Reduction in Microbial Load of Farmed Freshwater Scampi (*Macrobrachium rosenbergii*) by Application of Permitted Food Preservatives

K.V. LALITHA, G.R. UNNITHAN and P.K. SURENDRAN
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682 029, India

Farmed freshwater scampi (*Macrobrachium rosenbergii*) from four different farms in Kerala were examined for their microbial quality. Total bacterial counts (TPC) were of the order of 10^6-10^7.g^{-1}, *Escherichia coli*, 10^2-10^4.g^{-1} and faecal streptococci, 10^2-10^5.g^{-1}. Presence of excessively high TPC and total faecal streptococci has been a major quality problem, since freezing did not reduce the TPC below 10^5.g^{-1} which is a requirement as per EU and USFDA regulations. Permitted food preservatives like sodium chloride, potassium sorbate, citric acid and chlorine were tried as dips to reduce the bacterial load. TPC, faecal streptococci, total coliforms, faecal coliform and *Escherichia coli* were estimated before and after treatments and the data were statistically analysed. A 15 min dip in a chilled aqueous solution of a combination of 0.5% potassium sorbate and 0.2% citric acid was found to be very effective in reducing both TPC and faecal streptococci considerably.

Key words: Microbial load, food preservatives, *Macrobrachium rosenbergii*

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), commercially known as scampi, has now emerged as an important commodity commanding good domestic and export market. Freshwater prawn farming is a very important commercial activity in several states of India. Andhra Pradesh producing 20,910 t per annum enjoys the number one position, followed by West Bengal (2270 t), Orissa (400 t) and Kerala (300 t). Export of frozen scampi in various product forms such as head-on, headless-shell-on (both IQF and block frozen) increased from 1796 t in 1994 to 2678 t in 1999. European Union and the USA are the major markets for frozen scampi (MPEDA, 2001). These products have to meet the importing country's standards of quality and safety. Even if raw scampi is refrigerated, the total microbial load does not reduce considerably. Also, reduction of bacterial load during freezing depends on the qualitative composition of the bacterial flora.

The present EU/USFDA standards require that TPC of fresh frozen shrimp should be less than $5x10^5$ cfu.g^{-1} (Table 1).

Table 1. EU / USFDA standards for fresh frozen shrimp

Microbiological parameter	Permitted levels
TPC at 37°C	5.00,000.g^{-1}
Escherichia coli	20.g^{-1}
Coagulase positive Staphylococci	100.g^{-1}
Salmonella	Absent in 25 g
Vibrio cholerae	Absent in 25 g

Surveys on the microbiological quality of cultured shrimp have shown that they harbour high microbial load. Pond fertilization through organic sources (cow dung, poultry manure) and supplementary feeding with animal protein sources like clams, and livestock wastes result in a high probability of contamination of the farmed prawn. Sunarya *et al.* (1992) reported increase in the total plate count in cultured shrimp on harvesting, handling and processing. During transportation to the processing unit, sorting, deheading and arranging in the pan before freezing, bacterial counts go up.

Efforts to reduce bacterial load on cultured brackishwater shrimp have been made by storage in ice, application of chlorine (Sunarya *et al.* 1992; Sunarya & Iskander, 1992), modified iced storage (Jiang & Lee, 1988) and treatment with organic acids and their salts (Zhuang *et al.*,1996; Al-Dagal & Bazarra, 1999). The anti-microbial effect of chemical preservatives have been evaluated using model food systems and microbiological media (ICMSF,1980; Lück & Jager, 1997). Much work has been done to determine the effect of food preservatives on growth of different microorganisms (Lück, 1977; Sofos & Busta, 1981; Liewan & Marth, 1985). The antimicrobial activity of sodium chloride, sorbic acid and citric acid is well documented (ICMSF, 1980; FAO, 1989; Sofos, 1992; Davidson, 1997). Wide concentration ranges of organic acid salts such as sodium acetate (0.5-10.0% w/w), potassium sorbate (0.1-10.0%) and sodium citrate (8.0-10.0%) have been used, alone or in combination, to extend the shelf life of fresh meat and seafoods (Ho *et al.*, 1986., Ward *et al.*, 1982., Mendocina *et al.* 1989., Al-Dagal & Bazarra, 1999). No single preservative is active against all the spoilage microorganisms present in the food. Therefore, combinations of preservatives have been employed in food technology to provide broader

