The Bioscan 12(1): 75-79, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

SURVIVAL AND GROWTH OF *AEROMONAS SPP* IN FISH MINCE AND FILLETS FROM MARINE EMPEROR FISH (*LETHRINUS LENTJAN*) UNDER DIFFERENT STORAGE CONDITIONS

G. RAJESH*, P. VELAYUTHAM, D. SUKUMAR, S. ATHITHAN, S. A. SHANMUGAM AND B. CHRISOLITE
Department of Fish Processing Technology,
Fisheries College and Research Institute, Tamil Nadu Fisheries University, Thoothukudi - 628 008, INDIA
e-mail: rajesh.bfsc@gmail.com

KEYWORDS
Survival
growth of *Aeromonas spp*
Fish mince
fillet

Received on :
08.12.2016

Accepted on :
31.01.2017

***Corresponding author**

ABSTRACT
The pattern of survival and growth of *Aeromonas spp* (*A. hydrophila, A. caviae, A. sobria* together) in fish mince and fillets of Emperor Fish (*Lethrinus lentjan)* under refrigerated storage for a period of 15 days and frozen storage for a period of 4 months were investigated. Aerobic Plate Count and native *Aeromonas* counts in control samples *i.e.* mince/fillet of marine fish increased under refrigerated storage and declined in frozen storage. In the intentionally contaminated mince sample, *Aeromonas* counts increased from 7.44 to 9.36 log cfu/g under refrigerated storage, while APC increased from 8.17 to 10.23 log cfu/g. However, under frozen storage, *Aeromonas* counts reduced to the level of 4.00 log cfu/g in mince from the initial level of 7.30 log cfu/g and correspondingly fillet samples. The result obtained showed that the frozen storage studies were effective to reduce the microbial load of mince and fillets when compare with refrigerated stored sample although small differences were found among fish mince and fillet sample. The temperature storage was the main factor to reduce the microbial growth. *Aeromonas spp* was unable to grow at frozen storage while significant growth at refrigeration.

INTRODUCTION

Aeromonads are gram negative, facultative anaerobic rod shaped motile bacteria (Nordmann and Poirel, 2002) widely distributed in fresh water, estuarine and marine environments (Hazen *et al*., 1979). Motile *Aeromonas spp*. (*A. hydrophila*, *A. sobria* and *A. caviae*) were recognized as causative agents of various infections in humans, the main types are gastroenteritis, wound infections and systemic infection (Khardori and Fainstein 1988; Janda 1991). However most of *Aeromonas spp*. is exist psychrotrophic and can grow at refrigerated temperature in addition studies have also shown that *Aeromonas spp*. are able to survive and multiply at low temperatures in food products such as beef, roasted beef and pork stored between -2 and - 10ºC (Krovacek *et al*., 1992; Mano *et al*., 2000), and can produce toxins even at these low temperatures (Mateos *et al*., 1993; Eley *et al*., 1993). Therefore seafood products are among the ideal substrates for proliferation of *Aeromonas* (Janda, 1991; Pinto *et al*., 2011). The *Aeromonads* implicated in human illnesses include *A. hydrophila*, *A. caviae*, and *A. veronii* (biotype *sobria*) (Janda and Abbot, 1998). Over the past twenty five years, *Aeromonas* have received increasing attention as an emergent agent of foodborne gastrointestinal disease (Pablos *et al*., 2009). The ability of *Aeromonas* to grow at refrigeration temperatures may have great impact on refrigerated stored foods (Daskalov, 2006). Although the number of food-borne outbreaks caused by *Aeromonas sp*. have been quite limited so far (Altwegg *et al*., 1990; Krovacek *et al*., 1991; Mattick and Donovan, 1998). It has been isolated from a variety of fresh foods including vegetables, fish, milk, cheese, ready to eat foods and also from drinking water sources (Sen and Rodgers, 2004; Janda and Abbott, 2010), 61 to 62% *Aeromonas spp* isolated from fish carrying crates (Sumerhassan *et al*., 2011), the presence of *Aeromonas sp*. in the food chain should not be ignored as their presence of in refrigerated and frozen storage could increase the food hazard risk. Das *et al*. (2012) reported the surveillance of *A. sobria* and *A. hydrophila* in commercial food stuffs, similarly (Provincial *et al*., 2013) survival of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in sea bream (*Sparus aurata*) fillets packaged under enriched CO_2 modified atmospheres. Popoff (1984) studied on the stability/survival of *A. hydrophila* to freezing or freeze-drying and found that only 20% of *A. hydrophila* isolates survived freeze-drying in skim milk. Earlier studies have shown that this organism can grow and produce toxins at refrigerated temperature which is of great importance in refrigerated food products that have an extended shelf life at this temperature. Therefore the study was taken up to determine the survival and growth of *Aeromonas spp*. in fish mince and fillets prepared from marine fish under different storage condition.

