

**Detection of *Salmonella* sp. in Sri Lanka's export
shrimp**

By

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Abstract

Shrimp industry is an important foreign exchange earner for Sri Lanka. This research was conducted in 12 major shrimp production areas in North Western Province where the black tiger shrimp (*Penaeus monodon*) is used in brackish water shrimp culture. Strict microbiological control is imposed by importing countries such as Japan and European Union. Therefore this research is valuable to check the microbial quality of the finished frozen shrimps which is exported from Sri Lanka. Moreover, the whole chain from farm until export was checked for the microbial quality that is total number of microorganisms, *E.coli* and *Salmonella*.

55 samples each of pond water, pond sediment, raw shrimps from the same ponds and the finished products (frozen shrimp) in relation to the sampled raw shrimps were analyzed for the total viable count, coliforms, *E.coli* and *Salmonella*. In addition to these, 12 samples each of ice used for transport of raw shrimp to the factory, water and ice used for processing in the factory were examined for the total viable count, coliforms and *E.coli*. At the time of sampling, physical parameters such as temperature, p^H and salinity of the pond water were recorded.

Microbiological parameters were analyzed according to the methods specified in the Sri Lanka standards. Species identification of *Salmonella* was done at the Medical Research Institute, Colombo, Sri Lanka. Confirmation of *Salmonella* isolates were carried out using Polymerase chain reaction (PCR) technique using *Salmonella* specific primers. Rapid detection and sensitivity of *Salmonella java* in artificially contaminated frozen shrimp product also were done by using PCR technique.

Log mean total plate count of raw shrimp is higher than the log mean total plate count of finished frozen product. In all the frozen finished products analyzed the total plate count range between 2.88×10^3 cfu/g to 3.33×10^6 cfu/g and all the results satisfy the ICMSF (1986) limits. The aerobic plate count of frozen shrimp ranges from 10^3 - 10^6 cfu/g and the bacterial counts of most samples ranges from 10^5 - 10^6 cfu/g (55%).

E.coli was not detected in any of the ice used for the transport, ice used in the factories and the water used for processing. The mean total plate count of the transport ice was between 1.322 cfu/ml – 3.322 cfu/ml, factory water and ice was 0 – 0.919 cfu/ml, 0 – 3.3222 cfu/ml. High numbers of coliforms have been observed in the pond water and sediment samples. Mean *E.coli* count of shrimp (3.05MPN/g) was higher than that of pond water (1.82MPN/100ml). In frozen shrimp samples *E.coli* count range between 0-11MPN/g. This range is below the ICMSF (1986) standards. In this study, *E.coli* count of raw shrimp is higher than the frozen shrimp.

Out of 220 samples analyzed, 5 samples were positive for *Salmonella*. In that four were from sediment samples and the other was raw shrimp. *Salmonella* was absent in all the 55 pond water samples analyzed. The *Salmonella* positive raw shrimp sample was not from the four ponds in which the sediment was found to be positive for *Salmonella*. The isolate from one raw shrimp and one sediment sample were identified as *Salmonella java*. Out of five samples analyzed as positive for *Salmonella*, only two samples were positive for *E.coli*. In the present study *Salmonella* was not detected in any of the frozen finished products analyzed. This satisfies the ICMSF (1986) microbiological limits which *Salmonella* should be absent in the final product. In the sediment samples analyzed, *Salmonella* and *Escherichia coli* were independent from each other at 95% confidence interval and in the raw shrimp samples analyzed, *Salmonella* and *E.coli* were dependent at 5% significant level. *Salmonella* isolates were confirmed by the PCR technique which yielded 457bp size product in the agarose gel electrophoresis. From the frozen shrimp sample *Salmonella java* had identified successfully. 1.6×10^{-1} cfu/g of the *Salmonella java* in frozen shrimp sample enriched in buffered peptone water had given the specified product (457bp) of *Salmonella* by the PCR technique.

The mean of temperature, salinity and p^H of all the pond water analyzed were 30.86°C , 19.8% and 8.4 respectively. The temperatures of pond water were observed in the range of 27°C to 38.9°C . There is a weakly positive correlation was observed between the temperature versus *E.coli* count and temperature versus coliform count in pond water. *E.coli* has been observed in the p^H range of 7.68-9.56 in pond water. There was a weakly positive correlation between p^H versus *E.coli* count and p^H versus coliform count in pond water. Weakly negative correlation was found between

salinity versus *E.coli* count and salinity versus coliform count in pond water. In this study, pond water salinity which was between 13% to 25%.

The overall microbiological quality (total number of organisms, coliforms, *E.coli* and *Salmonella*) of the finished frozen shrimp product which was analyzed is found to be satisfactory.