

Bacterial Levels in the Muscle of Post-Harvested Shrimp

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Abstract

Bacterial levels in shrimp muscle during harvest were determined for shrimps from four ponds. The shrimps were sampled immediately after harvest to obtain initial bacterial levels, then sampled again 30 minutes after an ice bath and once more during sorting. Bacterial levels for the total number of *Vibrio* sp. and the total number of *Aeromonas* sp. were calculated and varied between 1.0×10^3 and 1.4×10^5 CFU/g. After sorting for size, the shrimps were dipped in chlorinated ice water (10 ppm) for a few seconds and washed with clean ice water before being stored in ice (2-5°C) for transport to the processing plant. The shrimps were sampled again after being dipped in chlorinated water at 0, 4, 12, 24, 36 and 48 hrs. The bacterial levels in the muscle decreased over time in shrimps from all four ponds and no bacteria were detected 24 hrs after the shrimps were dipped in chlorinated ice water.

Introduction

Shrimp aquaculture in Thailand developed through the 1980s. Initially it started as a semi-intensive culture systems, and rapidly changed to intensive systems in the latter part of the decade. Shrimp became a major export commodity as well as a major source of income among people involved in the industry. The production of black tiger shrimp reached 230,000 metric tons in 1995. The Thai shrimp aquaculture industry has an excellent record in the production of safe products of consistent quality which have gained acceptance in various markets worldwide. However, as the industry continues to expand, the Royal Thai Department of Fisheries is alert to factors which may have a

detrimental impact on the safety of shrimp products. Specific problems include contamination of shrimp flesh by bacterial pathogens, and the presence of antimicrobial drug residues and residues from other aquaculture chemicals and environmental contaminants which are considered hazardous to public health.

Among the problems cited, microbiological hazards pose a major concern for processors and exporters since such contaminants can cause many food borne diseases among humans. *Salmonella* and *Vibrio cholerae* are well recognised potential hazards in shrimp flesh, therefore most importing countries will not accept raw frozen shrimp. However, in a survey in southern Thailand to investigate the prevalence of *Vibrio cholerae* and *Salmonella* in pond soil, water and shrimp did not recover *Salmonella* from any sample. *V. cholerae* 01 was found in this study in 2% of samples whereas *V. cholerae* non-01 was found in up to 33% of the samples. The results indicate that *V. cholerae* non-01 is ubiquitous in shrimp farm environments (Dalsgaard *et. al.* 1995). The significance of non-01 *V. cholerae* to human health needs to be investigated as quickly as possible.

Vibrio spp. is part of the normal bacterial flora of the shrimp farm environment, and may become a secondary pathogen of stressed shrimp, causing outbreaks of infectious diseases. Therefore, the presence of contaminating *Vibrios* in shrimp flesh may be due to infections of the live animal. An alternative route of contamination could be environmental contact during the harvesting process.

This study was carried out to determine the bacterial level in shrimp muscle during and after harvest. The results from this study would help increase the level of awareness of farmers and processors of the potential sources of contamination and precautionary measures to reduce contamination of shrimp products by potential pathogens.

Materials and Methods

Shrimp sampling

Samples were collected from four harvest ponds between July 1997 and January 1998. Five kilograms of shrimp were randomly sampled during harvest. Immediately after sampling, five shrimps were taken for initial bacterial count. The shrimps were sampled again 30 minutes after being stored in ice and during sorting for size. After size sorting, the shrimps were dipped in chlorinated ice water (10 ppm, calcium hypochlorite 60%) for a few seconds and washed with clean, fresh ice water before being stored in ice for transport to the laboratory. Shrimp samples were again taken immediately after dipping in chlorinated water and then at 4, 12, 24, 36 and 48 hrs.

Bacterial counting

The shrimps were washed in sterilised distilled water and carefully peeled. Cephalothorax and intestines were removed from the muscle portion to avoid

contamination from the digestive tract. One gram of muscle from each shrimp was mixed with one millilitre of 2% NaCl and homogenized to make a suspension for the bacterial count. A viable count was performed using a standard plate count described by Collins *et al* (1989) on thiosulfate citrate bile sucrose agar (TCBS Agar, Difco) for *Vibrio* count and RS-medium (Shotts and Rimler, 1973) for *Aeromonas* count.