spectrum of action, increased antimicrobial action and lower concentration of individual substances. Microbial inhibition can often be enhanced when sorbic acid is combined with other chemical preservatives (Sofos, 1992). Use of veterinary drugs and antibiotics to decontaminate harvested *M. rosenbergii* may result in antibiotic residues leading to food safety problems and such use is banned in India. This study was undertaken with a view to developing methods to reduce the microbial load on farmed *M. rosenbergii* to acceptable levels.

Materials and Methods

Macrobrachium rosenbergii samples were collected from freshwater farms from three districts of Kerala (India), namely Trichur, Kottayam and Alleppy. The prawns were comparatively large sized, weighing 60-90 g each. The prawn samples were aseptically transferred to sterile polythene bags and transported under ice, to the laboratory within 2-4 h of harvesting.

The experiments to study the effect of various types of handling and preservative treatments were conducted as follows:

Effect of washing, chlorination, icing and freezing on the microbial load of the prawns

Each prawn sample was divided into two lots. First lot was kept as whole and the prawns of the second lot were deheaded. Each lot was further divided to 5 groups. Each group was given the following treatments.

Group I : Control sample

Group II : Washed with clean potable water

Group III : Kept under crushed ice (1:1 ratio) for 5 days

Group IV : Frozen at –40°C and stored at –20°C for one month

Group V : Dipped in potable water having 10 ppm available chlorine

Samples from each group were analysed for microbial parameters.

Effect of preservatives on the microbial load of the prawns

Each prawn sample was divided to two lots. First lot was given the dip treatments as given in Table 2 for whole shrimp and deheaded shrimp. Second lot was given similar treatments, but the solution was chilled to 2±1°C. After a dipping time of 15 min the prawns were drained and analysed

for microbiological parameters. In the case of the second lot, the samples were stored in crushed ice in sterile polythene bags for 5 days to study the effect of low temperature storage on bacterial parameters.

Table 2. Types of treatments given with various combinations of permitted food preservatives

Treatment code	Treatment	pH of the treatment solution
C	Control	-
T1	2% sodium chloride and 0.2% citric acid	<3.8
T2	0.5% potassium sorbate and 0.2% citric acid	4.6
T3	2% sodium chloride, 0.5% potassium sorbate and 0.2% citric acid	4.1
T4	2% sodium chloride, 0.2% citric acid and 10 ppm chlorine	<3.8
T5	2% sodium chloride, 0.5% potassium sorbate, 0.2% citric acid and 10 ppm chlorine	4.4
T6	0.5% potassium sorbate and 2% sodium chloride	6.0
T7	2% sodium chloride and 10 ppm chlorine	5.4

Microbiological analysis

Microbiological examinations of the shrimp samples were done by the USFDA methods. Total bacterial count, faecal streptococci count and *Staphylococcus aureus* count were determined by the plating method and total coliforms, faecal coliforms and *Escherichia coli* was determined by the three tube MPN method (USFDA, 1995). Student 't' test was used to evaluate the significance of difference between means of microbial counts performed for various treatments.

Results and Discussion

The Total bacterial count (TPC), faecal streptococci count, total coliforms, faecal coliforms, *Escherichia coli* and *Staphylococcus aureus* counts of *M. rosenbergii* from the four farms are presented in Tables 3 and 4. The TPC of whole shrimp samples ranged from 10^6 to $10^7.g^{-1}$, while that of headless prawn was in the range of 10^5-$10^6.g^{-1}$. The faecal streptococci counts were very high in both whole and headless prawn, being in the range of 10^3-$10^5.g^{-1}$. Total and faecal coliform and *E. coli* counts were also very high, being in the range of 10^3-$10^5.g^{-1}$.

