A Comparative Quality Assessment of Five Types of Selected Fishes Collected from Retail Market in Sri Lanka

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Abstract   The chemical and microbiological quality and safety of fresh fish obtained from the retail and supermarkets of Sri Lanka were examined. Fresh fish samples (n = 155) of yellowfin tuna (*Thunnus albacares*), sailfish (*Istiophorus platypterus*), salaya/ sardine (*Sardinella gibbosa*, shrimp (*Fenneropenaeus indicus*) and squids sp (*Loligo duvaucelii*) were collected from 19 places around the country and evaluated in the laboratory. Samples were analysed for several chemical and microbial quality including total volatile base nitrogen, histamine, total mercury and total plate count. Furthermore, the presence of selected pathogens such as *Escherichia coli* and *Salmonella* spp. were detected. In microbiological analysis of samples total plate counts were obtained in the range of 7×10^3-1.2×10^8 cfu/g and 28% of the samples had $< 5 \times 10^5$ cfu/g. Eight percent of the samples contained $> 10^3$ MPN/g of *E. coli* and 1 sample contained 500 MPN/g and rest of the samples had < 200 MPN/g with 33% of the samples without E. coli. Twelve percent of samples were positive for Salmonella spp. The range of total volatile base nitrogen (TVB-N) was 15-4883 mgN/100 g and 33% samples were exceeded the maximum acceptable levels. Further considering histamine and mercury level of yellowfin tuna and sailfish, 7% and 4% of samples exceeded the maximum acceptable levels respectively. When considering both microbiological and chemical results it can be seen that majority of the samples are not fit for the human consumption and that counted as; 35% of yellowfin tuna, 47% of sailfish, 35% of sardine, 100% of squids and 53% shrimp.

Keywords: fresh fish, chemical and microbial quality, retail market, Sri Lanka

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1. Introduction

Sri Lanka is a small tropical island in the Indian Ocean off the southern tip of India and having an exclusive economic zone (EEZ) area of 517,000 km^2. Capture fisheries produced 293,170 MT and total fisheries production including aquaculture was 339,170 MT in 2009 (MOFAR, 2011a). This was an increase in seafood production by 6.8% compared to the previous year. The fisheries sector contributed a significant income to the national economy. The total contribution of the fisheries sub-sector to the gross domestic production (GDP) was 1.7% in 2009 (MOFAR, 2011). Fisheries sector is an important source of local employment generation and provides about 475,000 employment opportunities directly and indirectly. This is about 8.5% of total employment in the country (MOFAR, 2011).

Fish can be finfish, shellfish (mollusks and crustaceans), or any other form of marine or freshwater animal life that can be used for human or domestic animal consumption (Mahmuda *et al.*, 2010). Nutritional and protein plays an important role in the life of man and nation, fish are known for their high nutritional quality they are relatively low in fat, saturated fat, and cholesterol, and high in polyunsaturated fatty acids, protein and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Gamal and Shamery, 2010). Meat from of fresh fish flesh is the most common source of high protein food and an important source of protein in human nutrition in Sri Lanka.

Raw fish and seafood is a highly perishable commodity compared to other fresh meat commodities and have short lifetimes even at refrigeration temperature (Lauzon *et al.*, 2010, Popovic *et al.*, 2010, Can, 2010). To maximize its value, freshness quality must be maintained and shorter lifetime is a big hurdle. The spoilage process begins immediately after capture. Harmless, natural spoilage bacteria on the skin and in the slime of the fish quickly invade the muscle blocks. However, for the first couple of days, changes in the fish are predominantly due to the intrinsic enzymes in the flesh. To maximize freshness quality, fish should be held at the temperature of melting ice i.e. 0°C. In addition, raw material quality, cooling methods, processing, packaging, transporting and storage

conditions should maintain freshness and shelf life extension (Lauzon *et al.*, 2010, Jayasinghe and Rajakaruna, 2005). The quality of fish at retail outlets depends on both its temperature and time.

