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Fish, being a highly perishable commodity, has to be handled and processed under good hygienic conditions. Though usually absent in freshly caught fish, the bacteria of public health significance like *Escherichia coli*, faecal streptococci, coagulase-positive staphylococci, salmonellae and *Vibrio parahaemolyticus* may contaminate fishery products at the different stages of processing, depending upon the level of hygiene and sanitation in the processing establishments. The available scientific information on the incidence, distribution, sources of contamination, growth pattern and behaviour of the organisms of public health significance appears to be insufficient to enable the processor to fully ensure consistent production of bacteriologically sound fishery products. A study was, therefore, undertaken to cover these aspects. The samples for the study were collected from the fish processing factories situated at Cochin.
The utensils used in the shrimp processing factories were found to be contaminated with organisms of public health significance. Faecal streptococci, *E. coli*, coagulase-positive staphylococci and salmonellae were isolated from 43%, 16%, 10% and 2% respectively of the utensils tested. Compared to utensils, the hands of the workers showed higher incidence of coagulase-positive staphylococci i.e. 34%. Palms of 50% of the workers were found to be contaminated with faecal streptococci, while only 10% of them contained *E. coli*. None of the palms examined showed the incidence of *Salmonella*. Incidence of faecal streptococci and *E. coli* in samples of water used in processing units was found to be 34% and 25% respectively. Similarly, the incidence of faecal streptococci and *E. coli* in the ice samples was recorded as 55% and 18% respectively. However, only 1% of the water samples and 3% of the ice samples were found to contain coagulase-positive staphylococci. *Salmonella* was detected in 1% of the water samples examined. Incidence of *Salmonella* in the water used in shrimp processing factories does not seem to have been reported previously. The total bacterial counts of the water and ice samples were also found to be
very high: 60% of the water samples and 68% of the ice sample showed bacterial counts more than 1000 per ml.

Incidence of faecal streptococci was very high in frozen shrimps i.e. 92% in headless (HL) shrimps, 94% in peeled and deveined (PD) shrimps and 96% in peeled undeveined (PUD) shrimps. The incidence of this organism was comparatively less (56%) in cooked frozen shrimps. Hundred percent of the frozen lobsters, 96% of the frozen cuttle fish, 80% of the frozen squids, 50% of the frozen cat fish, 55% of the frozen seer fish and 95% of the frozen froglegs tested contained faecal streptococci, the incidence of \textit{E. coli} in these samples being comparatively less. None of the squid samples and the red snapper analysed contained \textit{E. coli} whereas the occurrence of this organism in HL, PD and PUD shrimps was 13%, 26% and 30% respectively. Six percent of the cooked frozen shrimps yielded \textit{E. coli}.

Coagulase-positive staphylococci was found to be absent in all the test samples of lobsters, cuttle fish, cat fish, seer fish, froglegs and the red snapper. One of the notable observations was the
higher incidence (i.e., 38%) of coagulase-positive staphylococci in cooked frozen shrimps. In the case of HL, PD and PUD shrimps, the incidence of coagulase-positive staphylococci was 6%, 12% and 14% respectively. Compared to faecal indicator organisms, coagulase-positive staphylococci posed a minor problem in raw frozen fish and shell fish.

Only squids and cooked shrimps were free from salmonellae. In general, the incidence of *Salmonella* in fish was found to be higher than in shrimps. Salmonellae were found to be present in 10% of HL shrimps, 12% of PD shrimps, 14% of PUD shrimps, 17% of lobsters, 14% of cuttle fish, 25% of cat fish, 20% of seer fish and 35% of froglegs tested. Apparently there is no previous report on the incidence of *Salmonella* in frozen lobsters, cuttle fish, squids, seer fish and red snapper.

Incidence of *V. parahaemolyticus* was 5% in HL shrimps and 3% in PUD shrimps.

The bacterial load on frozen shrimps and frozen lobsters was found to be heavier than that on frozen
fish and frozen froglegs. Eighty-four percent of frozen cuttle fish, 100% of frozen squids, 88% of frozen cat fish, 90% of frozen seer fish and 92% of frozen froglegs had total bacterial count less than $2.0 \times 10^5$ per gram. On the other hand, only 78% of the frozen HL shrimps, 54% of frozen PD shrimps and 40% of frozen PUD shrimps were found to carry the bacterial load within the above range, the remainder yielded higher counts. Because of the heat treatment received, 96% of the frozen cooked shrimps showed total bacterial count less than $2.0 \times 10^5$ per gram. None of the cooked frozen shrimps, frozen squids, frozen cat fish, frozen seer fish or frozen froglegs had total bacterial count more than $1.0 \times 10^6$ per gram; but this was not the case with frozen HL shrimps, PD shrimps and PUD shrimps.