MATERIALS AND METHODS

Sample collection

Sample of marine emperor fish (*Lethrinus lentjan*) was procured from Thoothukudi fishing harbor and immediately

transferred to the laboratory aseptically for microbiological analysis.

Bacteriological media

Nutrient agar (NA), Aeromonas starch DNA agar base, Ampicillin supplement. These were prepared using either individual ingredients according to standard formulation as suggested in the methods of dehydrated media supplied by M/S. Himedia, Mumbai.

Culture

Standard bacterial cultures such as *Aeromonas hydrophila* (MTCC 1739), *Aeromonas caviae* (MTCC 7725) and *Aeromonas sobria* (MTCC 3613) were procured from Microbial Type Culture Collection, Institute of Microbial Technology (IMTech), Chandigarh and used in the present study.

Preparation of bacterial inoculum

Stock culture of *Aeromonas hydrophila, A. caviae* and *A. sobria* was maintained in nutrient agar slants. Cultures were reactivated by streaking on NA plate with 24 h incubation at 30ºC. Ten milliliter of sterilized NB medium was inoculated with reactivated bacterial cultures individually and incubated at 30ºC/24 h. Cells were pelleted by centrifugation at 5000 rpm/15 min, washed in 10 ml of physiological saline (0.85% Nacl) method followed as per (Brandi *et al*., 1999). The process was repeated twice finally the pellet was suspended in 1 ml of saline, which contained 10^8 cells/ ml of *A. hydrophila, A. caviae* and *A. sobria* together. From this, required volume of inoculum was taken for use.

Preparation, packing and storage of mince and fillet

Fish mince was prepared by using of mechanical deboner and filleted manually with a sharp knife. Samples unit of 10 g of mince and fillet was packed individually in pre-sterilized zip lock cover aseptically using sterile gloves inside the laminar air flow chamber. Mince and fillet 10 set of packs was inoculated with 1 ml of bacterial suspension and stored in a refrigerator (5ºC). A set of each 10 packs were maintained as control. Similarly, in another mince and fillet 10 set of packs were inoculated with bacterial suspension and stored in a freezer (-18ºC) and a set of each 10 packs were maintained as control. Samples were analysed from stored samples of refrigerated condition were drawn for microbiological analysis at the interval of 5 days and the samples from frozen storage were tested in 30 days interval is done as per (Provincial *et al*., 2013).

Microbiological analysis

Whole 10 g of sample in the pack was transferred to sterile pestle and mortar and homogenized with 90 ml physiological saline (0.85%). Serially diluted sample was plated for aerobic plate count and *Aeromonas* count by spread plate technique. Aerobic plate count was estimated by spread plate methods using NA medium. Plates were incubated for 24 h/30ºC and the counts were taken and the results expressed as the colony forming units /gram (cfu/g). *Aeromonas* count was analyzed by spread plating technique on *Aeromonas* starch DNA agar with ampicillin supplement. The plates were incubated at 30ºC/24 h. Counts were taken and the results were expressed as the colony forming units /gram (cfu/g). The methods followed as per APHA (1988).