Whole fish storage at subzero temperatures may extend shelf life. Modified atmosphere (MA) bulk storage of whole fish under chilled conditions also generally contributes to longer shelf life, but the extent and textural defects resulting may differ among fish species (Lauzon *et al.*, 2010). Temperature control is important to maintain fish quality. The use of ice as an efficient and economical cooling medium to quickly lower whole fish temperature and to evaluate its effect on to maintain freshness and shelf life extension fish products. However the private water sources used for ice production of the Sri Lanka and is produced from them are not up to the microbiological standards for potable water and therefore not fit to be directly used in food items intended for human consumption (Kariyawasam *et al.*, 2007). This should be carefully considered during the selection of ice for processing of fresh fish products while transporting or exporting to foreign or local markets. The use of modified atmosphere packaging (MAP) generally resulted in an increased sensory shelf life when compared with traditional ice storage, but the magnitude of the increase depended on various factors such as the composition of the gas mixture, storage temperature, raw material quality and pack size. Lower levels of carbon dioxide are generally used to reduce water loss and textural defects (Lauzon *et al.*, 2010).

The human sensory assessment remains the fastest and most accurate way of assessing fish freshness. Nonhuman techniques exist but these can't be representing totally in human sensory. Chemical analysis including total volatile bases (TVB) or (TMA) is also time consuming to get a result and can prove inaccurate until the fish is well past the point of acceptability. Microbiological testing is also used to determine freshness quality.

In Sri Lanka, it has been estimated that post-harvest loss of fresh produce varies between 30 to 50 percent of the total production at any point between farmer and consumer in the supply chain, depending on the commodity. Poor post-harvest handling during storage, improper packaging and transportation, diseases and inadequate storage facilities are some of the major reasons for such high post-harvest losses. Although it is generally known that the post-harvest wastage of locally produced perishable fish is substantially high there is very little data available on the proportion of such losses and their causes. The few available research also concerns the target land area, physical loss, but now many of the consumers also concern about the quality of fish. There is a need in Sri Lanka to improve the nutritional status and food security of the people by increasing the national fish production by minimizing post-harvest losses and improving quality of the fish products to acceptable standards.

The aim of this work was to evaluate the chemical and microbial characteristics of selected marine and freshwater fish species in the retail markets of Sri Lanka.
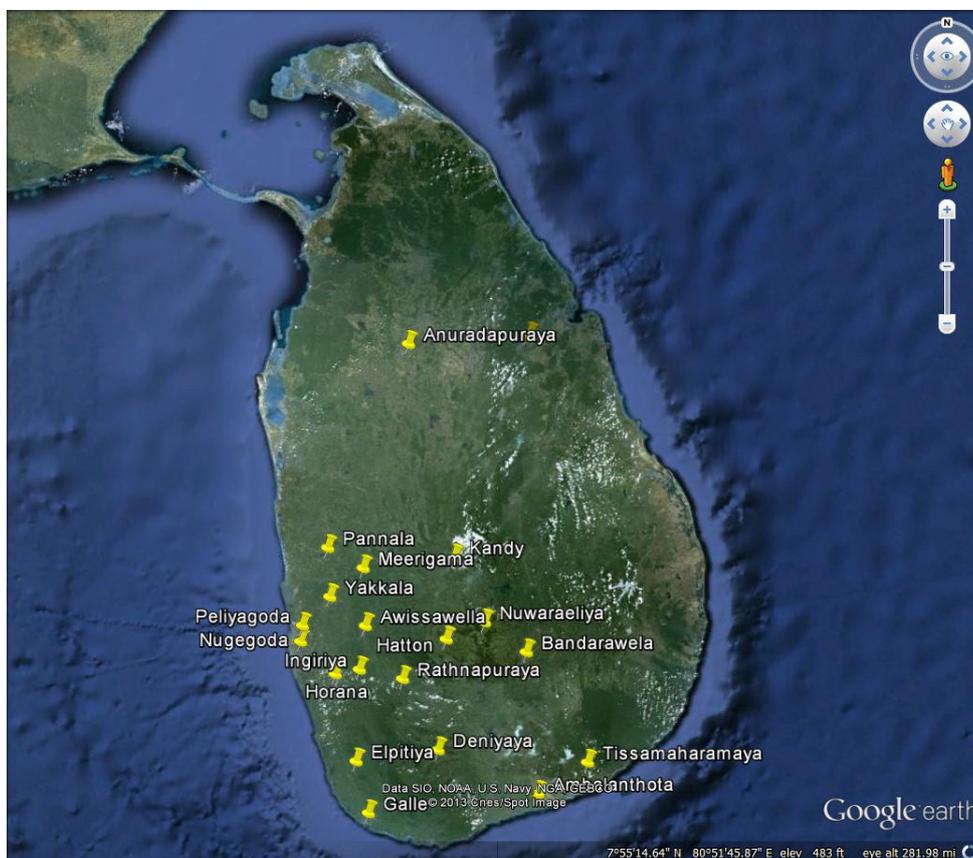
2. Methods