An important observation was the incidence of Salmonella in cases where both faecal streptococci and E. coli were absent. Results of the present work pointed out that neither E. coli nor faecal streptococci could indicate the presence of Salmonella in fishery products. Isolation of Salmonella from
a sample of frozen froglegs with a total bacterial count as low as 724 organisms per gram deserves special mention.

During the processing of cooked frozen shrimps, improper hygiene of workers, delay in processing, contaminated utensils and ice, polluted cooling water and reglazing water were found to affect the bacterial quality of the final product. In the processing of fresh frozen shrimps, the bacterial load on shrimps was found to increase considerably during beheading and peeling. Eventhough grading and washing decreased the bacterial count of the material, these values increased again when the material was kept overnight in ice, followed by reduction in the count during freezing.

Amongst the media compared, Tergitol-7 agar, KF agar and Baird-Parker medium were found to be the best for the enumeration of E. coli, faecal streptococci and coagulase-positive staphylococci respectively in fishery products.
About 90 to 98% of *E. coli* inoculated into raw peeled and deveined shrimps and cooked shrimps were destroyed during freezing at -40°C. There was gradual destruction of the organism during subsequent storage at -20°C and within four months most of the organisms studied lost their viability. On the contrary, under similar conditions, faecal streptococci were comparatively more resistant to freezing and subsequent cold storage. Only 25 to 30% of these organisms were destroyed during freezing and, of the residual flora, 75% were viable even after an year of storage at -20°C. Results indicated that, in frozen shrimps, faecal streptococci are better indicators of hygiene and sanitation compared to *E. coli*. There was a considerable difference between the destruction rate of coagulase-positive staphylococci in raw peeled and deveined shrimps and cooked shrimps. In cooked shrimps, the destruction during freezing at -40°C was between 8% and 15%. There was a gradual decrease in count during subsequent storage at -20°C and, in about 6 months, most of the test strains lost viability. In raw peeled and deveined shrimps, on the other hand, the
destruction during freezing was between 40% and 50%. Compared to cooked frozen shrimps, the rate of destruction of these organisms in raw frozen shrimps, during frozen storage, was rapid, with the result that, in about 5 months, all the strains studied lost their viability. All the test strains of Salmonella inoculated into cooked shrimp-homogenate were resistant to freezing at -40°C but, during subsequent frozen storage, there was some difference between the serotypes with regard to their viability. S. paratyphi B was the most resistant which survived up to 9 months while S. saintpaul was the least resistant having survival up to 5 months only. S. typhimurium, S. bareilly, S. salford, S. waycross and S. poona remained viable up to 8 months whereas S. roan, S. newport and S. weltevreden survived up to 7 months. Among the organisms studied V. parahaemolyticus was the least resistant to sub-zero temperatures: nearly 99.9% of the total number of this organism lost its viability during freezing at -40°C and none of the test strains survived the subsequent storage at -20°C for more than 7 days.
Shrimp-homogenate was found to be a good medium for the growth of *E. coli*, faecal streptococci and coagulase-positive staphylococci. All the test strains of these organisms grew luxuriantly at 37°C and 28°C. At 6-7°C, both *E. coli* and coagulase-positive staphylococci increased slowly in numbers and, after 6 to 7 days in the case of *E. coli* and 9 to 10 days in the case of coagulase-positive staphylococci, the numbers declined steadily. On the contrary, at these temperatures, all the test strains of faecal streptococci recorded steady increase in numbers throughout the period of study. However, none of the strains of *E. coli*, faecal streptococci and coagulase-positive staphylococci multiplied in the shrimp-homogenate medium at 0°C.

Another important observation in this study was the inability of coagulase-positive staphylococci to grow in competition with *E. coli* or faecal streptococci in shrimp-homogenate at 28°C, 10°C or 0°C. When higher numbers of *E. coli* or faecal streptococci were present in the homogenate, the growth of staphylococci was suppressed to a greater extent. The temperature of incubation also markedly
influenced the extent of such inhibition in the
growth of staphylococci. The natural bacterial
flora present in shrimps were also found to suppress
the growth of coagulase-positive staphylococci at
28°C, 10°C and 0°C. However, *E. coli* and faecal
streptococci exhibited no competition between each
other for growth in shrimp-homogenate.

During the present work, 27 different serotypes
of *Salmonella* were isolated from various fishery
products, the total number of serotypes obtained
from frozen froglegs being higher than that obtained
from any other individual source. The major
serotype isolated from frozen shrimps was
*S. weltevreden*. *S. roan* and *S. larocheille* were
isolated from frozen froglegs for the first time in
India. A few serotypes, rarely reported in India,
were also isolated in this study. These include
*S. orion* from frozen fish, *S. waycross* and *S. matopeni*
from frozen froglegs and *S. heidelberg* from frozen
froglegs and raw shrimps.