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Assessment of microbiological and bio-chemical quality of fish in a supply chain in Negombo, Sri Lanka

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Abstract

This study aimed to investigate quality of fish landed in Negombo area and distributed in suburban areas in Western province of Sri Lanka. Hundred samples of large fish (*Katsuwonus pelamis* and *Euthynnus affinis*) and 60 samples of small fish (*Amblygaster sirm, Pterocaesio chrysozona, Stolephorus commersoni,* and *Sardinella albella*) were sampled from different stages of a supply chain at five and six sampling visits, respectively. All fish samples (N=160) were analysed for aerobic plate counts (APC) at 37ºC, Coliforms, feacal coliforms, *Escherichia coli, Salmonella* spp., *Listeria monocytogenes*, total volatile base nitrogen (TVB-N) while 130 were analysed for histamine. Water from fishery harbor basin, fishery harbour, ice manufcaturing plants and ice used in multiday boats were also analysed for microbiological parameters. Large and small fish contained APC in the range of 2.0 $x10^2$ - 2.0 x 10^6 and 8.0×10^3 - 2.0×10^8 cfu/g, respectively. Faecal coliform counts ranged between not detected (ND) and 90 MPN/g in large fish and between ND and >1100 MPN/g in small fish. 5% of large fish were contaminated with *E.coli* and ranged from ND to 15 MPN/g. *E.coli* was present in 70% of small fish samples and ranged from ND to >1100 MPN/g . Of the 160 fish samples, tested *Salmonella* spp were detected in nine occassions. Of the 160 fish samples, *L. monocytogenes* was found in eight *Katsuwonus pelamis* and one *Sardinella albella* fish. TVB-N of large fish were found at range of 1-67 mgN/100 g and 79% samples contained unaccptable levels. Small fish contained about 25.10-104.30 mgN/100 g

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while 78% samples exceeded acceptable levels. Histamine level of large and small fish, 26% and 83% of samples exceeded the maximum acceptable levels, respectively. Harbour basin water was heavily contaminated with *Salmonella* spp. (50%), *Faecal streptococci* (100%), Faecal coliforms and *E.coli* (100%). Ice samples (20%) from one ice plant were found contaminated with *Salmonella* spp.

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Keywords: Chemical quality; Fish quality; Microbiological quality

1. Introduction

Fish is more perishable than other proteinacious animal food and its freshness is the most important criteria for judging the quality[1]. Proper post-harvest handling of catch is the most crucial step in the production of a high quality finished fishery product to meet the consumer demand[2]. Contamination of fish with pathogenic bacteria reflects use of un-cleaned utensils, contaminated water and ice, inadequate amount of ice and unhygienic handling practices[3].

The deteriorative changes occurring in fish results in the gradual accumulation of volatile and carbonyl compounds in the flesh due to the effect of varieties of biochemical and microbial mechanisms. Total volatile basic amines (TVB) are one of the most widely used measurements of seafood quality[4]. Quantification of these compounds can provide a measure of the progress of deterioration[5].

Negombo is one of the main food fish supply location in the Western province of Sri Lanka. Annual fish supply from Negombo is about 41,000 mt. Generally, large fish is supplied from multi-day boats whereas small fish caught in day-boats. Fish from both multi-day and one-day boats are landed at Fishery Harbour (Lellama) landing centre while other fishing crafts are landed at shore (Wella) in Negombo. Recent observations made by government officials and complain by consumers on low quality fish indicates poor handling practices along fish supply chains. However, scientific information on fish quality along supply chains in the area is not abundant. This study aimed to conduct scientific study on microbiological and biochemical quality of fish landed in Negombo.

2. Methodology

2.1 Sample fish species and fish supply chains

Present study was conducted with fish samples obtained in two fish supply chains begun at fish landings in Negombo from April to June 2014. One chain consisted of the distribution of large fish along four consecutive stages: 1) From multiday boats anchored at Negombo fishery harbour 2) Auction place at pier of fishery harbour 3) Immediately after transporting of fish in a vehicle to the sales destination 4) At fish retailing stall. The other chain included small fish which were caught in day boats and destined over two successive stages: 1) Day-boat at near shore (Wella) in Negombo and 2) During retailing of fish at a fish market near show in Negombo.

2.1. Simulation of present handling practices and field sampling of large fish

Large fish were sampled in five field visits including *Katsuwonus pelamis* and *Euthynnus affinis* in four and one visits, respectively. Initially, twenty fish were purchased directly from a multiday boat and fish were randomly seperated in to four groups as to contain five fish in each group. All fish samples were moved along the fish supply chain by following commonly adhered handling practices. All samples units in chill storage were transported to Quality Control Laboratory of Institute Post Harvest Technology at NARA in two hours.

