Quality assessment of seafood sold in selected retail outlets of the Western Province, Sri Lanka

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Abstract

Quality of seafood, sold in retail market is a major concern. Bacterial counts, presence of pathogenic bacteria, formation of nitrogenous volatile compounds and histamine are key indicators of seafood quality. A total of 174 seafood samples including tuna species, cuttlefish, prawn, sailfish, Indian mackerel, goldstripe sardinella, herring, rockfish, flyingfish, trevally, anchovy and Indian scad were collected from 37 retail outlets in the Western province of Sri Lanka to analyse the quality of seafood they sell. Samples were analysed for Aerobic Plate Count (APC), *Salmonella* spp., *Staphylococcus aureus,* coliforms, feacal coliforms and *Escherichia coli* to check the microbial quality and histamine and Total Volatile Base Nitrogen (TVB-N) content were examined to assess chemical quality. In respect of microbial quality of samples, APC was found in the range of 2.5×10^2 to 1.0×10^8 cfu/g. About 43% of the samples had less than 5.0×10^5 cfu/g, 14.5% of the samples contained greater than 1.0×10^7cfu/g and 42.5 % of samples contained between 5.0×10^5cfu/g - 1.0×10^7cfu/g of APC. Thirty four percent of the samples contained greater than 10^3 MPN/g of total coliforms and 7% of samples had greater than 10^3 MPN/g of feacal coliforms. Sixty nine percent of the samples had *E.coli* content less than 11 MPN/g and 3% of the samples contained greater than 500 MPN/g. *Salmonella* spp. was present in 13% of the samples and all the samples were negative for *S. aureus.* TVB-N was found in the range of 15 – 803 mgN/100 g with 14% of samples containing greater than 35 mgN/100 g. One percent of the samples contained greater than 100 mg/kg of histamine. Only 21% of the samples comprised with acceptable values for all the analysed parameters. When considering the quality of seafood with both microbiological and chemical aspects it can be stated that quality should be improved.

Keywords: APC, Pathogenic bacteria, Histamine, TVB-N

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Introduction

Although seafood is a nutrient rich healthy food, there is a potential risk of consuming contaminated seafood (Iwamoto *et al*., 2010) and therein getting food borne illnesses. Therefore it is very important to assess the quality of seafood we consume. Quality of seafood can be assessed microbiologically and chemically since microorganisms and enzymes play major roles in the spoilage of seafood. According to the Food and Agriculture Organization (2014) preliminary estimates, world per capita fish consumption has increased from an average of 9.9 kg in the 1960's to 19.2 kg in 2012. Total fish production in the year 2012 (provisional estimates) is 158.0 million tons and world human fish consumption is 136.2 million tons (FAO, 2014). In Sri Lanka total fish production was 486,170 Mt and annual per capita fish consumption was 14.5 kg/year in the year 2012. Further, fish contributes 55% of total animal protein intake (Ministry of Fisheries, Sri Lanka, 2014).

Centre for Science in the Public Interest, USA (2013) reported that the seafood was responsible for the second most food related outbreaks of food borne illness and there had been 38.6% of scombroid outbreaks 4% *Staphylococcus* outbreaks and 3.3% of *Salmonella* outbreaks due to consumption of seafood, taken place in the USA in 2001 – 2010. Most of the countries in the world do not report each and every food borne illness. Estimates show that there's only 1% of actual food borne illnesses being reported (Mossel, 1982).

Contamination of seafood may occur at any occasion from the time of harvest or even before and throughout the supply chain till it is being prepared by the consumer. The type and amount of pathogens present in seafood depend upon the environment, way of handling, processing storage, transportation and final preparation. Presence of pathogens in time and temperature abused seafood due to the delays between harvest and refrigeration is more evident (Iwamoto *et al*., 2010).

Escherichia coli, *Salmonella* spp. and *Staphylococcus aureus* are most common food borne infectious and toxin forming bacteria. Those bacteria can be naturally found in raw food of animal origin or introduced to food while handling of food by man (Da Silva *et al.,* 2010).

