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Post-harvest Bacterial Quality of *Lethrinus lentjan* (Lacepede, 1802)

Femeena Hassan[1]*, Treesa Gracious[1], V. Geethalakshmi[1], S. Sanjeev[1,2]
Central Institute of Fisheries Technology, P. O. Mastyapuri, Cochin - 682 029, India

Abstract

Food safety and microbiological quality particularly in high moisture foods like seafood have gained significant attention among consumers. Compromise with regard to quality standards for the retail trade of fish and fish products is decreasing the quality of fish. Present study was conducted to detect the bacterial progression fish from harbour to retail shop and market. *Lethrinus lentjan* (white snapper) was collected from three different strategic locations, *viz.,* harbour, retail shop and market on the same day and quantitative and qualitative bacteriological analyses were carried out. Samples collected from market exhibited 12.61% more mesophilic count than that of harbour samples and 9.5% more than retail samples. The increasing trend in bacterial counts was noticed in psychrophiles, enterobacteriaceae, faecal streptococci, hydrogen sulphide producers, *Brochothrix thermosphacta* and histamine producing bacteria. Pathogens like Salmonella, *Vibrio cholerae, V. parahaemolyticus* and *Listeria monocytogenes* were absent in the analysed samples.

Keywords: *Lethrinus lentjan*, microbial quality, harbour, retail market

Introduction

Fish is a source of easily digestible proteins and contains many nutrients and is regarded as a healthy food. It is a highly perishable food item and has to be handled, stored and marketed with extreme care in minimum possible time. Proper hygiene measures have to be implemented in fish handling areas for prevention of contamination and loss of quality of fish. Coastal water bodies and estuaries are the major sources of seafood in India and are often contaminated by anthropogenic activities of adjoining population and seafood harvested from such areas often contain pathogenic microorganisms. In addition, poor sanitation in landing centers and open fish markets exacerbates the situation (Kumar, 2001). Major factors contributing to the poor quality of fish in retail trade is unhygienic handling and storage leading to off-smell, physical damage, building up of bacterial load and contamination with dirt and objectionable microorganisms (Sugumar et al., 2004). It has been reported that quality of fish sold in domestic market in India is poor compared to that of export trade and are mostly contaminated with pathogenic microorganisms (Nambiar & Iyer, 1990). Even though epidemiological evidence on outbreak of food borne diseases are scarce, there are indications that food could be contaminated to unsafe levels at the points of consumption with flora from handlers, equipments/ utensils and raw food materials (Edema et al., 2008). Food borne illness can be avoided to a great extent and safety can be ensured by effective hygiene control through bacteriological testing.

Usually fish is landed in harbour from where it is distributed and marketed in retail shops and other markets. There is very limited data available on the quality of fishes from various locations. Hence the present study was undertaken with the aim to assess the microbial quality of an economically important fish *L. lentjan* (white snapper) collected from three different strategic locations in Kochi *viz.,* harbour, retail shops and market to understand the quality of fish and bacterial progression in fish as a consequence of holding time, unhygienic practices and improper storage.

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* *E-mail: femeenahassan@rediffmail.com*

[2] *Present Address: Plot No. 143, Karthika, Kumarasan Nagar, P. O. Kadavanthra, Cochin - 682 020*

Materials and Methods

Fish was collected at weekly intervals for one month at random from Cochin fishing harbour, retail shops and markets (n=5). The route of fish distribution was traced and the fish, from the same catch was collected on the same day from retail shop and market in Cochin, located within a radius of 8 km. Fish samples were iced in 1:1 proportion and brought to the laboratory under aseptic conditions within an hour.

Fifty grams of fish flesh was aseptically transferred to a stomacher bag (Seward, London, UK), 450 ml of sterile Butterfield's Phosphate buffer was added and blended for 2 min in a stomacher (Lab blender 400, Seward, Medical, London, UK) to obtain the original homogenate fluid (10^{-1} dilution).