The excessively high bacterial load in the farmed freshwater prawn is a major quality problem. The USFDA and EU standards for frozen shrimp

(Table 1) stipulate that the TPC at 37°C should not exceed $5x10^5.g^{-1}$, *E. coli* $20.g^{-1}$ and coagulase positive staphylococci $100.g^{-1}$. The farm fresh scampi had TPCs at least 10 to 100 times more than the stipulated limits (Tables 3 & 4). The counts of *E. coli* and coagulase positive staphylococci were also very high. Deheading of the scampi could bring about only marginal (up to one log cycle) reduction in TPC as well as other microbial parameters.

Table 3. Microbial quality of farmed *Macrobrachium rosenbergii* (whole) obtained from Alleppey, Kottayam and Trichur districts (Kerala)

Microbial parameters	Mean microbial count			
	Vallakom (Kottayam)	TV Puram (Kottayam)	Vayalar (Alleppey)	Ashtamichira (Trichur)
TPC.g^{-1}	$8.4x10^6$	$1.65x10^7$	$2.0x10^7$	$1.0x10^7$
Faecal streptococci .g^{-1}	$7.2x10^3$	$5.5x10^4$	$5.9x10^4$	$5.5x10^5$
Staphylococcus aureus.g^{-1}	Not detected	$7.2x10^3$	Not detected	$4.0x10^1$
Total coliforms, MPN.g^{-1}	4500	140000	140000	14000
Faecal coliforms, MPN.g^{-1}	2500	140000	140000	14000
Escherichia coli, MPN.g^{-1}	2500	140000	140000	14000

Table 4. Microbial quality of farmed *Macrobrachium rosenbergii* (headless) obtained from Alleppey, Kottayam and Trichur districts (Kerala)

Microbial parameters	Mean microbial count			
	Vallakom (Kottayam)	TV Puram (Kottayam)	Vayalar (Alleppey)	Ashtamichira (Trichur)
TPC.g^{-1}	$4.0x10^5$	$6.2x10^5$	$8.4x10^5$	$4.5x10^6$
Faecal streptococci .g^{-1}	$1.1x10^4$	$1.7x10^5$	$1.8x10^5$	$4.6x10^5$
Staphylococcus aureus.g^{-1}	Not detected	$4.0x10^2$	Not detected	$2.0x10^2$
Total coliforms, MPN.g^{-1}	4500	2500	140000	14000
Faecal coliforms, MPN.g^{-1}	1400	400	140000	14000
Escherichia coli, MPN.g^{-1}	1400	400	140000	14000

Effect of washing, chlorination, icing and freezing on the microbial load of the prawns

Effect of washing, dipping in 10 ppm chlorine water, icing and freezing (-40°C) on the microbiological parameters of farm fresh scampi are presented in Tables 5 and 6. Washing in potable water reduced the TPC of the whole scampi by 54% and of the headless scampi by 45%. Similar reduction

(40-45%) was noticed in *E. coli* and *S. aureus* counts. Faecal streptococci counts were also reduced by 33-36%. However, the microbial counts even after washing were above the stipulated limits. Similar reduction in microbial counts was noticed by Al-Dagal & Bazarra (1999) after washing shrimp in sterile water. Washing in potable water having 10 ppm residual chlorine is an approved practice followed in farms to reduce the bacterial load. These steps generally bring down the total bacterial population. Table 5 and 6 shows that dip in 10 ppm chlorinated water for 15 min reduced the TPC (82-85%), faecal streptococci (64-78%), *E. coli* (99%) and *S. aureus* (90%). However, still the TPC was above $10^6.g^{-1}$, but *E. coli* and *S. aureus* counts were reduced to the approved limits. Sunarya *et al.* (1992) noticed 46% reduction in TPC and 83% reduction in *E. coli* on cultured brackishwater shrimp, *P. monodon* soaked in 10 ppm chlorine for 30 min. In another study, Sunarya & Iskander (1992) observed 98% reduction in TPC of cultured prawn washed with 10 ppm chlorinated chilled water.