Figure 1. Map of the sampling place

A total of 155fresh fish samples (37 of yellowfin tuna, 36 of sailfish, 26 of sardine, 36 of shrimp and 20 of squid) were collected from 19 randomly selected sites from February 2012 to November 2012 (Figure 1). Three types of retail outlets were used in each city to take samples; co-operation outlets, supermarkets and open market. Nearly 1 kg of each fish sample was collected in sterile polythene bags, recorded surface temperature, stored in ample amount of ice in insulated boxes, and transported to Institute of Post-Harvest Technology, National Aquatic Resources Research and Development Agency. All the samples were tested for total volatile base nitrogen (TVB-N), Aerobic Plate Count (30°C), *E. coli*, and *Salmonella*. Only yellowfin tuna and sailfish were tested for total mercury and histamine content.

Aerobic plate count (APC); this method was followed from the Sri Lanka standard Institute (SLS) 516: PART 1:1991. Ten grams of the sample was weighed aseptically into a sterile stomacher bag and 90 mL of diluents (maximum recovery diluents-Oxoid) was added and blended in a stomacher blender for 1-2 minutes to make up the 10^{-1} dilution. Four dilutions were used starting from the 10^{-2} dilution for plating. 1 mL of 10^{-2} dilution was transferred to each of the 2 sterile petri plates using sterile pipettes. Same procedure was repeated with the other dilutions. About 15 mL of standard plate count agar (Oxoid) medium at 45 ± 0.5°C was poured into each petri plate and mixed with the inoculums and allowed to solidify. Plates were incubated at 30°C for 72 hours. After the incubation period bacterial counts were taken.

E. coil count of the fish samples was analysed according to the SLS 516: PART 3:1982. Ten grams of the sample was weighed in a sterile stomacher bag and 90 mL of the diluent (maximum recovery diluents-Oxoid) was added and blended. Then 10 mL of the 10^{-1} dilution was inoculated into each three tubes containing 10 mL of double strength Macconkey broth purple (Oxoid). Then 1 mL of each 10^{-1} dilution was inoculated to each three tubes containing 10 mL of single strength Macconkey broth purple. The same procedure was repeated for 10^{-2} and 10^{-3} dilutions. Tubes were incubated at 36 ± 1°C to 24 and 48 hours. Tubes were examined for acid and gas production. Tubes showing acid and gas production after incubation is considered are positive for presumptive coliform. Positive tubes were sub cultured into tubes containing 10 mL of Brilliant Green bile Broth and incubated 44 ± 0.1°C, 48 hours. Tubes with gas production were considered as positive for faecal coliforms. From the positive f. coliform tubes subculture onto eosin methylene blue agar (Oxoid) by streaking plates were incubated at 36 ± 1°C for 24 hours. Typical *E. coli* colonies were examined and colonies were inoculated into tubes containing peptone water and incubated at 44 ± 0.1°C for 24-48 hours. Production of indole was tested by adding indole reagent. Cultures showing indole production were considered as *E. coli* positive cultures. A calculation was made by using the MPN table.