Mesophiles, psychrophiles, Pseudomonas, Brochothrix, hydrogen sulphide producing bacteria, *Staphylococcus aureus*, histamine forming colonies, *Escherichia coli*, Enterobacteriaceae and faecal Streptococci were analyzed quantitatively while qualitative analysis was performed for *Salmonella* spp., *V. cholerae, V. parahaemolyticus* and *L. monocytogenes.*

Plate Count Agar (Difco 247940) was used for the enumeration of mesophiles and psychrophiles according to the American Public Health Association (APHA, 2002). Inoculated plate count agar plates were incubated for 48±2 h at 35°C for aerobic plate count (USFDA, 2001) and for 7 days at 5°C for psychrophilic count (APHA, 1992).

Tergitol anionic 7 agar, T_7 (Himedia M616) supplemented with triphenyl tetrazolium chloride was used for *E. coli* isolation (IS-5887 part-1), 1976). Presumptive *E. coli* colonies were confirmed by IMViC tests. Violet red bile glucose agar media (Difco 218661) was used for enterobacteriaceae (Mossel et al., 1979) enumeration. All the plates were examined for typical colonies after 24 ± 2 h at 35°C incubation.

The selective medium used for *S. aureus* was Baird Parker Agar (Difco 276840) with potassium tellurite-egg yolk supplement and for feacal Streptococcus, Kenner Feacal agar (Difco 249610) was used. After 48 h of incubation at 35°C inoculated plates were examined for presumptive colonies of *S. aureus*, USFDA, 2001 and Streptococcus (Kenner et al., 1961).

Streptomycin-thallus-acetate–actidione agar (Oxoid CM0881) supplemented with STA supplement was used for the isolation and quantitative enumeration of *Brochothrix thermosphacta* (Gardner, 1966). Hydrogen sulphide producers were cultured on peptone iron agar (Gram et al., 1987). White or semi-transparent convex colonies on STAA plates and black colonies formed on Peptone iron agar (Himedia M440) plates by the production of H_2S were enumerated after 5 days of incubation at 20°C.

To isolate histamine forming bacteria, differential agar fortified with L-histidine was used (Niven et al., 1981). The inoculated differential agar plates were incubated for 36-72 h at 35°C, and histidine decarboxylating colonies that appear in violet colour were enumerated after the period of incubation. Pseudomonas colonies were enumerated from Kings B agar Media (Himedia M1544) after 2 days of incubation at 20°C (King et al., 1954).

Microbial data were transformed into logarithms of the number of colony forming units/gram. Averages and standard deviations of the transformed values were then estimated, to take the variability in bacterial cell counts into consideration. All plates were examined for typical colony characteristics and morphology associated with each growth medium.

Samples were also analyzed for detecting *V. cholerae, V. parahaemolyticus*, *Salmonella* spp. and *Listeria* spp. (USFDA, 2001).

The data on microbiological parameters was subjected to statistical analysis like correlation, ANOVA and Liner Discriminant analysis using SAS (ver.9.3).

Results and Discussion

The progressive trend of microbiological growth in white snapper collected from different strategic locations are shown in Table 1.

Total microbial count is an important criterion for quality evaluation in fresh and frozen seafood products. The maximum acceptable microbial limit in fresh and frozen fish is log 10^5 cfu g^{-1} (FSSAI, 2011). Heterotropic bacterial load of about log 10^6-10^7 cfu g^{-1} was reported in fish in retail trade at Cochin (Nambiar & Iyer, 1990) and a load of up to 10^8 from fishes marketed in Karachi (Shamshad et al., 1992). In the present study, among the samples examined from different strategic

Table 1. Microbial load (Mean±SD) in white snapper from different strategic locations