Table 5. Effect of washing, icing, freezing and chlorine treatment on bacterial profile of farmed *Macrobrachium rosenbergii* (whole)

Microbiological parameter	Mean microbial count				
	Before washing (control)	After washing	After icing (5 days)	After freezing and frozen storage (1 month)	After Chlorine treatment
TPC.g^{-1}	$1.5x10^7$	$6.9x10^6$ (54)	$1.2x10^6$ (92)	$1.6x10^6$ (89)	$2.7x10^6$ (82)
Faecal streptococci .g^{-1}	$5.9x10^4$	$4.0x10^4$ (33)	$1.0x10^4$ (80)	$6.4x10^5$	$1.3x10^4$ (78)
Escherichia coli, MPN.g^{-1}	15000	9000 (40)	1400 (91)	9.5 (99.9)	40 (99.8)
Staphylococcus aureus.g^{-1}	$3.6x10^3$	$2.0x10^3$ (45)	$4.6x10^2$ (78)	$4.0x10^1$ (89)	$3.0 x10^2$ (92)

Numbers in parenthesis shows % reduction in count from the control

After 5 days of iced storage, a reduction has been noticed in TPC (80-90%) and *E. coli* counts (91%) of whole and headless shrimp (Tables 5 & 6). This was probably due to the washing effect on the surface microorganisms. Faecal streptococci and *S. aureus* counts were not significantly affected by icing. The bacterial numbers were still high in each case.

Freezing at –40°C followed by frozen storage at –20°C for one month caused a reduction in TPC by 70-90%, *E. coli* by 99% and *S. aureus* by 90%, but faecal streptococci count was not at all significantly affected by freezing (Tables 5 and 6). Since the faecal streptococci count were in the range of 10^4 to $10^5.g^{-1}$, their resistance to freezing is very significant in

Table 6. Effect of washing, icing, freezing and chlorination on bacterial profile of farmed *Macrobrachium rosenbergii* (headless)

Microbiological parameter	Mean microbial count				
	Before washing (control)	After washing	After icing (5 days)	After freezing and frozen storage (1 month)	After Chlorine treatment
TPC.g^{-1}	$5.4x10^5$	$3.0x10^5$ (45)	$9.7x10^4$ (82)	$1.5x10^5$ (73)	$8.1x10^4$ (85)
Faecal streptococci .g^{-1}	$1.3x10^5$	$9.0x10^4$ (36)	$1.5x10^4$ (90)	$1.3x10^5$	$4.5x10^4$ (65)
Escherichia coli, MPN.g^{-1}	14000	4500 (68)	1100 (93)	250 (99)	30 (99.9)
Staphylococcus aureus.g^{-1}	$3.0x10^2$	$2.0x10^2$ (33)	$1.5x10^2$ (50)	$2.6x10^1$ (92)	$2.0x10^1$ (90)

Numbers in parenthesis shows % reduction in count from the control

determining the residual bacterial count after freezing. Similar observations were made earlier by Simmonds & Lamprecht (1985). Gram-negative organisms are generally more sensitive to freezing than Gram-positive ones. Enterococci are more resistant to freezing. *S. aureus* has been found to die off during frozen storage, rather rapidly at first but more slowly as time progressed so that significant numbers survive after several months (Simmonds & Lamprecht, 1985). Sunarya *et al.* (1992) noticed 93% reduction in TPC and 99.7% in *E. coli* on cultured *P. Monodon* during frozen storage. Similar data on freshwater prawn is lacking and probably this is the first report.