Salmonella; was determined based on SLS 516: PART5:1992.Twenty five grams of the sample was weighed into a flask containing 225 mL of pre enrichment medium (buffered peptone water-Oxoid) and incubated at 37°C for 18-24 hours. After incubation period 0.1 mL of the culture was transferred to Rappaport-Vassiliadis-Oxoid (RV) medium and 1mL was transferred to Selective Cystine-Oxoid (SC) medium. Incubation RV medium at 42°C for 18-24 hours and SC medium at 37°C for 18-24 hours. After incubation loop-full from both cultures were streaked onto plates containing brilliant green bile agar and Xylose Lysine Deoxycholate (XLD) agar-Oxoid, and incubated at 37 ± 1°C for 18-24 hours. Plates were examined and typical colonies were selected and biochemical and serological testing was done to confirm *Salmonella*.

TVB-N was determined based on an adaptation of the current official European steam-distillation method (EU/EC, 1995, Malle and Poumeyrol, 1989). The method is based on the extraction of TVB using alkaline solution and the titration of the recovered ammonia as follows: fishsamples were homogenized with a laboratory blender for 1 min and weighed 100 g of fish into the beaker. Then 200 mL of 7.5% trichloro acetic acid (TCA) was added and the extract was homogenized for 2 min. After that, the mixture was filtered using Whatman no 2 filter paper to obtain a clear solution for analysis. Then, 25 mL of fish extract was placed in the distillation flask in VELP mark aperture (model UDK-6, Milan, Italy). Then 30 mL of 10% NaOH solution was added, the apparatus immediately sealed and the end of the steam distillate collected in a flask containing 25 mL of 4% boric acid and few drops of mixed indicator (methyl red/methylene blue 2:1). The steam distillation procedure was continued until 5 min and distillate was collected. The obtained basic solution was titrated against 0.025 N H_2SO_4 to the endpoint indicated by a green to pink colour change. The TVB-N content was determined after blank correction that has been determined by the steam distillation with 25 mL of distilled water sample.

The total mercury (T-Hg) was determined using a cold vapor system (CV-AAS), based on AOAC 971.21 (AOAC, 1990) with Varian VGA 77 (Varian, Mulgrave, Australia). All chemicals used were at least analytical reagent grade. Standard solutions of Hg, having a concentration of 1000 mg/L was obtained from Fluka, Switzerland. All glassware used were first soaked overnight in a liquid detergent solution in tap water. The glassware then thoroughly rinse with tap water and then soaking 10% (v/v) HNO_3 overnight. Subsequent rinsing was performed using de-ionized water. Then all glassware were oven dried and plasticware were air dried prior to use. Approximately 1 g of fish (yellowfin tuna and sailfish) sample was weighed into microwave vessels mineralized using closed vessels acid digestion method. Portion of samples with 10 mL of Conc.HNO_3 acid (65%,'AR'-Sigma) was added to 100 mL digestion bomb and allowed for 15 minutes in the fume hood for pre digestion. Two reagent blanks, two spiked samples were prepared for each batch of analysis in a similar manner as samples without adding samples to the digestion vessels. Each sample was analysed in duplicate. Pre-digestion samples were digested under pressure using a microwave oven (CEM-Mass XP-1500+, Matthews, USA). The digestion conditions were 400 PSI pressure, 200°C temperature and 10 min. holding times. The digested samples were quantitatively transferred into 50 mL volumetric flasks and it was made up to 50 mL with deionised water. Using the standard metal solution series, a calibration curve for each metal was obtained. After the standard curve was made reagent blanks, samples and spikes were placed in AAS and readings were taken.