Statistical analysis

Results of the analyses were statistically processed using standard deviation (Microsoft Office EXCEL 2012).

RESULTS

Microbial changes in fish mince and fillet control sample under refrigerated and frozen storage condition

Quantitative changes of APC and native *Aeromonas* count in marine fish mince and fillet under refrigerated storage made each sampling days shows (Table 1). Aerobic plate count was increased from 4.14 to 6.41 log cfu/g in fish mince and fillet sample increased from 3.82 to 6.27 log cfu/g during refrigerated storage. Native *Aeromonas* count in fish mince and fillet increased from 4.23 to 6.38 and 3.93 to 6.20 log cfu/g respectively. During storage period it was found that both of APC and native *Aeromonas* count in fish mince and fillets

Table 1: Quantitative changes of APC and native *Aeromonas* count in marine fish mince and fillet control samples under refrigerated storage condition (log cfu/g)

Storage period(Days)	Fish Mince APC	*Aeromonas*	Fish Fillet APC	*Aeromonas*
0	4.14±0.04	4.23±0.08	3.82±0.12	3.93±0.13
1	4.07±0.02	4.17±0.09	3.71±0.09	3.84±0.09
5	5.32±0.30	5.14±0.09	5.44±0.14	4.68±0.18
10	6.20±0.19	5.20±0.15	6.17±0.17	5.11±0.01
15	6.41±0.30	6.38±0.20	6.27±0.07	6.20±0.05

Table 2: Quantitative changes of APC and native *Aeromonas* count in marine fish mince and fillets control samples under frozen storage condition (log cfu/g)

Storage period (Months)	Fish Mince APC	*Aeromonas*	Fish Fillet APC	*Aeromonas*
0	4.43±0.21	4.38±0.30	3.34±0.14	4.17±0.07
1	4.97±0.07	4.27±0.17	3.25±0.25	4.00±0.20
2	3.90±0.10	3.17±0.10	3.32±0.12	4.34±0.04
3	3.27±0.07	3.97±0.17	3.23±0.13	3.41±0.21
4	3.23±0.08	3.04±0.04	2.14±0.04	3.25±0.25

Table 3: Survival and growth of APC and *Aeromonas* count in intentionally contaminated fish mince and fillets samples under refrigerated storage condition (log cfu/g)

Storage period(Days)	Fish Mince APC	*Aeromonas*	Fish Fillet APC	*Aeromonas*
1	8.17±0.10	7.44±0.14	7.25±0.25	7.43±0.31
5	8.27±0.12	7.99±0.14	8.27±0.07	7.00±0.20
10	9.39±0.09	8.07±0.07	9.44±0.43	7.14±0.10
15	10.23±0.13	9.36±0.06	9.98±0.08	8.46±0.26

Table 4: Survival and growth of APC and *Aeromonas* count in intentionally contaminated fish mince and fillets samples under frozen storage condition (log cfu/g)

Storage period (Months)	Fish Mince APC	*Aeromonas*	Fish Fillet APC	*Aeromonas*
0	7.41±0.11	7.30±0.10	7.39±0.34	7.41±0.21
1	7.23±0.03	6.17±0.07	6.25±0.25	6.39±0.30
2	6.41±0.20	6.04±0.04	6.17±0.17	5.34±0.28
3	5.1±0.10	5.00±0.05	6.97±0.10	5.20±0.05
4	5.95±0.15	4.00±0.10	5.25±0.05	4.92±0.02

gradually increased border line of spoilage.