Effect of preservatives on the microbial load of the prawns

Compared to the untreated control, treatment of prawn (whole and headless) with various combinations of potassium sorbate, citric acid, sodium chloride, chlorine and also with various combinations of sodium chloride, citric acid and chlorine in water at ambient temperature (30°C) caused a reduction in the natural microflora (Tables 7 & 8). However, the differences in TPC and faecal streptococci counts were significant ($p< 0.05$) only for samples treated with potassium sorbate and citric acid (T2). For treatments T1 and T7, significant differences were noticed only for TPC ($p<0.05$) of headless prawn whereas for T3 and T4, significant differences were noticed only for TPC ($p<0.05$) of whole prawn. Among the 7 lots of treated samples, significant differences were noticed ($p<0.01$) in the streptococci counts of whole and headless prawn samples treated with potassium sorbate and citric acid (T2) only. For T6 and T7, difference in the streptococci count was significant at 5% level only for whole prawn.

Table 7. Mean total plate counts of *Macrobrachium rosenbergii* treated with preservatives in water at 30°C

	Treatments	Mean total plate count (log cfu.g^{-1}) Whole	Headless
C	Control	6.295	5.9341
T1	2% sodium chloride and 0.2% citric acid	6.0216	5.4941
T2	0.5% potassium sorbate and 0.2% citric acid	5.8004	5.1649
T3	2% sodium chloride, 0.5% potassium sorbate and 0.2% citric acid	5.7306	5.6546
T4	2% sodium chloride, 0.2% citric acid and 10 ppm chlorine	5.8686	5.4398
T5	2% sodium chloride, 0.5% potassium sorbate, 0.2% citric acid and 10 ppm chlorine	5.9486	5.8966
T6	0.5% potassium sorbate and 2% sodium chloride	5.9862	5.6741
T7	2% sodium chloride and 10 ppm chlorine	6.0124	5.8212

Table 8. Mean Faecal Streptococci count of *Macrobrachium rosenbergii* treated with preservatives in water at 30°C

	Treatments	Mean faecal streptococci count (log cfu.g^{-1}) Whole	Headless
C	Control	4.7196	4.6531
T1	2% sodium chloride and 0.2% citric acid	4.7922	4.7532
T2	0.5% potassium sorbate and 0.2% citric acid	3.5379	3.8288
T3	2% sodium chloride, 0.5% potassium sorbate and 0.2% citric acid	4.1274	4.5182
T4	2% sodium chloride, 0.2% citric acid and 10 ppm chlorine	4.7356	4.0498
T5	2% sodium chloride, 0.5% potassium sorbate, 0.2% citric acid and 10 ppm chlorine	4.6299	4.0221
T6	0.5% potassium sorbate and 2% sodium chloride	5.1769	5.1531
T7	2% sodium chloride and 10 ppm chlorine	4.3944	4.6862

Treatments which were found effective on whole and headless prawn at ambient temperature were repeated using ice-cold solutions, followed by storage in ice for 5 days. The TPC was significantly reduced from log 7.32 to log 6.49-6.43 in whole prawn samples treated with potassium sorbate and citric acid (T2) and with sodium chloride, citric acid and chlorine (T4). A similar reduction in TPC was also noticed on headless prawn (Fig. 1). Similarly, faecal streptococci counts also reduced considerably in T2 samples.