Parameters	Sources		
	Harbour	Retail shop	Market
APC (log_{10} cfu g^{-1})	$5.89^a \pm 0.0416$	$6.1^b \pm 0.03$	$6.74^c \pm 0.05$
Psychrophiles (log_{10} cfu g^{-1})	$4.94^a \pm 0.04$	$5.36^b \pm 0.06$	$5.54^c \pm 0.09$
S. aureus (cfu g^{-1})	<10	<10	<10
Enterobacteriaceae (log_{10} cfu g^{-1})	$3.18^a \pm 0.17$	$3.94^b \pm 0.03$	$4.21^c \pm 0.03$
E. coli (cfu g^{-1})	<10	<10	10
Histamine forming bacteria (log_{10} cfu g^{-1})	$3.47^a \pm 0.061$	$3.79^b \pm 0.06$	$4.3^c \pm 0.01$
Pseudomonas (log_{10} cfu g^{-1})	$4.67^a \pm 0.03$	$5.46^b \pm 0.025$	$5.46^b \pm 0.08$
Brochothrix thermosphacta (log_{10} cfu g^{-1})	$3.56^a \pm 0.055$	$3.89^b \pm 0.015$	$4.52^c \pm 0.075$
Faecal Streptococci (log_{10} cfu g^{-1})	$2.77^a \pm 0.04$	$2.86^a \pm 0.041$	$3.46^b \pm 0.07$
H_2S producers (log_{10} cfu g^{-1})	$3.46^a \pm 0.07$	$3.5^a \pm 0.055$	$3.7^b \pm 0.04$

(n=5). Means within and between the column with different superscripts are significantly different at 5% level of significance. Numbers within parenthesis indicate standard deviation.

locations, market samples exhibited higher aerobic plate count (6.74 log_{10} cfu g^{-1}) and least in harbour samples (5.89 log_{10} cfu g^{-1}). Retail samples showed 9.5% lower counts from that of market samples. The flesh of newly caught fish is sterile since the immune system of the fish prevents the bacteria from growing in the flesh, but when the fish dies, the immune system collapses and consequently during storage, bacteria invade the flesh. So harbour samples exhibited relatively lower counts than the samples from other two locations as it was collected immediately after landing and aerobic plate count showed a significant increase ($p<0.05$) with three different locations.

Psychrophilic bacteria are the major group of micro-organisms responsible for spoilage of fresh sea foods (Adams et al., 1964). Market samples gave a count of 5.55 log_{10} cfu g^{-1} for psychrophilic bacteria which is 11.2% more than that given by harbour samples. Harbour to retail samples also exhibited an increase in count by 7.85%. Retail to market samples also exhibited an increase in psychrophilic count but the variation was comparatively less (3.6%). The psychrophilic bacteria exhibits proteolytic activity (Singh et al., 1993) and have the ability to produce fat splitting enzyme lipases, which give rises to the quality changes in the chilled stored food (Gobbett et al., 1996).

The presence of Enterobacteriaceae in fish and their spoilage potential are important when fish is collected from polluted water or if there is a delay in chilling after catch (Bahmani, 2011). Most members of Enterobacteriaceae, can reduce TMAO to TMA (Barrett & Kwan, 1985) leading to spoilage. Harbour samples gave a count of 3.18 log_{10} cfu g^{-1} but when it reached market the Enterobacteriaceae population increased by more than 1 log_{10} cfu g^{-1}. Retail and market samples reported slight variation of 6.4% while retail samples exhibited an increase of 19.3% in Enterobacteriaceae counts compared to samples from the harbour. The rise in microbial counts may be due to improper icing which favors the growth of micro flora on the fish surface or through unhygienic handling practices.

According to Thampuran et al., (2005), *E. coli* is commonly associated with seafood contamination in the tropics, where it is encountered in high number. *E. coli* occurrence in seafood is considered as a sanitary case and may represent a risk to the consumers if the strains are to pathogenic, especially diarrheagenic *E. coli*. However, the presence of *E. coli* in fish and shellfish is important from public health aspect, since this bacterium is recognized as an indicator of faecal contamination, possibly indicating the presence of other enteric pathogens (Renata, 2013). Present study showed *E. coli* <10 cfu g^{-1} in harbour and retail samples but market samples reported positive results (10 cfu g^{-1}). Though the acceptable limit for *E. coli* in raw fish is 20 cfu g^{-1} (FSSAI, 2011), presence is an indication of post-harvest contamination and it could be due to poor

quality of water and ice, unhygienic handling or contaminated fish contact surfaces. Rao et al., 2006 have reported that *E. coli* and coagulase positive Staphylococci were present in 45 and 30% of samples respectively from Visakhapatnam harbour.