Quantitative changes of APC and native *Aeromonas* count in fish mince and fillets control samples under frozen storage condition result shown in table 2. Initially, APC count was 4.43 log cfu/g in marine fish mince and 3.34 log cfu/g in marine fish fillet. During frozen storage, there was about 1 log cfu/g reduction in both samples. Native *Aeromonas* count slightly decreased from 4.38 to 3.04 log cfu/g in marine fish mince and from 4.17 to 3.25 log cfu/g in marine fish fillet. In frozen storage condition microbial count was decreased during the storage period of 4 month.

Survival and growth of APC and *Aeromonas* count in intentionally contaminated samples under refrigerated storage condition.

Survival and growth of APC and *Aeromonas* count in intentionally contaminated fish mince and fillets samples under refrigerated storage condition are presented in (Table 3) After inoculation, APC increased from 8.17 to 10.23 log cfu/g in marine fish mince and from 7.25 to 9.98 log cfu/g in marine fish fillet. Over the storage period APC increased by about 2.06 log cfu/g in marine fish mince and 2.73 log cfu/g in marine fish fillet . *Aeromonas* count was 7.44 log cfu/g in marine fish mince and 7.43 log cfu/g in marine fish fillet after inoculation of *Aeromonas spp*. Upon storage, there was about 1.92 log cfu/g increase in *Aeromonas* count in fish mince samples and 1.03log cfu/g increased in marine fish fillet. Overall refrigerated storage period there was not much growth occurred at the same time not declined. Survived the both microbes in low temperature storage not grow well.

Survival and growth of APC and *Aeromonas* count in intentionally contaminated samples under frozen storage condition.

Survival and growth of APC and *Aeromonas* count in intentionally contaminated fish mince and fillets samples under frozen storage condition are given (Table 4). After inoculation of *Aeromonas spp* in fish mince and fillet the APC count was 7.41log cfu/g in fish mince and 7.39 log cfu/g in fish fillet initially. It was decreased slowly both the sample 2 log cfu/g during storage period of four months. *Aeromonas* count was decreased from 7.30 to 4.00 log cfu/g in fish mince and in fish fillet decreased from 7.41 to 4.92 log cfu/g throughout storage period.

DISCUSSION

Microbial changes in fish mince and fillet control sample under refrigerated and frozen storage condition

Microbial growth in fresh seafood is the main factor associated with quality deterioration, spoilage and economic loss (Zhuang *et al*., 1996). Aerobic plate count was increased from 4.14 to 6.41 log cfu/g in fish mince and 3.82 to 6.27 log cfu/g in fish fillet during refrigerated storage. Present study results are in accordance with earlier reports (Garg and Stephen, 1982; Reddy and Srikar, 1991; Ali and Karunasagar, 1992; Du *et al*., 2001) which is revealed that the total bacterial counts raised by 2 log cfu/g in tuna, carp, kati and pink perch stored in ice. These results conformed to the results of Shalini *et al*. (2001) that the total plate count increased by 3 log units in *Lethrinus lentjan* fillets stored under refrigeration temperature (4ºC) in 9 days. *Aeromonas* count was 4.23 log cfu/g in marine fish mince initially. Neyts *et al*. (2000) who reported that number of *Aeromonas* organisms in food products varies from $< 10^2$ to 10^5 cfu/g, which is also confirmed by Palumbo *et al*. (1987). Upon storage period of 15 days there was about 2 log cfu/g raise in marine fish mince. Palumbo *et al*. (1985) who observed the *Aeromonas* counts raised by 1-3 log cfu/g over 7 days of storage at 5ºC. Native *Aeromonas* count in fish fillet increased from 3.93 to 6.20 log cfu/g respectively. Boari *et al*. (2008) reported *Aeromonas* count was 2 log cfu/g in fresh tilapia fillet.