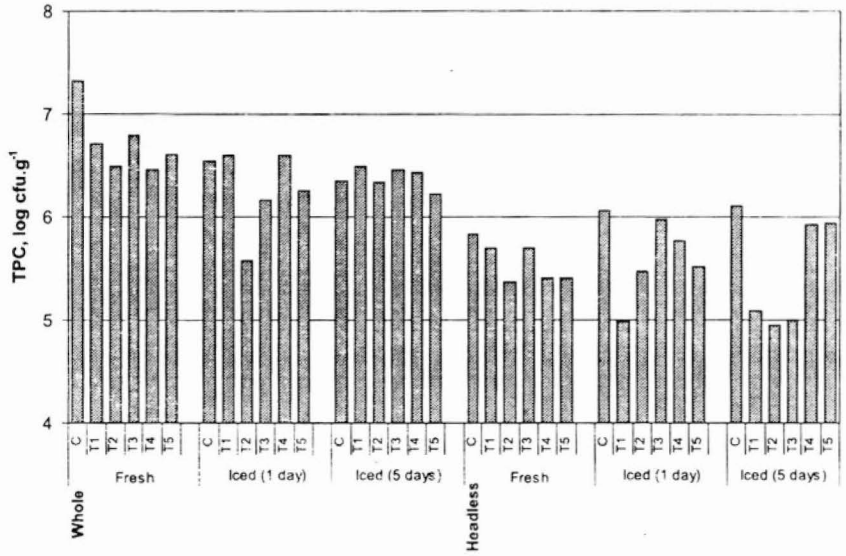

Fig. 1. Mean TPC of whole and headless *M. rosenbergii* treated with preservatives in chilled water and stored for 5 days

A reduction of 1-1.5 log (88-97%) was observed in faecal streptococci counts only on whole and headless prawn treated with potassium sorbate and citric acid (T2) (Fig. 2). Ice-cold treatment solution having a combination of potassium sorbate and citric acid was significantly more effective in suppressing bacterial growth.

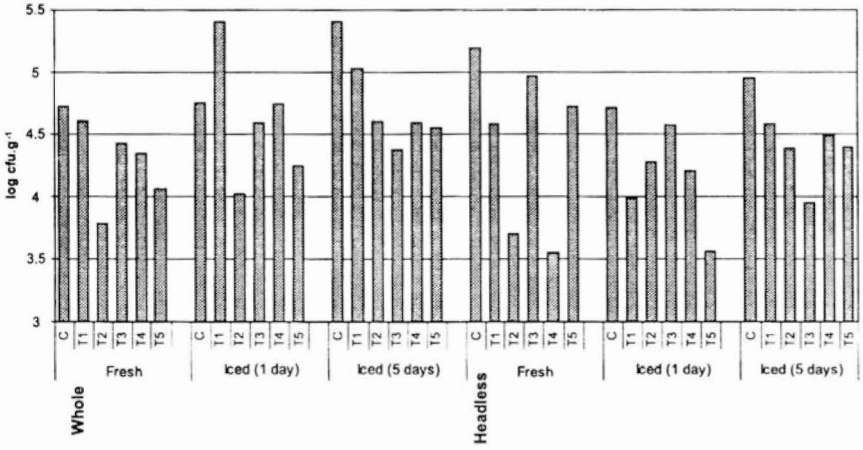

Fig. 2. Mean faecal streptococci counts of whole and headless *M. rosenbergii* treated with preservatives in chilled water and stored for 5 days

The TPC of whole prawn treated with potassium sorbate and citric acid reduced from log 7.32 to log 5.9 cfu.g^{-1} with one day ice storage. Subsequent frozen storage of iced prawn treated with potassium sorbate and citric acid (T2) caused a further reduction in TPC by 89%. Therefore, shelf life of sorbate treated freshwater prawn was longer when they are refrigerated. Similar observations were made earlier by Sofos (1992).

The antimicrobial activity of sorbic acid may also be influenced by the pH and the type of acid naturally present or added to foods during processing. Inorganic acids are generally less effective than acetic and citric acid. In the present study, significant reduction in TPC was noticed in T2 treated samples and it can be attributed to the enhanced antimicrobial activity of sorbate at pH 4.6. The antimicrobial activity of sorbic acid is enhanced as the pH approaches its dissociation constant i.e., pH 4.6 (Sofos, 1992). Al-Dagal & Bazarra (1999) reported extension of microbiological shelf life of whole shrimp by 3 days after treatment with potassium sorbate and bifidobacteria. The inhibitory effect has been attributed to the production of acids by bifidobacteria thereby lowering the pH. A similar additive interaction occurred in the present study when potassium sorbate was combined with citric acid by lowering the pH to 4.6 and the microbial counts were significantly lowered.