Results and Discussion

Initial *Vibrio* levels from four harvest ponds varied between 1.30×10^3 and 1.44×10^5 CFU/gm whereas *Aeromonas* levels varied between 1.34×10^3 and 4.00×10^3 (Tables 1 and 2).

Levels of both bacteria decreased gradually over time. Most of the bacteria from the harvested shrimp could not be isolated 24 hours after the ice bath. Dipping the shrimp in chlorinated ice water before storage in ice did not reduce much bacterial levels in shrimp muscle. However, this may have an effect on bacteria on the surface of the shrimp. Careful washing after dipping is necessary to eliminate any residue remaining in the product. In the future to avoid chemical residues, it may be possible to eliminate this step from farm practice as bacterial levels were influenced by low temperatures rather than chlorine treatment.

Consumption of raw shrimp should be avoided as bacterial levels indicated in the table are relatively high. This is particularly important if the shrimps

Table 1. Number of *Vibrio* sp. in shrimp muscle.

Period	Harvested			
	I	II	III	IV
After harvest	1.08×10^4	1.44×10^5	1.30×10^3	7.36×10^3
After bath in ice 30-45 min.	2.80×10^3	5.26×10^3	2.10×10^3	2.12×10^3
Sorting sizes	2.44×10^3	1.12×10^3	2.86×10^3	2.62×10^3
After dipping in chlorine	1.30×10^3	3.60×10^2	4.20×10^2	5.60×10^2
After bath in ice 3-6 hr.	1.72×10^3	3.20×10^2	6.00×10^1	8.40×10^2
After bath in ice 12 hr.	-	1.48×10^2	8.80×10^2	1.14×10^2
After bath in ice 24 hr.	4.00×10^1	-	3.20×10^2	-
After bath in ice 36 hr.	-	-	-	-
After bath in ice 48 hr.	-	-	-	-

Table 2. Number of *Aeromonas* sp. in shrimp muscle.

Period	Harvested			
	I	II	III	IV
After harvest	4.00×10^3	2.90×10^3	1.34×10^3	1.72×10^3
After bath in ice 30-45 min.	7.60×10^2	2.58×10^3	2.30×10^3	1.42×10^3
Sorting sizes	1.16×10^3	2.06×10^3	4.36×10^3	8.40×10^2
After dipping in chlorine	1.60×10^2	4.00×10^1	7.20×10^2	1.02×10^3
After bath in ice 3-6 hr.	8.00×10^1	-	4.40×10^2	2.40×10^2
After bath in ice 12 hr.	-	6.00×10^1	1.04×10^3	6.80×10^2
After bath in ice 24 hr.	-	-	1.36×10^2	-
After bath in ice 36 hr.	-	-	-	-
After bath in ice 48 hr.	-	-	-	-

are not properly chilled, as some bacteria may survive and cause health problems. *Vibrio* species should be further investigated since the survey results from southern Thailand showed a high prevalence of *V. cholerae* non-01 (Dalsgaard et. al., 1995).

Aeromonas species were also found in the shrimp muscle because the shrimps were cultured in low salinity. The presence of this bacteria was not surprising as the bacteria is known as a normal flora in freshwater and brackishwater.

Conclusion

Vibrio species found in shrimp muscle were higher than *Aeromonas* species. Both levels of bacteria gradually decreased over time, due to the low temperature during storage rather than due to dipping in chlorinated iced water. The bacteria could not be isolated from most of the shrimps 24 hours after being chilled. However, chlorinated iced water may play an important role in reducing surface contamination.

References

- Collins, C.H., P.M. Lyne and J.M. Grange. 1989. Collins and Lyne's Microbiological Methods. Sixth edition. Butler & Tanner, U.K. 409 p.
- Dalsgaard, A., H.H. Huss, A. H. Kittikun, and J.L. Larsen. 1995. Prevalence of *Vibrio cholerae* and *Samnonella* in a major shrimp production area in Thailand. *Int. J. Food. Microbio.* 28, 101-113.
- Shotts, Jr., E.B. and R. Rimler. 1973. Medium for the isolation of *Aeromonas hydrophila*. *Applied Microbiology*, 26, 550-553.