On contrary to the refrigerated storage, APC counts declined by about 1 log unit both in fish mince and fillet kept under frozen storage from the initial level of 4.43 log cfu/g. These observations are in agree with the findings of Agrawal *et al*. (2013) who observed that there was a continuous reduction in APC from 4.5×10^5 to 8.3×10^4 cfu/g in *Nandus nandus*, 4×10^5 to 6.6×10^3 in *Channa marulius* and 3.3×10^5 to 3×10^4 cfu/g in *Puntius sarana*, which were stored at -20ºC for a period of 180 days of storage. Native *Aeromonas* count slightly

decreased from 4.38 to 3.04 log cfu/g in marine fish mince and from 4.17to 3.25 log cfu/g in marine fish fillet. This initial count of *Aeromonas* result was similar to that by (Ali, 2011). However, pseudomonas /*Aeromonas* count of 2.75 log cfu/g in frozen shrimp had been reported by (Ali, 2011). In frozen oyster, Liobrera *et al*. (1986) recorded 5.3 – 33.3% *Aeromonas* group among the total bacterial population. Ali (2011) observed the total psychrotrophic count of 4.19 log cfu/g and Pseudomonas/*Aeromonas* count of 3.65 log cfu/g in fresh fish fillet. This result was similar to the present study.

Survival and growth of *Aeromonas spp* in intentionally contaminated samples under refrigerated storage condition.

Aeromonas spp are able to survive and multiply at low temperature in a variety of food products stored between -2ºC and -10ºC such as beef, roast beef and pork (Krovacek *et al*., 1991; Mano *et al*., 2000). *Aeromonas* count was 7.44 log cfu/g in marine fish mince and 7.43 log cfu/g in marine fish fillet after inoculation of *Aeromonas spp*. Upon storage, there was about 1.92 log cfu/g increase *Aeromonas* count in mince and 1.03 log cfu/g fillet. In the present study, the observation on survival and multiplication of *Aeromonas* organisms 1-2 log cfu/g raise in both the samples at refrigerated condition are in accordance with Herrera *et al*. (2006) reported that *Aeromonas* count ranged between 2.29 and 7.20 log cfu/g in marine fish fillet and streak. Conclusively, the inoculated *Aeromonas* along with native *Aeromonas* population not only survived but also grew well on storage at 5ºC with an increase of 2 log cfu/g approximately.

Survival and growth of *Aeromonas spp* in intentionally contaminated samples under frozen storage condition.

Aeromonas count was decreased from 7.30 to 4.00 log cfu/g in mince and fillet decreased from 7.41 to 4.92 log cfu/g throughout storage period. This reduction in counts is obviously due to the cold storage temperature of -18ºC which created an unfavorable condition for further growth of microorganisms as reported by Obemeata *et al*. (2011). Altwegg *et al*. (1990) reported that psychrophilic strains having an optimum growth temperature of 15 to 20ºC, may grow at temperatures as low as 0 to - 5ºC. Published reports are not much available on the survival of *Aeromonas spp* in fish mince and fillets under frozen storage condition. However, Das *et al*. (2012) have recorded the prevalence of *A. sobria* and *A. hydrophila* in commercial food stuffs including frozen fish, freshwater fish and fish pickle *etc*.

ACKNOWLEDGEMENT

The authors are thankful to Tamil Nadu Fisheries University for encouragement towards research.

REFERENCES

Agrawal, R., Das, M., Upadhyay, A. K. and Danish, M. 2013. Estimation of microbial flora in mincemeat collected from *Nandus nandus*, *Channa marulius* and *Puntius sarana* during six months storage at -20ºC. *Afr. J. Microbiol. Res*. **7(28):** 3704-3710.

Ali, A. and Karunasagar, I. 1992. Bacteriological changes during iced storage of the tropical freshwater carp, *Labeo rohita*. *Fish. Res*. **13:** 189–197.

Ali, F. H. M. 2011. Quality evaluation of some fresh and imported frozen seafood. *Adv. J. Food Sci. Technol*. **3(1):** 83-88.

