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Bacteriological Quality of Fresh and Ice-stored Farmed *Macrobrachium rosenbergii* from Central Kerala

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Farmed freshwater Scampi (*Macrobrachium rosenbergii* de Man) collected from four different farms in central Kerala were analysed for bacteriological quality immediately after harvest and after icing for two weeks. Aerobic mesophilic bacteria (TPC), faecal streptococci, faecal coliforms, *Escherichia coli* and *Staphylococcus aureus* were enumerated. Total aerobic mesophilic bacterial counts (TPC) on fresh prawn (whole) were of the order of 10^6 to 10^7 cfu g^{-1}. The TPC of fresh and ice stored prawn (whole and headless) were within the acceptable limit. The levels of faecal streptococci and faecal coliforms in fresh M. rosenbergii were above the acceptable limits. However, faecal coliform and E. coli numbers were within the limit (<100 cfu g^{-1}) in deheaded prawn during iced storage. Presence of excessively high TPC and faecal streptococci numbers has been a major microbiological problem in freshwater prawn. This study revealed that *A. hydrophila, A. veronii biovar sobria* and Enterococci grew well at refrigerated temperatures. *A. hydrophila, A. veronii* biovar *sobria*, S. *putrefaciens* and *Pseudomonas* were identified as potent spoilers of farmed *M. rosenbergii.*

Key words : Microbial quality, spoilage flora, Fresh, iced-stored, *Macrobrachium rosenbergii*, Kerala.

Freshwater prawn (*Macrobrachium rosenbergii* de Man) commercially known as "scampi", has good demand in export and domestic market (Marine Products Development Authority, MPEDA, 2001). Microbial growth and activity in fresh seafood are the main factors associated with quality deterioration, spoilage and economic loss (Zhuang *et. al.*, 1996). Therefore, identification of the spoilage associations facilitates the development of methods to improve quality and to extend shelf life of these products (Dalgaard, 2000; Leisner & Gram, 2000). It is common practice to store shrimp in ice (at 0°C) to extend the shelf-life.

Spoilage of fresh or refrigerated fish and prawn under aerobic storage conditions is attributed largely to growth and metabolic activities of bacteria (Cann, 1977., Reilly *et. al.* 1984., Reilly & Dangla, 1986., Leitào & Rios, 2000., Shamshad *et. al.*, 2000) mainly Gram-negative psychrotrophic organisms such as *Pseudomonas, Shewanella* and *Flavobacterium* spp. (Hubbs,1991). The microflora responsible for spoilage of fresh fish/shrimp changes with changes in storage temperature (Gram *et. al.*, 1987). Farming practices such as fertilization and supplementary feeding imposes a high probability of contamination on the cultured prawn.

The microflora of farmed fresh *M. rosenbergii* have been reported (Surendran *et. al.*,

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1995., Jayasekharan & Ayyappan, 2002., Lalitha & Surendran 2003, 2004). The changes in biochemical and sensory quality characteristics during ice storage and shelf life have been reported on wild caught (Joseph *et. al.*, 1992) and cultured scampi (Angel *et. al.*, 1981, 1985., Rodrigues *et. al.*, 2000., Ninan *et. al.*, 2003). However, a few studies have been published on the changes in the microflora associated with ice stored cultured scampi from Israel (Angel *et. al.* 1981, 1985), Brazil (Leitào & Rios, 2000) and India (Lalitha &Surendran, 2006). The actual spoilage process and spoilage microflora associated with the freshwater prawn has not been extensively studied. Since the culture of the freshwater prawn is a commercial activity in Kerala and in several other parts of India, the information on the spoilage flora associated with *M. rosenbergii* reared in freshwater farms in Kerala is necessary in order to develop methods to control spoilage and to improve quality and to extend shelf life.

The purpose of the present study was to determine the microbial associations with fresh and ice stored farmed *M. rosenbergii* and to identify the spoilage flora to develop methods to improve the quality and to extend the shelf life.