2.2. Simulation of present handling practices and field sampling of small fish

Small fish species were sampled in six different occassions comprising *Amblygaster sirm*, *Pterocaesio chrysozona*, *Stolephorus commersoni* and *Sardinella albella* in 3, 1, 1, and 1 seperate field visits, respectvely. Fish were purchased from day-boats while fish were in the boat deck. Five out of ten samples were taken directly into sterile plastic sampling bags and stored in ice as above. Other five fish grups were washed with coastal water and displayed for two hours in a fish market in Negombo. At the end of two hours, fish sampled as above.

2.3. Microbiological analysis of fish

Five sample units per one sample have been analyzed for the following parameters. Fish samples (n=160) were tested for APC at 37ºC, Coliforms, Faecal coliforms, *E.coli*, *Salmonella* spp. and *Listeria monocytogenes*. Dehydrated culture media manufactured by Oxoid were used in all analysis work. APC were tested by the pour plate technique on plate count agar (SLS 516: part 1:1991). Counts of coliforms, Faecal coliforms and *E.coli* were done by MPN-technique (SLS 614: part 2:1983). ISO: 11290:1996 standard method was used to detect *L. monocytogenes*. *Salmonella* spp. was tested according to SLS 516: part 5:1992.

2.4. Bio-chemical analaysis of fish

Same fish samples were used for testing of biochemical quality. All fish (n=160) were investigated for Total Volatile Nitrogen (TVN) conent using Kjeldhal distillation method (EC/149/1995). Histamine levels of fish (n=130) except in *Pterocaesio chrysozona*, *Stolephorus commersoni* and *Sardinella albella*) are also assessed using the Fluorometric method (AOAC official method 977.3).

2.5. Microbiological analysis of water and ice

Water near boat anchoring are of the fishery harbour, water from municipal water supply line in harbour prmises, water used to make ice in ice plants and ice available for sale for fish handling operations were sampled in to sterile containers (500 ml), stored with ice in chill bins and transported to laboratory at NARA in 2 h. Water and ice samples were analysed for APC at 37 ºC, Coliforms, faecal coliforms, *E.coli*, *Faecal streptococci* (SLS 516: Part 4: 1982) and *Salmonella* spp.

2.7 Statistical analysis

Statistical analysis was performed through oneway ANOVA test using Minitab 17 software package.

3. Results and discussion

3.1 Fish samples from multiday fishing boats

APC ranged between 2.0 x10^2 and 2.0 x 10^6cfu /g in large fish along the distribution channel and the differences were not significant ($p>0.05$). Ganegama Arachchi et al., [6] has also found that APC of *Katsuwonus pelamis* were in the range of 10^4-10^6 cfu/g along the distribution channel in Negombo Sri Lanka.

As shown in Table 1, 15% of fish were contaminated with faecal coliforms and ranged from ND to 90 MPN/g whereas *E. coli* were detected in 5% of fish samples and ranged from ND to 15 MPN/g. In a previous study conducted in Negombo it has been found in 92% of *Katsuwonus pelamis* from multiday boats were contaminated with faecal coliforms and ranged from <3 to 1100MPN/g. *E.coli* were detected in 68% of samples and ranged from <3 to 21 MPN/g[4]. Of the 100 fish samples tested one *Katsuwonus pelamis* and two *Euthynnus affinis*, were found to

be positive for *Salmonella* spp (3%). *L. monocytogenes* was found in 8 *Katsuwonus pelamis* fish samples (8%). Presence of pathogenic bacteria shows that there is a cross contamination. This can occur either from water used to wash fish, surfaces fish stored or trucks used to transport fish.

Histamine level has incresed along fish distribution channel. Though the histamine content shows the increase levels with increasing time and temperature the differences were not significant ($p > 0.05$). This can be due to wide variation in histamine level of analyzed fish samples. TVB-N content didn't show any relationship between the increasing time and temperature (Table 2) and the differences were not significant ($p>0.05$).

Table 1. Analysis of pathogenic bacteria in fish samples from multiday fishing boats.

Sample set	**Number of samples analysed**	**Total coliforms (range)**	**Faecal coliforms (range)**	***E. coli* (range)**	***Salmonella* spp.**	***Listeria monocytogenes***
Samples before washing	25	ND -9	ND-4	ND -4	Absent	16% positive
Samples afterdisplaying at the pier for two hours	25	ND -23	ND	ND	Absent	Absent
Samples after transported to the stall	25	ND ->1100	ND-40	ND-15	4% positive	8% positive
Samples after displaying at the stall for two hours	25	ND -1100	ND-90	ND-9	8% positive	8% positive

ND-Not Detected

3.2 Analaysis of small fish from one day fishing boats

In 83% and 70% of small fish samples faecal coliform and *E.coli* was present and ranged from ND to >1100 MPN/g. From the results obtained (Table 3) it can be seen that the APC of both sample sets i.e. before washing and after washing had no significant difference ($p>0.05$). But the prevelence of *salmonella* spp. has increased and the *L. monocytogenes* has decreased. Number of *E.coli* (>500) is also decreased in the second sample set. Of the 60 fish samples tested four *Amblygaster sirm* one *Epinephelus malabaricus* and one *Sardinella albella* fish samples were found to be positive for *Salmonella* spp (10%). *L. monocytogenes* was found in one *Sardinella albella* sample. Though the differences were not significant ($p>0.05$) histamine levels and TVB-N has increased along fish distribution channel (Table 4).