Faecal Streptococci are non-pathogenic organisms but commonly occur in the intestines of man and other warm-blooded animals which make them a useful group of indicator of faecal contamination (Lin, 1974). Faecal Streptococci population also showed an increase by 19.9% in market samples than that of harbour samples. Retail and market samples gave a variation of 17.74%. However, harbour and retail samples showed no significant variation ($p>0.05$).

Spoilage is more often a result of the production of off-odors and flavors caused by specific spoilage organisms, which are only a fraction of the total microflora (Huss et al., 1974). H_2S producing bacteria have been reported as the specific spoilage bacteria in fish from tropical waters and fresh fish stored aerobically (Koutsoumanis & Nychas, 1999). The microbes that are able to produce sulphide and cysteine from thiosulphate i.e., *Shewanella putrefaciens* as well as sulphide-producing *Vibrio, Aeromonas* and *Enterobacterieaceae* spp., appear as black colonies after 24–48 h of incubation due to precipitation of iron sulphide (Gram et al., 1987). The detection reaction is thus directly related to the spoilage property of bacteria (Olaug et al., 2004). H_2S producing bacterial count was found to be lower than *Pseudomonas* spp., *Brochothrix themosphacta* and histamine forming bacteria. H_2S producing bacterial count in fish samples showed a progressive trend from harbour to market samples but no significant variation was noticed. Harbour samples exhibited 6.5% variation in counts from that of market samples.

With regard to *B. thermosphacta*, a bacterium more common in meat products, counts were significantly more ($p<0.05$) in fish collected from market compared to the other two samples. Fish samples showed an increasing trend in *B. thermosphacta* count, of about 21.23% from harbour to market.

Pseudomonas were found to be a dominant flora in the present study. Liston (1980) reported *Pseudomonas* is able to utilize a variety of compounds including NPN in the fish muscle quickly and efficiently as one of the characteristics to ensure dominance. *Pseudomonas* counts of harbour sample were found to be 14.5% lower than that of retail and market samples (5.46 $\log_{10}$ cfu g^{-1}).

Histamine formation in raw fish is primarily by Gram negative enteric bacteria (Lopez sabater et al., 1994; Lopez sabater et al., 1996; Gingerich et al., 1999; Kim et al., 2001). *Clostridium perfringens, Morganella morganii, Hafnia alvei, Rauoltella planticola, M. psychrotolerans* and *Photobacterium phosphoreum* were found to be the bacteria associated with histamine formation in raw fish. Histamine forming bacterial count of harbour sample was 3.47 $\log_{10}$ cfu g^{-1}, which showed an increasing trend of 8.44% when it reached retail shops. Retail samples gave a count of 3.79 $\log_{10}$ cfu g^{-1}, which is 11.86% more than that reported by harbour samples. Significant increase ($p<0.05$) in histamine forming bacterial count was noted from harbour to market sample (19.3%). Manju (2005) reported *S. aureus* count of 1.2 $\log_{10}$ cfu g^{-1} in fresh pearl spot. In the present study *S. aureus* was found to be <10 cfu g^{-1}. The results were similar to that of the results of Santiago et al. (2007), in Coho salmon. Pathogens like *Salmonella, V. cholerae, V. parahaemolyticus and L. monocytogenes* were not detected in the collected samples from three locations in the study.