Materials and Methods

Farmed freshwater Scampi (*Macrobrachium rosenbergii*) were collected (ca. 20 prawn/kg) from four different farms in central Kerala. Samples were packed in sterile polythene pouches, kept on ice in insulated boxes and transported within 3hrs after being caught. On arrival at the laboratory, each prawn sample (*M. rosenbergii)* was divided to two lots. First lot was kept as whole shrimp. Second lot was deheaded. The two lots were iced in two insulated boxes in 1:1 ice to fish ratio. Ice was continuously replaced and water removed. Two independent storage experiments were conducted for each prawn sample. Bacteriological quality was analyzed for whole and deheaded prawn immediately after harvest and after icing for two weeks. On each sampling day, 4-5 prawn were analysed.

Samples of muscle tissue (10g) were aseptically taken and transferred to a stomacher bag (Seward Medical, London, UK), 90 ml of physiological saline (Nacl,0.85% w/v) was added, and the mixture was homogenized for 30s with a stomacher (Lab blender 400, Seward Medical). Samples (1ml) of serial dilutions of prawn homogenates were plated on agar or poured in to tubes for MPN method.

Samples (1ml) of serial dilutions of prawn homogenates were plated on Tryptone Soya Agar (TSA Oxoid, UK), incubated at 30°C for 2d for determination of total aerobic mesophilic counts. Counts of Faecal streptococci and *Staphylococcus aureus* were determined by the plating method and counts of total coliforms, faecal coliforms, *Escherichia coli* and *Clostridium perfringens* by the three tube MPN method (FDA,1998., West, 1989).

A total of 86 bacterial cultures were isolated from prawn. Colonies were picked from TSA plates sampled from the fresh and ice stored fish. All colonies from a sector of the plate or all colonies from a whole plate were isolated , purified and stored on TSA slants. The strains were tested for Gram reaction, catalase and oxidase reactions, motility oxidation/ fermentation test and presence of spores. They were then grouped according to the taxonomic schemes of Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984; Sneath *et. al.*,1986) and further tested for the most relevant characteristics of each group and identified using the schemes proposed by Dainty *et. al.*, (1979) and Valera & Esteve, (2002).

Results

The initial aerobic mesophilic bacterial counts (TPC) at 30°C on fresh whole and

headless prawn were in the range of 6.0-7.0 and 5.0-6.0 $\log_{10}$ cfu g^{-1} respectively (Fig. 1 and 2). After two weeks storage in ice, the mesophilic counts on whole and deheaded prawn were 5.7-6.3 x -10 ^{5} cfu g^{-1}. A reduction was noticed in the TPC (80-90%) on whole shrimp, probably due to the washing effect on the surface microorganisms.

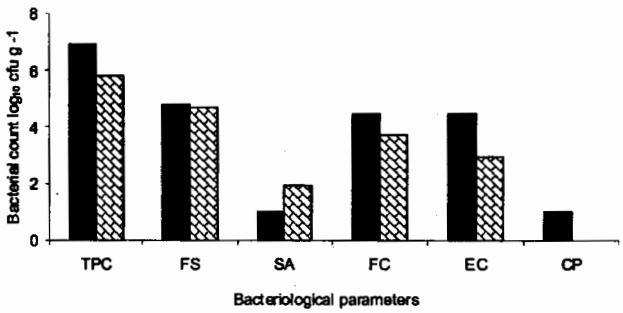

Fig. 1. Bacteriological quality of *Macrobrachium rosenbergii* (whole) from freshwater farms located at central Kerala

■ fresh ▨ Iced

TPC-Aerobic count, FS-faecal streptococci, SA-S. aureus, FC-Faecal coliforms, EC-*E. coli*, CP-C. *perfringens*