Altwegg, M., Steiger walt, A. G., Altwegg- Bissig, R., Luthy-Hottnstein, J. and Brenner, D. J. 1990. Biochemical identification of *Aeromonas* genospecies isolated from humans. *J. Clin. Microbial*. **28:** 258-264.

APHA. 1988. Standard Methods for the Examination of Water and Waste Water. 20th Edition. (Clesceri, L.S., Greenberg,A.E. and Eaton, A.D.). *American Public Health association, Washington,* DC.

Boari, C. A., Pereira, G. I., Valeriano, C., Silva, B. C., Morais, V. M., Figueiredo, H. C. and Piccoli, R. H. 2008. Bacterial ecology of tilapia fresh fillets and some factors that can influence their microbial quality. *Cienc. Tecnol. Aliment., Campinas*. **28 (4):** 863-867.

Brandi, G., Sisti, M., Giardini, F., Schiavano, G. F. and Albano, A. 1999. Survival ability of cytotoxic strains of motile *Aeromonas spp*. in different types of water. *Lett. Applied Microbiol*. **29:** 211-215.

Das, A., Vinayasree, V., Santhosh, C. R. and Sree Hari, S. 2012. Surveillance of *Aeromonas sobria* and *Aeromonas hydrophila* from commercial food stuffs and environmental sources. *J. Experimental Sciences*. **3(9):** 36-42.

Daskalov, H. 2006. The importance of *Aeromonas hydrophila* in food safety. *Food Control*. **17:** 474-483.

Du, W. X., Kim, J., Cornell, J. A., Huang, T. S., Marshall, M. R. and Wei, C. I. 2001. Microbiological, sensory and electronic nose evaluation of yellow fin tuna under various conditions. *J. Food Prot*. **64:** 2027-2036.

Eley, A., Geary, I. and Wilcox, M. H. 1993. Growth of *Aeromonas* species at 4ºC and related toxin production. *Lett. Applied Microbiol*. **16:** 36-39.

Garg, D. K. and Stephen, J. 1982. Ice storage studies of kati (*Pellona sp*). *Fish. Technol*. **19:** 45-47.

Hazen, T. C. 1979. Ecology of *Aeromonas hydrophila* in a South Carolina cooling reservoir. *Microbial Ecology*. **5:** 179-195.

Herrera, F. C., Santosotero, J. A. and Garcia-Lopez, M. L. 2006. Occurrence of foodborne pathogenic bacteria in retail prepackaged portions of marine fish in Spain. *J. Appl. Microbiol*. **100:** 527-536.

Janda, J. M. and Abbott, S. L. 2010. The genus *Aeromonas* : taxonomy, pathogenicity and infection. *Clin. Microbiol. Rev*. **23(1):** 35-73.

Janda, J. M. and S. L. Abbott. 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. *Clin. Infect. Dis*. **27:** 332-344.

Janda, J. M. 1991. Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with the genus *Aeromonas*. *Clinical Microbiology Review*. **4:** 397-410.

Khardori, N. and Fainstein, V. 1988. *Aeromonas* and *Plesiomonas* as etiological agents. *Annual Review of Microbiology*. **42:** 395-419.

Krovacek, K., Faris, A. and Mansson, I. 1991. Growth and toxin production by *Aeromonas hydrophila* and *Aeromonas sobria* at low temperatures. *Int. J. Food Microbiol*. **13:**165-175.

Liobrera, A. T., Bulalcao, M. L. and Sunas, N. 1986. Effect of storage on the microbial quality of slipper oysters, *Crassostrea iredalei*. **In:** Maclean, J.L, Dizon, L.B, Hosillos, L.V (Eds). Proceedings of the first Asian fisheries forum, Asian fisheries society, Manila, Philippines, pp. 437-442.

Mano, S., Ordonez, J. A. and Fernando, G. D. 2000. Growth/survival of natural flora and *Aeromonas hydrophila* on refrigerated uncooked pork and turkey packaged in modified atmospheres. *Food Microbiol*. **17:** 657-669.