Histamine of yellowfin tuna and sailfish samples were determined based on the AOAC official method 977.3, Histamine in seafood-Fluorometric method. The ground samples (10 g) were homogenized with 50 mL of methanol for 2 min. Then, transferred it to 100 mL volumetric flask and volume up with distilled water and heated to 60°C for 15 min in a water bath, cooled to room temperature and filtered through Whatman 2 filter paper. The filtered sample passed through the Bio-Rad AG 1-X8, 50 100 mesh resin and 1 mL of extract were derivartized with 0.1% O-Phthaldialdehyde solution. The fluorescence was determined with Shimadzu- xxx spectro flurophotometer, using an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Standard curves were automatically obtained by the spectro fluorometer from known solutions. The results were expressed as mg/kg wet weight of fresh fish muscle sample.

Experimental data was analyzed for the effect of various chemical and meteorological factors on the prevalence of bacterial population in fish statistically by using one-way analysis of variance (ANOVA). The Wilcoxon Signed-Rank test was used to detect the differences in the distributions of various chemical and microbiological variables. Data was performed using the Statistical Package for Social Sciences (SPSS 16, Chicago, USA) and Microsoft Excel 2011 software.

3. Results and Discussion

3.1. Display Temperature

The surface temperatures of studied fish were varying (Table 1) and many retailers were not maintaining the product temperature. To prevent seafood spoilage, product temperature must be controlled during storage and display time.

Table 1. Surface temperature and percentage of fish samples displayed below 4°C

	Avg. Surface temperature (°C)	% fresh fish samples displayed below 4°C
Yellowfin tuna	13.5	18.9
Sailfish	10.6	27.8
Sardine	12.8	15.4
Shrimp	13.5	16.7
Squids	11.4	25.0

Fish, shellfish and their products at retail should be received, handled, stored and displayed to consumers in a manner that minimizes potential food safety hazards and defects while maintaining essential quality. Chilled products should be stored in a hygienic manner at temperatures less than or equal to 4°C while frozen products should be stored at temperatures less than or equal to – 18°C (WHO, 2009) .Most microorganisms that cause food borne illness optimal temperatures of 5°C and 60°C. This is commonly referred to as the temperature danger zone (Chong, 2012). One of the main factors affecting the formation of histamine is cooling method and handling techniques. Basically maintenance of the temperature of fish affecting growth of histamine producing bacteria include the type and size of fish (Joshi and Bhoir, 2011). In this study, most of the fishes in the markets were kept outside the ice for sell for a considerable amount of time, which results in ambient temperature abuse of fish, and this gives an opportunity for histamine producing bacteria to proliferate.

3.2. Total Volatile Basic Nitrogen (TVB-N)

Total volatile basic amines (TVB) are one of the most widely used measurements of seafood quality (Zhong-Yi *et al.*, 2010). It is a general term that includes the measurement of tri-methylamine (produced by spoilage bacteria), di-methylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage.

Levels of total volatile basic nitrogen (TVB-N) in all fresh fish were analysed. According to the literature, TVBN amounts within 30 and 35 mg N per 100 g of muscles is considered acceptable for fresh fish (Amegovu *et al.*, 2012, EU/EC, 2008). The average, maximum and minimum TVB-N for studying fresh fish species are shown in Table 2 and average TVB-N levels of these species are difference significant ($p < 0.05$). The average TVB-N of yellowfin tuna and sardine was below the rejection limits. The TVB-N of one sample of sailfish was recorded as 1021 mg N/100 g of fish, without that sample the average TVB-N value of sailfish was 25 mg N/100 g. The TVB-N level of all samples of squids was the rejection level.

Table 2. Average, maximum and minimum TVB-N (mg N/100 g) values of five types of fish

Fish type	Average TVB-N	TVB-N range	% of exceeding the rejection level
Yellowfin tuna	31	16 - 234	5
Sailfish	52	16 - 1021	8
Sardine	27	15 - 82	12
Squids	1153	40 - 4883	100
Shrimp	119	19 - 3172	42

The main constituents of TVB-N are tri-methylamine, di-methylamine and ammonia. Its amount increases with time of storage in the unfrozen state (Bechtel *et al.*, 2010). Tri-methylamine originates from bacterial decomposition and therefore, considered as an indicator for bacterial growth, while the ammonia comes from decomposition of amino acids – thus reducing the quality of the available protein. Cephalopods contain more carbohydrate and less protein compared with other fin-fishes and crustaceans. Hence, the spoilage mechanisms associated with cephalopods are quite different from other fin-fishes and crustaceans. Spoilage pattern in cephalopods is also dominated by autolysis that leads to a shorter shelf life by sensory characteristics (Sykes *et al.*, 2009). Crustaceans and other shellfishes spoil more rapidly compared with other fishes, mainly because they are small in size.