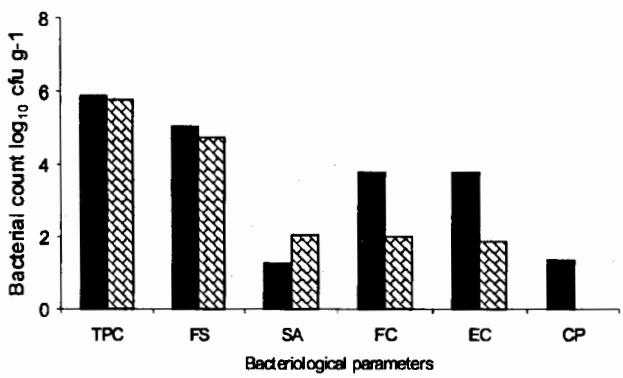

Fig. 2. Bacteriological quality of fresh and ice stored *Macrobrachium rosenbergii* (headless) from freshwater farms located at central Kerala

■ fresh ▨ Iced

TPC-Aerobic count, FS-faecal streptococci, SA-S. aureus, FC. Faecal coliforms, EC-E. coli, CP-C. perfringens

For frozen raw shrimp, the microbiological limits proposed by the ICMSF (1998) for aerobic plate counts at 30°C are m= 5.69 $\log_{10}$ and M=7.0 $\log_{10}$ cfu g^{-1}. The TPC values for farmed fresh prawn immediately after harvest were close to the upper limit of acceptability of frozen raw shrimp as defined by ICMSF even after keeping on ice and immediate transport to the laboratory and it is indicative of high bacterial load on fresh prawn. However, the metallic sheen, iridescence of the skin of prawn as well as glossy, bright red gills possessing shellfish odors indicate extreme freshness and good quality prawn. The high TPC values of freshwater prawn was not unusual when compared to other data from Israel (Angel *et. al.*,1981, 1985) and Brazil (Leitào & Rios, 2000). Scampi samples from a processing plant in Kakinada harboured high microbial load and APC was >10^5 cfu/g in 80% of samples of whole prawn and 50% samples of headless prawn (International Atomic Energy Agency IAEA 2005). High bacterial loads imply that the products need special handling and processing to reduce the bacterial load. Buras *et. al.* (1987) have shown that bacterial levels of pond-raised fish may increase considerably in edible tissues after a threshold level has been surpassed in the environment. In the present study, the aerobic mesophilic counts on prawn stored at 0°C were lower than the recommended limits (7.0 $\log_{10}$ cfu g^{-1}) even after 14 days storage. However, the head started to separate from the body after two weeks of storage at 0°C.

The faecal coliform and *Escherichia coli* counts on fresh prawn (whole and headless) ranged from 2- 4 $\log_{10}$ cfu g^{-1}. On icing, their numbers were reduced to < 2 $\log_{10}$ cfu g^{-1} in headless prawn (Fig.1 and 2.). Faecal coliforms and *E. coli* are considered particularly useful indicators of potentially hazardous contamination and mishandling of fish and fish products . *E. coli* limits proposed for frozen raw shrimp by the ICMSF (1998) are m=11 and M = 500 cfu g^{-1} or cm ^{2} and these limits are often considered as minimal reference values.

The high numbers of coliforms, faecal coliforms and *E. coli* in freshwater prawn samples indicated that the prawn farms were polluted. In contrast, these microorganisms were either absent or present in low numbers in

marine shrimp. The high indicator bacterial load on scampi could be attributed to the application of organic manure (cow dung, poultry manure) to fertilize the pond and also to feeding with animal protein sources like clam meat and trash fish. Angel *et. al.*, (1981) reported *Enterobacteriaceae* counts of 4.7 and 4.4 $\log_{10}$ cfu g^{-1} for fresh and ice stored (14 days) *M. rosenbergii* from Israel ponds. However, faecal coliforms (1 $\log_{10}$ cfu g^{-1}) were detected only on head and not in tail of fresh prawn and also in tail of ice stored prawn. *E. coli* was reported at levels of < 20 cfu/g in 50% of whole and 25% of headless Scampi samples from a processing plant in Kakinada (IAEA, 2005).