Scombrotoxin fish poisoning is caused by ingesting some species of marine fish containing high levels of histamine and other biogenic amines. Histamine is formed as a result of bacterial decarboxylation of free histidine when fish (e.g. tuna, mackerel, sardines and anchovy) is subjected to temperature abuse during or after harvest (FAO, 2012). U.S. Food and Drug Administration (2011) has stated that disease causing level of histamine in fish, in most incidences is above 200 mg/kg and frequently it is above 500 mg/kg. Histamine is heat stable and survives subsequent processing, including canning. Once the bacterial enzyme histidine decarboxylase is formed it cannot be destroyed even by cooking or retorting (Mc Lauchlin *et al*., 2005 and USFDA, 2011). Fish spoilage can also be determined by chemically analysing Total Volatile Base Nitrogen (TVB-N) content. TVB-N is a measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage. Trimethyl Amine (TMA) is a component of Total Volatile Bases (TVB) which is present in small amounts in fresh fish flesh, increases with time of storage. Amount of these compounds increase when spoilage proceeds (Malle and Poumeyrol, 1989 and Ruiz-Capillas and Horner, 1999). Previous study conducted in Sri Lanka has reported that TVB-N content of fish were significantly higher in samples collected from the retail market than from the boat and the jetty (Ganegama Arachchi *et al*., 2000).

The aim of this study was to assess the quality of seafood sold in selected retail outlets located in the Western Province of Sri Lanka by analysing them for microbiological (APC, coliforms, Feacal coliforms, *E.coli*, *Salmonella* spp. and *S. aureus)* and chemical (Histamine of selected species and TVB-N content) parameters and to issue recommendations on handling of seafood safely in terms of quality, based on obtained results.

A total of 174 commonly available seafood samples (tuna spp. (n=41), cuttlefish (n=09), prawn (n=15), sailfish (n=16), Indian mackeral (n=11), trevally (n=12), herring (n=16), Indian scad (n=12), goldstripe sardinella (n=15), anchovy (n=9), flyingfish (n=8) and rockfish (n=10)) were collected from 37 selected retail outlets located in the Western Province of Sri Lanka from April to December, 2013. Each sample weighed approximately 500 g. All the collected fish samples were immediately placed into sterile polythene bags and stored in insulated ice boxes and transported to the laboratory and sample analysis were carried out immediately.

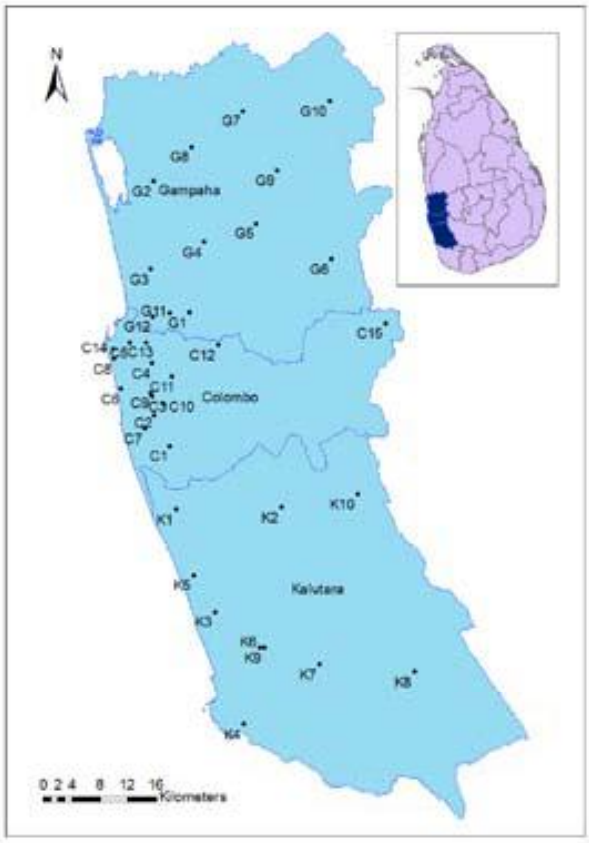

Fig 1. Sampling locations at Western Province

Table 1. Sampling locations

Sampling points	Colombo District	Sampling points	Gampaha District	Sampling points	Kalutara District
C1	Piliyandala	G1	Makola	K1	Panadura
C2	Boralasgamuwa	G2	Raddolugama	K2	Horana
C3	Nugegoda	G3	Welisara	K3	Kalutara
C4	Rajagiriya	G4	Ganemulla	K4	Darga town
C5	Malligawatte	G5	Yakkala	K5	Wadduwa
C6	Wellawatta	G6	Kiridiwela	K6	Dodangoda 1
C7	Ratmalana	G7	Diwulapitiya	K7	Mathugama
C8	Colpetty	G8	Minuwangoda	K8	Baduraliya
C9	Nugegoda (Kattia junction)	G9	Veyangoda	K9	Dodangoda 2
C10	Nawinna	G10	Mirigama	K10	Ingiriya
C11	Pelawatta	G11	Kelaniya	-	-
C12	Kaduwela	G12	Thorana junction	-	-
C13	Kolonnawa	-	-	-	-
C14	Slave Island	-	-	-	-
C15	Awissawella	-	-	-	-

Aerobic plate count (APC): APC was carried out using SLS 516: Part 1:1991. Ten grams of the sample was weighed aseptically into a sterile stomacher bag and 90 ml of diluent (maximum recovery diluent-Oxoid) was added and blended in a stomacher blender for 1 to 2 min. to make up the 10^{-1} dilution. Four dilutions were used starting from the 10^{-2} dilution for plating. One milliliter of 10^{-2} dilution was transferred to each of the two 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 37°C for 48 hours. After the incubation period bacterial count was taken.