High positive correlation was found between the microbiological parameters *viz.,* mesophiles, psychrophiles, faecal streptococci, *Pseudomonas,* Enterobacteriaceae, hydrogen sulphide producers, histamine forming bacteria and *Brochothrix thermosphacta*. The microbial activities with the resultant accumulation of microbial metabolites play the crucial role in the spoilage process (Burt & Hardy, 1992; Clancy et al., 1995). Linear Discriminant Function analysis of the data showed clear cut distinction between the three sources of fish samples *viz.,* harbour, retail shop and market with respect to the contamination due to the microbiological parameters studied (Fig. 1).

Microbial quality is a major factor determining the shelf life of a product. In all the cases, highest counts were reported from market samples and the least from harbour samples. The poor microbial quality of the fishes in the harbour could be due to the lack of Good handling process practices (GHP) on-board and in the harbour. The condition of fish market was not good with open drainages, filthy, slimy floors, fish waste and foul smell. There was no cleaning schedule in the market. Icing was also improper

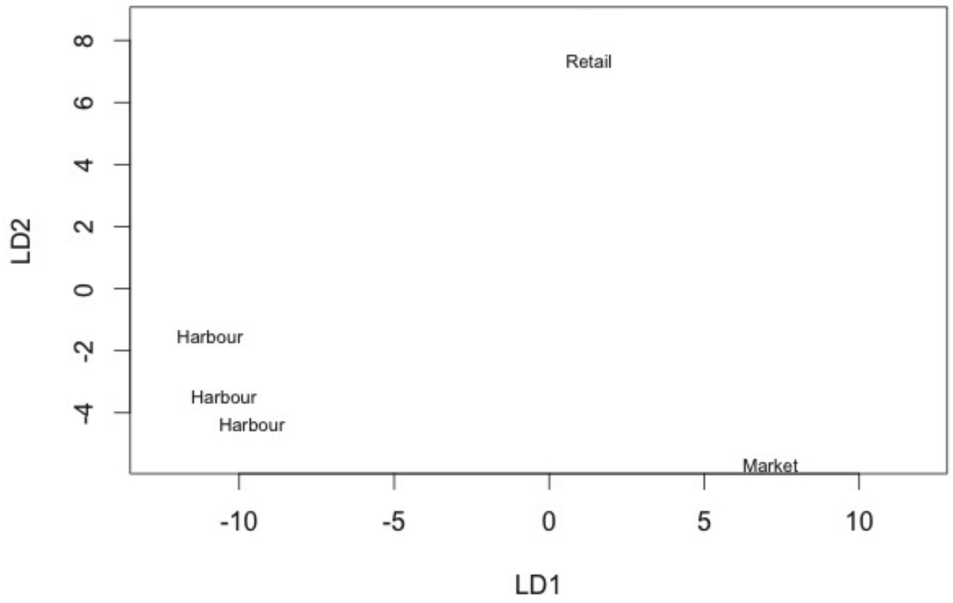

Fig. 1. Linear Discriminant Function plot – first two LDs explain 91% of between-class variance

which account for bacterial proliferation and the easy spoilage of fish. Retail shop was found to be better with cleaning schedule and proper storage facility, could be attributed to this and low microbial counts. It is apparent that for all organisms examined, microbial counts in market samples were higher than those obtained for harbour and retail samples. High bacterial load in the fish from market could be attributed to contamination during handling, transportation, contaminated food contact surfaces, utensils or due to the multiplication of inherent bacteria.

The quality of freshly caught fish is generally good but improper handling, storage and transportation can adversely affect quality and consumer acceptance. Fish handling practices have paramount importance in preventing contamination. Apart from the indigenous microorganisms that fishes have at the time of capture, unhygienic handling and contaminated equipment further added to microbial load. Domestic fish markets hold a huge potential but it is highly unorganized and unregulated. Quality of fresh fish from harbour to market varies so widely that there is an obvious need for developing standards in order to safe guard the health of the consumers. Improvement of fish marketing system and supply of quality fish under hygienic conditions is expected to increase the fish sales and per capita fish consumption in the country.

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