Mateos, D., Anguita, J., Naharro, G. and Paniagua, C. 1993. Influence of growth temperature on the production of extracellular virulence

factors temperature on the production of extracellular virulence factors and pathogenicity of environmental and human strains of *Aeromonas hydrophila*. *J. Appl. Bacteriol*. **74:** 111-118.

Mattick, K. L. and Donovan, T. J. 1998. The risk to public health of *Aeromonas* in ready to eat salad products. *Commun. Dis. Public Health*. **1:** 263-266.

Neyts, K., Huys, G., Uyttendaele, M., Swings, J. and Debevere, J. 2000. Incidence and identification of mesophilic *Aeromonas spp*. from retail foods. *Lett. Applied Microbiol*. **31:** 359-363.

Nordmann, P. and Poirel, L. 2002. Emerging carbapenemases in Gram- negative aerobes. *Clinical Microbiology and Infection*. **8:** 321-331.

Obemeata, O., Nnenna, F. P. and Christopher, N. 2011. Microbiological assessment of stored *Tilapia guidneesis*. *Afric. J. Food Sci*. **5(4):** 242-247.

Pablos, M., Rodrígez-Calleja, J. M., Santos, J. A., Otero, A. and García-López, M. L. 2009. Occurrence of motile *Aeromonas* in municipal drinking water and distribution of genes encoding virulence factors. *Int. J. Food Microbiol*. **135:**158-164.

Palumbo, S. A., Maxino, F., Williams, A. C., Buchanan, R. L. and Thayer, D. T. W. 1985. Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol*. **50:** 1027-1030.

Palumbo, S. A., Williams, A. C., Buchanan, R. L. and Phillips, J. G. 1987. Thermal resistance of *Aeromonas hydrophila*. *J. Food Prot*. **50:** 761-764.

Pinto, A. D., Terio, V., Pinto, P. D. and Tantillo, G. 2011. Detection of potentially pathogenic *Aeromonas* isolates from ready-to-eat seafood products by PCR analysis. *Int. J. Food sci. Technol*. **10:** 1365-2621.

Popoff, M. 1984. Genus III *Aeromonas* Kluyver and Van Niel. In *Bergey's Manual of Systematic Bacteriology*,. Williams and Wilkins, Baltimore, MD. **1:** 545-548.

Provincial, L., Guillen, L., Alonso, V., Gil, M., Roncales, P. and Beltran, J. A. 2013. Survival of *Vibrio parahaemolyticus* and *Aeromonas hydrophila* in sea bream (*Sparus aurata*) fillets packaged under enriched CO2 modified atmospheres. *Int. J. Food Microbiol*. **166:**141-147.

Reddy, G. V. and Srikar, L. 1991. Storage of pink perch (*Nemipterus japonicus*) in ice: chemical, microbiological and sensory assessment. *Indian J. Fish*. **38:** 55-59.

Sen, M. and Rodgers, M. 2004. Distribution of six virulence factors in Aeromonas species isolated from US drinking water utilities: a PCR identification. *J. Appl. Microbiol*. **97:** 1077-1086.

Shalini, R., Indra Jasmine, G., Shanmugam, S. A. and Ramkumar, K. 2001. Effect of potassium sorbate dip- treatment in vacuum packaged *Lethrinus lentjan* fillets under refrigerated storage. *J. Food sci. Technol*. **38(1):** 12-16.

Sumer Hassan, Qureshi, T. A., Bilal Ahmad and Susan Manohar. 2011. Microflora of ice in fish carrying crates from certain fish markets of Bhopal. The Bioscan. **5(1and2):** 81-83.

Zhuang, R. Y., Huang, Y. W. and Beuchat, L. R. 1996. Quality changes during refrigerated storage of packaged shrimp and catfish fillets treated with sodium acetate, sodium lactate or propyl gallate. *J Food Sci*. **61:** 241-244.