MPN method for Coliforms, Faecal coliforms and E.coli: For enumeration of coliforms, faecal coliforms and *E. coli* SLS 516: Part 3: 1982 method was used. Sample was prepared as same as in the APC method. Then 10 ml of the 10^{-1} dilution was inoculated into each three tubes containing 10 ml of double strength Mac-Conkey broth (Oxoid). Then the 10^{-1}, 10^{-2} and 10^{-3} dilutions were inoculated into tubes containing 10 ml of single strength Mac-Conkey broth. Tubes were incubated at 36±1°C to 24 - 48 hours and examined for acid and gas production. Positive tubes were sub cultured into two tube sets containing 10 ml of Brilliant Green Bile Broth (BGBB) and one set was incubated at 36±1°C for 48 hours for total coliforms and the other tube set was incubated at 44±0.1°C, for 48 hours for faecal coliforms. From the positive faecal coliform tubes loop full of cultures were streaked onto eosin methylene blue agar (Oxoid) and incubated at 36±1°C for 24 hours. Typical *E. coli* colonies were inoculated into peptone water tubes and incubated at 44±0.1°C for 24-48 hours. Cultures showing indole production were considered as *E. coli* positive cultures.

***Detection of Salmonella* spp.:** *Salmonella* spp. was determined based on the SLS 516: part 5:1992 method. 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. One milliliter of the culture was transferred to Selenite Cystine- Oxoid (SC) medium and 0.1 ml of the culture was transferred to Rappaport-Vassiliadis- Oxoid (RV) medium. Incubation time for RV medium is at 42°C for 18-24 hours and for SC medium is 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 were done to confirm *Salmonella*.

Enumeration of Staphylococcus aureus: Enumeration of *S. aureus* was carried out using SLS 516: Part 6: 1992. Ten grams of the sample was weighed aseptically into a sterile stomacher bag and 90 ml of diluents (maximum recovery diluent-Oxoid) was added and blended in a stomacher blender for 1-2 minutes to make up the 10^{-1} dilution. One milliliter of the 10^{-1} dilution was transferred on to Baird-Parker agar plates (90mm). Inoculum was spread carefully and allowed to dry. Then the plates were incubated at 37±1°C for 18-24 hours. After the incubation typical and atypical colonies were selected and confirmation was done using the coagulase test. Confirmed colonies were taken into account and the result was given as the number of *S. aureus* per gram.

***Detection of Histamine content*:** Fluorometric method (AOAC Official Method 977.13, 1998) was used to analyse the histamine content of fish samples. Five grams of the sample was taken into 50 ml beaker and 35 ml of methanol was added. Sample was blended using a homogenizer. Contents were transferred into 50 ml volumetric flask and heated in a water bath to 60ºC and let stand for 15 minutes at the same temperature. After 15 minutes flask was taken out from the water bath and allowed to cool to room temperature. Volume was made up to 50 ml by adding methanol and filtered through a folded paper. One milliliter of extract was pipetted onto the column (Bio-Rad ag 1-x8, 50 100 mesh) and 4-5 ml of H_2O was added. Column flow was immediately initiated into 50 ml volumetric flask containing 5 ml of 1 N HCl. Then H_2O was introduced into the column until 35 ml has eluted. Column flow was stopped and made the volume up to 50 ml by adding distilled water. Five milliliter from eluate was pipetted into 50 ml conical flask and then 10 ml of 0.1N HCl was pipetted in. Again 3 ml of 1N NaOH was pipetted in and mixed. Within 5 minutes 1 ml of OPT solution was pipetted in and mixed immediately. After exactly 4 minutes, 3 ml of 3.57N H_3PO_4 was pipetted and mixed immediately. Fluorescence was determined using Shimadzu 1501 photofluorometer with excitation wavelength at 350 nm and measuring emission at 444 nm.

***Determination of TVB-N content*:** TVB-N content was determined based on the adaptation of the current official European steam distillation method (Jinadasa, 2014).

Data analysis was done by using Microsoft excel software and Minitab statistical software (Version 17) was used to analyse the correlation.

