high product quality, which was disseminated through regular classes by the management as a part of educating the workers. The counts of coagulase positive *Staphylococcus* detected were much lower than those reported by Iyer and Shrivastava (1989a) in frozen shrimps.

Salmonella typhimurium was detected in one sample collected during March 1995. This prevalence is lower than those reported by Zuberi et al. (1983) who reported a 1% prevalence of *Salmonella* in shrimps collected from two processing plants. Iyer and Shrivastava (1989b) also reported a higher prevalence of *Salmonella* in frozen shrimps collected from processing environments. The source of the *Salmonella* isolate in the present study is suspected to be contaminated raw material rather than cross contamination during processing. Rattagool (1991) reported frequent isolations of *Salmonella* in farm raised tiger shrimp (*Penaeus monodon*). Reilly and Twiddy (1991) also reported widespread incidence of *Salmonella* in the environment of shrimp farms. Both studies reported the use of untreated chicken manure to fertilize the ponds as the main reason as well as the droppings of aquatic birds.

The number of samples of frozen cooked ready-to-eat shrimp analysed during each month and the results of bacteriological analysis are given in Table 3. The APC count was well below 10^4 in most of the samples. Philips and Peeler (1972) survey showed an APC values of 15000 cfu/gm for plants with good sanitary practices and 170000 cfu/gm in plants judged to be poor. The counts detected in the present study are a clear indication of good sanitary practices followed in the processing unit under study. While sporadic detection of coliforms were made in cooked

Table 3. Monthwise data of bacteriological analysis of individually quick-frozen cooked ready-to-eat shrimp during the period Jan 1994–Dec 1995

Year & month	Samples analysed	Percent of samples with indicated count levels/gm						*Staphylococcus*[a]	*E. coli*	*Salmonella*
		APC				Coliforms				
		10^2	10^3	10^4	10^5	10^1	10^2			
1994 Jan	13	54	46	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Feb	22	68	32	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Mar	25	83	17	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Apr	39	85	15	0·0	0·0	0·0	0·0	0·0	0·0	0·0
May	45	91	9	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Jun	14	36	64	0·0	0·0	64	0·0	0·0	0·0	0·0
Jul	35	22	63	37	0·0	20	0·0	0·0	0·0	0·0
Aug	40	78	22	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Sep	66	88	11	1	0·0	1	0·0	0·0	0·0	0·0
Oct	52	96	4	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Nov	41	78	15	7	0·0	0·0	0·0	0·0	0·0	0·0
Dec	64	91	9	0·0	0·0	2	0·0	0·0	0·0	0·0
1995 Jan	21	100	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Feb	4	100	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Mar	5	80	20	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Apr	21	71	29	0·0	0·0	0·0	0·0	0·0	0·0	0·0
May	28	86	14	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Jun	34	65	35	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Jul	31	58	42	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Aug	20	90	10	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Sep	58	78	19	3	0·0	2	0·0	0·0	0·0	0·0
Oct	77	68	30	2	0·0	3	1	0·0	0·0	0·0
Nov	86	77	21	2	0·0	1	0·0	0·0	0·0	0·0
Dec	69	87	13	0·0	0·0	0·0	0·0	0·0	0·0	0·0

[a]Coagulase positive staphylococci

shrimp samples, none of them tested positive for coagulase positive *Staphylococcus*, *E. coli* or *Salmonella*. The source of coliforms after the thermal processing step appears to be the processing environment particularly in regard to the adequacy of both sanitation procedures and temperature control (Buchanan 1991). The primary source of *Staphylococcus aureus* in foods that have received a thermal treatment is human contact, with more than 50% of humans harbouring this organism on their skin or in their respiratory tract (Garret 1988). Its presence is considered an excellent indicator of post-thermal contamination by food handlers in a variety of products, including cooked ready-to-eat shrimp and crab meat (NAS/NRC 1985, ICMSF 1978, 1980, 1988). The absence of this organism in the cooked ready-to-eat shrimp samples collected from the plant under investigation reflects excellent personal hygiene maintained by the workers in the processing environment.

The overall bacteriological quality was much superior to those reported earlier by Iyer and Shrivastava (1989a, b), who observed 38% incidence of coagulase positive *Staphylococcus*, 50% incidence of *E. coli* and 2% incidence of *Salmonella* in frozen cooked peeled and deveined shrimps. Furthermore, all the cooked ready-to-eat shrimp samples were within microbiological criteria recommended for cooked ready-to-eat shrimp by NACMCF (1990). The results of the present study reveals that the adherence to CGMPs and HACCP plan based processing greatly enhances the quality of seafood. APC values of less than 10^4 for uncooked and less than 10^3 for cooked ready-to-eat shrimps indicate very low, negligible level of contamination. The evidence indicates that the sanitary conditions of seafood processing plant correlate well with microbial quality of finished product (Philips and Peeler 1972, Duran et al. 1983).

In conclusion, it is realised that data from an investigation cannot be considered as a satisfactory basis for the formulation of factory control or regulatory bacteriological standards. It is however known that products with good bacteriological quality could be consistently produced by application of simple hygienic precautions during processing. Strict adherence to CGMPs and HACCP plan based processing can greatly enhance the product quality and should be enforced without delay in all the processing units by the regulatory agencies.

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