Faecal streptococci counts on prawn were not significantly reduced from 4-5 $\log_{10}$ cfu g^{-1} during two weeks storage at 0°C. Enterococci were identified as *Enterococcus faecalis* and *E. faecium*. Such high load of Enterococci in edible tissues of pond - raised prawn might have come from the environment after a threshold level has been surpassed as reported earlier for pond raised fish (Buras *et. al.*, 1987). Growth of these organisms in prawn becomes a safety issue if products are severely temperature abused.

Significant numbers of faecal coliforms and enterococci were previously reported in tiger prawn farms in India (Surendran *et. al.*, 1995) and Philippines (Reilly *et. al.*, 1984). When present in high levels in ready -to -eat foods, *Enterococcus* species may represent a health risk to consumers (Franz *et. al.*, 1999). Pathogenic Staphylococci and Enterococci cause severe infections in humans (Andrew & Mitchell, 1997) and the increasing emergence of acquired antibiotic resistance among these group of gram-positive cocci and transfer antibiotic resistance genes to other pathogens (Bonadio *et. al.*, 2000) is a major concern. *Enterococcus* species isolated from *M. rosenbergii* can harbor various antimicrobial resistance and virulence traits.

Staphylococcus aureus and *C.perfringens* counts were 10^1 cfu g^{-1} in whole prawn. Although staphylococci are considered mesophilic bacteria, significant growth (0.6- 0.8 log increase) of these organisms in freshwater prawn during storage at 0°C were noticed as reported earlier in trout (González-Rodríguez *et. al.*, 2001). Coagulase positive Staphylococci counts were within the limits (100 cfu g^{-1}) of acceptability of frozen raw shrimp as defined by ICMSF (1998). *S. aureus* count of 10^1 cfu g^{-1} was reported earlier for farm reared *M.rosenbergii* (Jayasekaran & Ayyappan, 2002). *C. perfringens* numbers were reduced during two weeks storage in ice.

A total of 86 strains were isolated from TSA plates (30°C) sampled from the fresh and ice stored *M.rosenbergii*. Of the 36 strains from fresh prawn, majority of the isolates were gram-negative rods. *Enterobacteriaceae, Aeromonadaceae* and genera *Pseudomonas* and *Moraxella* were dominant (Fig. 3). Gram-positives were also represented by genera *Enterococcus, Micrococcus, Bacillus, Corynebacterium* and *Arthrobacter*. *Enterobacteriaceae* were identified as *Enterobacter, Klebsiella* and *Citrobacter* . Bacteria of *Aeromonadaceae* family, *Aeromonas hydrophila, A. veronii* biovar *sobria, A. veronii biovar veronii* and *A. jandaei* spp. were predominant in the

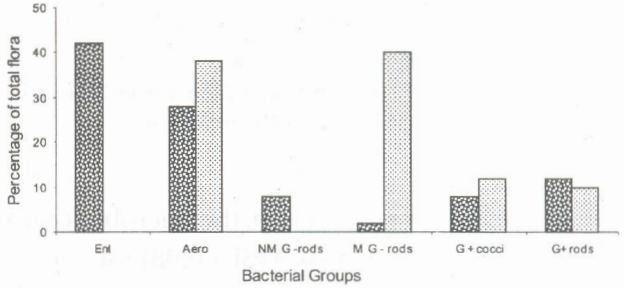

Fig. 3. Major bacterial groups on fresh and ice stored *Macrobrachium rosenbergii*

fresh Iced

Ent- Enterobacteriaceae, Aeromonadaceae, N MG-rods-non motile Gram- ive rods, MG-rods-motile Gram-ive rods, G+ cocci - Gram +ive cocci, G+rods-rods-Gram +ive rods

freshwater prawn microflora. They were proteolytic, amylolytic and hemolytic as reported earlier by González *et. al.*, (2001) in freshwater fish. *A. hydrophila* and *A. veronii* biovar *sobria* has been linked to gastrointestinal infections and cases of human wound infections by *A. hydrophila* has been reported (Kirov, 2001, Hanninen *et. al.*, 1997). *Enterobacter, Citrobacter, Enterococcus* and *Micrococcus* strains were capable of producing low amounts of hydrogen sulphide indicating low spoiling activity. The predominance of *Enterobacteriaceae* and *Aeromonadaceae* in the microflora was reported earlier for farmed freshwater prawn (Leitào and Rios, 2000) and for farmed freshwater fish (González-Rodríguez *et. al.*, 2001 González *et. al.*, 2001) The influence of rearing practices (pond fertilization using animal manure and feeding clam meat) and organic matter levels in rearing ponds on the microbial load and microflora associated with the farmed fish was reported earlier (González *et. al.*, 1999) and shrimp (Reilly *et. al.* 1985; Surendran *et. al.* 1995., Leitào & Rios, 2000).