Results

Table 2. Results of microbiological and chemical parameters of analysed samples; based on districts

Parameter	All Districts (n=174)	Colombo (n=68)	Gampaha (n=56)	Kalutara (n=50)
APC :cfu/g	2.5×10²-1.1 ×10⁸	4.0×10³ - 4.0×10⁷	2.5×10²- 4.0×10⁷	1.0×10⁴- 1.1×10⁸
Coliforms :MPN/g	ND - >1100	ND - >1100	ND - >1100	ND - >1100
F. coliforms :MPN/g	ND - >1100	ND - >1100	ND - >1100	ND - >1100
E.coli :MPN/g	ND - >1100	ND - >1100	ND - >1100	ND - 500
S. aureus cfu/g	<1.0×10¹	<1.0×10¹	<1.0×10¹	<1.0×10¹
TVN :mgN/100g	15.24 - 803	17.3 - 803	15.24 - 84.47	15.25 - 129.36
Histamine :mg/kg	0.29 - 250	1.1 - 250	0.29- 54.04	1.24 - 78.72

ND – Not Detected

In this study, APC of all the samples analysed were found in the range of 2.5×10^2 to 1.1×10^8 cfu/g having ranges from 4.0×10^3 – 4.0×10^7 cfu/g in Colombo District, 2.5×10^2-4.0×10^7 cfu/g in Gampaha District and 1.0×10^4- 1.1×10^8 cfu/g in Kalutara District (Table 2.). According to International Commission on Microbiological Specification for Foods (ICMSF, 1986), APC levels of fresh and frozen fish less than 5.0×10^5cfu/g are acceptable, APC levels between 5.0×10^5cfu/g and 1.0×10^7cfu/g are marginally acceptable and APC levels more than 1.0×10^7cfu/g are unacceptable. Fig.2. shows that in all three districts (of all the samples analysed) 43% of the samples had less than 5.0×10^5cfu/g of APC, which can be considered as acceptable, 42.5 % of samples contained APC between 5.0×10^5cfu/g -1.0×10^7cfu/g which are marginally acceptable and

14.5% of the samples contained greater than 1.0×10^7cfu/g of APC which exceeds acceptable quality. Percentage of acceptability of fish district wise is shown in the Fig. 2.

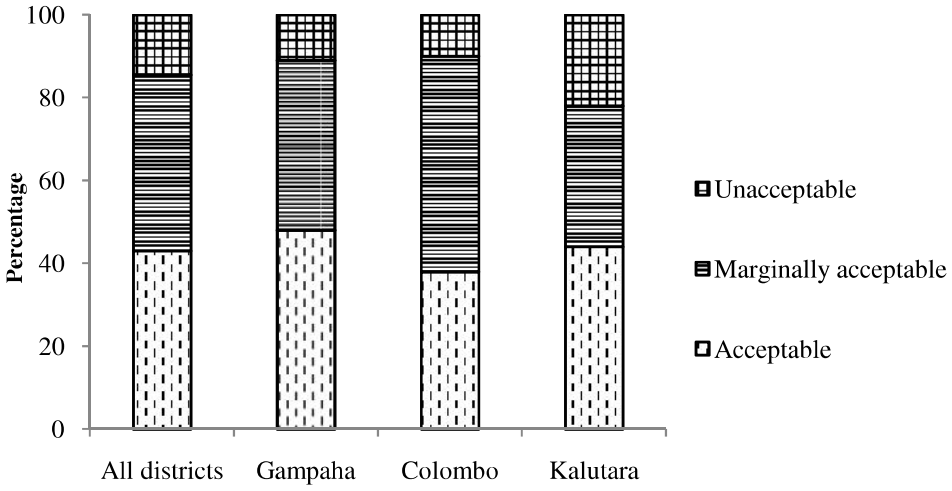

Fig 2. Acceptability percentages of fish on the basis of APC

Total coliforms, faecal coliforms and *E.coli* content of the analysed samples ranged from not detected to greater than 10^3MPN/g. According to ICMSF, (1986) recommendations fresh and frozen fish containing *E.coli* less than 11 MPN/g are acceptable, in between 11-500 MPN/g are marginally acceptable and more than 500 MPN/g are unacceptable. 34% of the samples contained greater than 10^3 MPN/g of total coliforms and 7% of samples had greater than 10^3 MPN/g of feacal coliforms. When considering these recommendations it can be stated that 69% of the samples had *E.coli* content less than 11 MPN/g which are acceptable, 28% of samples had between 11-500 MPN/g of *E.coli* which are marginally acceptable and 3% of the samples contained greater than 500 MPN/g which is unacceptable. Fig. 3. shows the acceptability percentages of fish based on the *E.coli* content in each district.