Table 2. Microbiological analysis of small fish from one day fishing boats

Sample set	Number of samples analysed	Mean APC cfu/g Range	Total Coliofrms (range) MPN/g	Faecal coliforms (range) MPN/g	*E.coli* (range) MPN/g	*Salmonella* spp.	*Listeria monocytogenes*
1	30	2.0×10^7 Range 8.0×10^3-2.0×10^8	ND -3% >500–23%	ND -13% >500 – 16%	ND –26% >500 – 6%	3.3% positive	3.3%
2	30	2.0×10^7 Range 6.0×10^4-1.0×10^8	ND -3% >500–16%	ND -10% >500 – 13%	ND -10% >500 – 0%	10% positive	Absent

Sample set 1- Before washing with coastal water
Sample set 2- After washing with coastal water and displaying for two hours in the fish stall

Table 4. Analysis of TVB-N and Histamine in small fish from one day fishing boats

Sample set	Number of samples analysed	TVN-N (mgN/100g) Range	Mean TVB-N (mgN/100g)	% above the Maximum permissible limit	Number of samples analysed	Histamine ppm	Mean Histamine ppm	% above the Maximum permissible limit
1	30	29.57-65.91	45	70%	15	24-2056	572	67%
2	30	25.10-104.30	43	73%	15	132-2565	914	100%

3.3 Analysis of Water

From the results (Table 5) it can be seen that the levels of APC, Coliforms, faecal coliforms , *E.coli* , *F. streptococci* and *Salmonella* spp. of harbor basin water are very high. Therefore it is not recommended to wash fish and other utensils using harbour basin water as it may lead to cross contaminate fish. Although the tap water contains lesser amounts of pathogenic bacteria and APC it doesn't contain the quality of drinking water. Therefore, it is not recommended to use this water as well. Water of ice plant also contains high amounts of bacteria. Therefore, measures should be taken to reduce the bacterial load as this can directly affect the quality of ice. Water from tube well located at the Negombo fish market also contained a high bacterial load. From the results of water obtained it can be stated that the quality of water should be improved immediately.

Table 3. Analysis of water samples.

Sample set	Number of samples	APC cfu/ml	Total Coliofrms MPN/100ml	Faecal coliforms MPN/100ml	*E. coli* MPN/100ml	Faecal streptococci MPN/ml	*Salmonella* spp./100ml
Harbor basin water	12	1.4×10^6 Range 4.0×10^4-9.0×10^6	900 -16000	800-25000	800-5500	1100->1100	66% positive
Water from tap (Negombo fisheries harbour)	5	6.0×10^3 Range 2.0×10^1-3.0×10^4	ND -5	ND-2	ND-2	ND->1100	Absent
Water from ice palnt	5	1.7×10^4 Range 6.0 -7.0×10^6	ND ->1100	ND-40	ND-15	ND-20	Absent
Water from tube well of Negombo fish market	1	8.0×10^4	1600	900 900	900 900	>1100	Positive >1100

3.4 Analysis of Ice

From the results (table 6) it can be seen that the levels of APC, Coliforms, faecal coliforms , *E.coli* and *Faecal streptococci* are very high. 20% of ice samples from one ice plant contained *Salmonella* spp. Therefore, it can be stated that the quality of ice is not in good condition. Quality of ice directly affects the quality of fish. Therefore measures should be taken to make good quality ice.

Table 4. Analysis of Ice.

Sample set	**Number of samples**	**Mean APC CFU/ml**	**Total Coliofrms MPN/100ml**	**Faecal coliforms MPN/100ml**	***E. coli* MPN/100ml**	***Faecal streptococci* MPN/ml**	***Salmonella* spp./100ml**
Ice from boat	5	1.6×10^5 Range 2.0×10^3-6.0×10^5	4 -180000+	ND-55000	ND-1300	70->1100	Absent
Ice from ice plant 1	5	2.0×10^7 Range 3.7×10^3-1.0×10^8	1- 1800 +	ND-1800+	ND-1600	2.3->1100	Absent
Ice from ice plant 2	5	2.8×10^5 Range 3.0×10^3-1.0×10^6	ND -1800 +	ND-1800+	ND-900	2.3->1100	20% positive

Presence of pathogens and coliform bacteria in fishes indicates the contaminant environment, poor post harvest processing and handling of fisherman. Thus, the following recommendations are made: fishes should be appropriate handled, cleaned, washed and cooked before consumption, fishermen should be educated on the adverse effect of lack of proper personnel, environmental hygiene, sanitation and the public health authorities should inspect the landing fishes before sold to the consumers. Therefore, precautions should be taken to prevent contamination during harvesting as well as post harvest handling of fishes.

4. Conclusions

Present study indicated that a great propotion of fish handled in the distribution channel were not in the required quality standards.

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