In the present study, 92% reduction in TPC was noticed in samples, dipped in 0.5% potassium sorbate and 0.2% citric acid (T_2). Among bacteria, the catalase-positive are inhibited more than the catalase-negative by sorbic acid, the strictly aerobic bacteria the most and both lactic acid bacteria and Clostridia the least (Lück & Jager, 1997). Inhibitory action of sorbic acid on Gram-positive cocci has been reported and the minimum inhibitory concentration reported ranged from 500-1500 ppm (0.05% to 0.15%) for *Micrococcus* (Liewen & Marth, 1985; Sofos, 1992). Significant reduction observed in the streptococci counts of *M. rosenbergii* may be attributed to inhibitory action of sorbic acid.

The results of the present study showed that disinfection of cultured *M. rosenbergii* for processing is essential because of high initial bacterial loads. Potassium sorbate when combined with citric acid exhibited significant potential for reducing the microbial loads on shrimp. A 15 min dip in ice-cold aqueous solution of 0.5% potassium sorbate and 0.2% citric acid was very effective in reducing the TPC, faecal streptococci and *E. coli* of farmed

freshwater scampi to acceptable levels. Removal of head before such dip enhanced the effectiveness of such treatment.

The authors are thankful to the Director, CIFT for the facilities provided and permission to publish this work.

References

Al-Dagal, M.M. and Bazarra, W.A. (1999) *J. Food Protect.* **62**, 51

Davidson, M.P. (1997) in *Food Microbiology: Fundamentals and Frontiers*, (Doyle, M.P., Beuchat, L.R. and Montville, T.J., Eds.), p. 520-556, ASM Press, Washington

FAO (1989) *Food Safety Regulations Applied to Fish by Major Importing Countries*, FAO Fisheries Circular 825, FAO, Rome

Ho, M.L., Cheng, H.H. and Jiang, S.T. (1986) *Nippon Suisan Gakkaishi* **52**, 479

ICMSF (1980) *Factors Affecting Life and Death of Microorganisms*, International Commission on Microbiological Specification of Foods, Academic Press Inc., London

Jiang, S.T. and Lee, T.C. (1988) *Nippon Suisan Gakkaishi* **54**, 1415

Liewan, M.B. and Marth, E.H. (1985) *J. Food Protect.* **48**, 364

Lück, E. (1977) *Chemische Lebensmittelkonservierung-Stoffe, Wirkungen, Methodeen*, Springer-Verlag, New York

Lück, E. and Jager, M. (1997) *Antimicrobial Food Additives: Characteristics, Uses, Effects*, 2nd edn, Springer-Verlag,.

Mendocina, A.F., Molins, R.A., Kraft, A.A. and Walker, H.W. (1989) *J. Food Sci.* **54**, 302

MPEDA (2001) Marine Products Export Development Authority Newsletter **6**, 21

Simonds, C.K. and Lamprecht, E.C. (1985) in *Microbiology of Frozen Foods* (Robinson, R.K., Ed.), p. 169-208, Elsevier Applied Science Publishers Ltd., England

Sofos, J.N. (1992) in *Encyclopedia of Microbiology*, Vol. 4 (Lederberg, J., Ed.), p. 43-52, Academic Press, New York

Sofos, J.N. and Busta, F.F. (1981) *J. Food Protection.* **44**, 614

Sunarya, S. and Iskander, I. (1992) in *FAO Fisheries Report No. 470*, p. 72-76, FAO, Rome

Sunarya, S., Betty, S.L., Winarti, J., Rahayu, P. and Wiguna, W. (1992) *FAO Fisheries Report No. 470*, p. 24-28, FAO, Rome

USFDA (1995) *Bacteriological Analytical Manual*, 8th edn., AOAC International, Gaithersburg, MD 20877, USA

Ward, D.R., Butler, P.F., Hopson, D.J. and Daniels, J.A. (1982) *Shelf life extension of fresh finfish and scallops with potassium sorbate as a function of concentration and method of application*, Paper presented at 42nd Annual Meeting, Inst. of Food Technologists, 22-25 June 1982, Las Vegas

Zhuang, R.Y., Huang, Y.W. and Beuchat, L.R. (1996) *J. Food Sci.* **61**, 241