The microbiological associations of fresh and ice stored whole prawn were found to vary considerably. *Shewanella, Moraxella* and *Pseudomonas* constituted a small proportion of the total aerobic flora of fresh prawn. Majority (78%) of the strains isolated after two week in ice were gram negative (Fig. 3). The population of *Aeromonas* spp. increased. However, the abundance of *Enterobacteriaceae* group, which represented a consistant part of the microflora of fresh prawn, decreased during ice storage. There was again a shift in the microflora after 2 weeks in ice and nearly 80% of the total flora belonging to genera *Aeromonas, Shewanella, Moraxella* and *Pseudomonas*. Of the 50 bacterial isolates identified from ice stored prawn, 19 belonged to *Aeromonadaceae* (*A.hydrophila* and *A. veronii* biovar *sobria*), 8 were identified as *Shewanella putrefaciens* and twelve were identified as *Pseudomonas*. Motile Gram-negative rods (aeromonads, *S. putrefaciens, Pseudomonas*), the most prolific organisms during the ice storage of fish and pond reared *P.monodon* (Reilly *et al.*, 1984., Reilly & Dangla, 1986) were found in significant numbers after two weeks storage. This may be the reason for the discolouration inside the shrimp cephalothorax and separation of the head from the body after two weeks on ice storage even when the aerobic mesophilic counts remained 10^6-10^7 cfu g^{-1}. Those bacterial groups were psychrotrophic and proteolytic which indicates their role in the spoilage of ice stored freshwater prawn. The minimum growth temperature of *A.hydrophila* varied from 0.1 to + 1.2°C (Walker, 1990). Exotoxin production by *A.hydrophila* has been reported (Kirov, 2001) in food slurries (prawn, scallop, fish) held at refrigeration temperatures. The role of *A.hydrophila* in food-borne gastroenteritis is not firmly established. On the other hand, potent enzyme activity, growth at low temperature and ability to utilize sulphur containing amino acids coupled with offensive off-odours and production of H_2S establish *A.hydrophila* and *A. veronii* biovar *sobria* as potent spoilers of ice stored freshwater prawn. Motile aeromonads (*A. hydrophila* and *A. veronii* biovar *sobria*) are well established as a component of the spoilage flora of freshwater fish (González *et. al.*, 2001) and pond reared shrimp *Penaeus monodon* (Reilly *et. al.*, 1984; Reilly & Dangla, 1986) at refrigeration temperatures.

Presence of excessively high TPC, faecal streptococci, faecal coliform and *E. coli* counts has been a major microbiological quality problem in farmed freshwater prawn. However, numbers of faecal coliform and *E. coli* were within the limit (<100 cfu g^{-1}) and *C. perfringens* was significantly reduced in deheaded prawn during iced storage. The present study has shown that *A. hydrophila, A. veronii* biovar *sobria* and Enterococci grew well at refrigerated

temperatures and therefore, additional preservation techniques are necessary to arrest the growth of these organisms on ice storage of freshwater prawn. Our results also indicates that *A. hydrophila, A. veronii* biovar *sobria, S. putrefaciens* and *Pseudomonas* that are the potent spoilers of fish, are present in abundant numbers on farmed *M. rosenbergii.* It is noteworthy that at the end of storage period of 14 days, there was no indication of off- odors. This points to the need for a better understanding of the complex interaction between SSO and substrate, to throw more light on the spoilage pattern of *M. rosenbergii.*

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