Secondly, as their guts are not removed immediately after harvesting, shellfishes are prone to early autolytic spoilage (Ashie *et al.*, 1996).

3.3. Histamine

In examining the histamine level of yellowfin tuna and sailfish (Table 3), the average histamine concentration of yellowfin tuna was 29.08 mg/kg, followed by sailfish (28.02 mg/kg). These results indicate higher occurrence of histamine levels observed in a few times in the retail market; however, most of the samples are also in accordance with the limits (100 mg/kg) established by European Union (CE, 1991); indicating that good quality tuna and sailfish was commercialized in Sri Lanka. Few samples showed the highest level of histamine. This higher level could result from the additional handling and processing; longer holding at higher temperatures associated with processing and from the increased surface area of the grated compared to the fish, which could facilitate contamination and enzyme substrate interaction.

Table 3. Average, maximum and minimum histamine (mg/kg in wet weight basis) values

Fish type	Average Histamine	Histamine range	% of exceeding the rejection level
Yellowfin tuna	29	ND-317	8
Sailfish	28	ND-347	6

ND: Not detected, the minimum detectable level was 1 mg/kg of histamine

The histamine-producing bacteria are usually considered part of the normal microflora of the gut, skin, or gills of fish. Aguilar *et al*, 1998 mentioned that post catch contamination may be a very important source of histamine-producing bacteria. Most microorganisms described as histamine producers belong to the Enterobacteriaceae family. Histamine-producing enteric bacteria will not be expected to proliferate at 0°C; on the other hand, enzymatic decarboxylation of histidine to produce histamine has its optimal temperature at 20–30°C and pH 6.5 (Aguilar *et al.*, 1998). In the previous study in Sri Lanka, the level of histamine in commercially processed yellowfin tuna, was recorded as 19 + 11 mg/kg (Somasiri *et al.*, 2007).

3.4. Total Mercury (T-Hg)

The highest mean T-Hg concentration was recorded form yellowfin tuna (0.28 mg/kg, wet weight) and lowest followed by sailfish (0.25 mg/kg, wet weight). However, the maximum acceptable limits of Hg in yellowfin tuna and sailfish are exceeded in only single sample from each fish. In 19% of total yellowfin tuna and 12% of sailfish analysed, T-Hg concentrations were over 0.5 mg/kg, near the half of the European legislation limit of 1 mg/kg.

Table 4. Average, maximum and minimum T-Hg (mg/kg in wet weight basis) values

Fish type	Average T-Hg	T-Hg range	% of exceeding the rejection level
Yellowfin tuna	0.28	ND-1.18	4
Sailfish	0.25	ND-1.14	4

ND: Not detected, the minimum detectable level was 1 mg/kg of T-Hg

The size of a fish is known as determining factor of its Hg burden and the size of fish has not considered in this study since practical reason. Fin fish is the major source of total mercury and methyl mercury intake for humans (Kojadinovic *et al.*, 2007). Specially, large predatory fish at the top of the food chain, such as the fish like swordfish, yellowfin tuna and marlin are significant sources of human exposure to methyl mercury. When T-Hg levels of yellowfin tuna of this study were compared with previous studies in Sri Lanka, an increase in the total mercury levels of YFT could be observed between 2002 and 2012. (Senadheera, 2005) recorded total mercury levels in yellowfin tuna samples ranging from 0.01 to 0.08 mg/kg with mean value of 0.05 mg/kg. In another study, the T-Hg levels of yellowfin tuna recorded the range of not detected to 0.98 mg/kg (mean = 0.30 ± 0.18 mg/kg) in Sri Lanka (Jinadasa *et al.*, 2012).

3.5. Aerobic Plate Count (APC)

Average value and range of APC of five fish species are given in Table 5. These APC values were significantly different from each other (P = 0. 05). The APC levels were analyzed based on APC of fresh and frozen fish by International Commission on Microbiological Specifications for Food (ICMSF) and it specifies the upper (rejectable) and lower (marginal) levels of acceptability as 1×10^7 and 5×10^5 cfu/g respectively. According to the APC value, 21% of yellowfin tuna, 28% of sailfish, 11% of sardine, 20% of squids and 8% of shrimp in Sri Lankan retail markets were not fit for human consumption.

Table 5. Average, maximum and minimum % of acceptability based on APC (cfu/ g) values of five types of fish

Fish type	Average APC	APC range	% of m	% of M
Yellowfin tuna	1.02×10^7	$5.20 \times 10^4 - 1.20 \times 10^8$	14	65
Sailfish	1.57×10^7	$8.00 \times 10^3 - 1.00 \times 10^8$	19	53
Sardine	9.36×10^6	$2.50 \times 10^4 - 1.00 \times 10^8$	35	54
Squids	7.74×10^6	$7.00 \times 10^3 - 8.60 \times 10^7$	55	25
Shrimp	3.93×10^6	$1.40 \times 10^4 - 5.00 \times 10^7$	39	53

m-Maximum recommended for good quality fish
M-Maximum recommended for marginal quality

Table 6. Average, maximum and minimum percentage of acceptability based on *E. coli* (MPN/ g) values of five types of fish

Fish type	Average *E. coli*	*E. coli* range	% of m	% of M
Yellowfin tuna		ND - > 1100	24	70
Sailfish		ND - > 1100	28	58
Sardine		ND- > 1100	63	27
Squids		ND- > 1100	25	70
Shrimp		ND- > 1100	83	14

3.6. Escherichia Coli

Since enterohemorrhagic *E. coli* O157:H7 was first identified as a food borne pathogen in 1982, this microorganism has become one of the most important foodborne pathogens because it has been found in a wide variety of foods and can cause potentially life-threatening illness (Benjamin and Datta, 1995). The average value, range and percentage of maximum recommended for good quality and marginal quality of fish considering the *E. coli* (MPN/g) are given in Table 6. *E. coli* counts were found to be in the range of ND to > 1100 (MPN/g) in all studied fish. Very low numbers of some faecal origin microbial agents are enough to cause illness (Ganegamaarachchi *et al.*, 2000). Hence, presence of *E. coli* in small numbers will be at risk when food safety is considered.

3.7. Salmonella

Salmonella is highly pathogenic and this is the major reason for isolation of such bacteria from sample fishes. In the present study, *Salmonella* was examined qualitatively. The analysis of *Salmonella,* 16% of yellowfin tuna, 19% of sailfish, 8% of sardine and 8% of shrimp showed the positive results, but none of the squid samples show the positive results. Aquatic environments are the major reservoirs of *Salmonella* and fish and shellfish appears to be passive carriers of Salmonella. Therefore, fishery products have been recognized as a major carrier of food-borne pathogens (Upadhyay *et al.*, 2010, Novotny *et al.*, 2004).

4. Conclusions:

This study gives a clear perspective on the variation of chemical and bacterial quality comparatively in five fishes collected from retail markets of Sri Lanka. Considering the analysed chemicals and microbiological parameters, 35% of yellowfin tuna, 47% of sailfish, 35% of sardine, 100% of squids and 53% shrimp samples were not good for human consumption. From the result, it can be concluded that the fish sold in retail markets are not standard to consume since the observed chemical and microbial levels are generally higher than the recommended levels. To overcome this situation, it is necessary to follow the code of practice concerning handling of the catch, icing, post-harvesting procedures and storage including depuration and hygienic measures. The proper hygienic condition should be maintained at every step of catching, landing and transportation, processing and marketing following HACCP steps for good quality of fish and fishery products for the consumer.

Acknowledgement

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