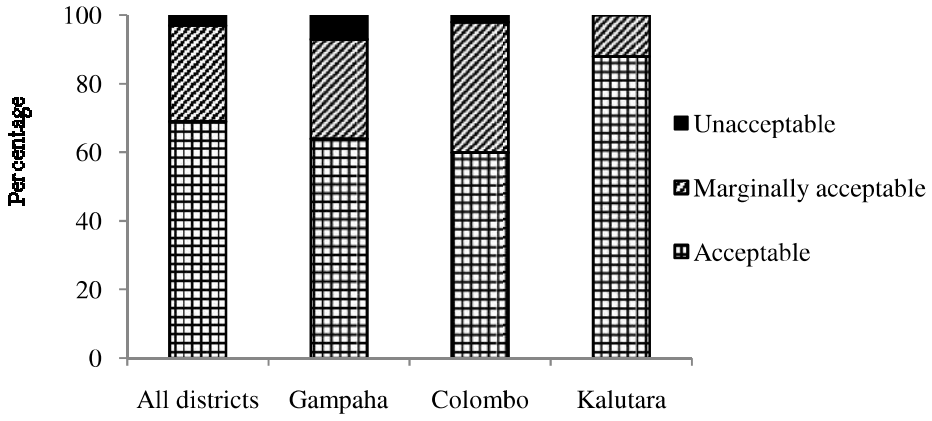

Fig. 3. Acceptability percentages of fish on the basis of *E.coli* in each district

Salmonella spp. was detected in 13% of the samples. Highest percentage was recorded in Gampaha District which is 25% (Fig. 4.). According to ICMSF, (1986) *Salmonella* spp. should not be present in any of the sample.

S. aureus was not detected in any of the sample analysed in this study.

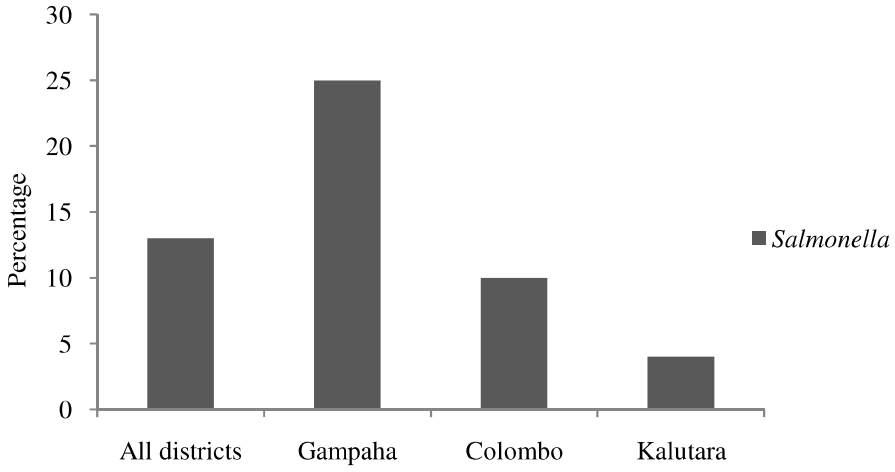

Fig. 4. Presence of *Salmonella* spp. in samples from each district.

TVB-N was found in the range of 15-803 mgN/100 g with 14% of samples containing greater than 35 mgN/100 g. Rest of the samples showed TVB-N content less than 35 mgN/100 g having 44% upto 25 mgN/100 g, 30% between 25 - 30 mgN/100 and 12% between 30-35 mgN/100. A cuttlefish sample from Colombo District recorded the highest value (803 mgN/100 g) for TVB-N. Although this sample contained a high

amount of TVB-N it had a value of 1×10^5 cfu/g for APC which is within the acceptable range for APC. When considering APC and TVB-N values there wasn't any correlation ($p>0.05$) between the two parameters. The average TVB-N values are greater than 35 mgN/100 g in cuttlefish, prawns and tuna spp., whereas cuttlefish samples contained the highest amount of TVB-N average value (Table 3.). Sixty percent of the prawn and 56% of cuttlefish samples exceeded the TVB-N rejection limit.

One percent of the samples contained greater than 100 mg/kg of histamine and rest of the samples contained histamine content of less than 100 mg/kg. Fig. 5 shows the percentages of acceptable samples with regard to histamine and TVB-N levels. Temperatures of fish ranged from 2°C -18.5°C. According to EU legal limits Histamine in food should be less than 100 mg/kg. Therefore it can be seen that 99% of the samples are within the histamine safe limit.

Table 3. Average TVB-N values obtained and percentage of unacceptable samples based on TVB-N levels

Fish type	**TVB-N content in mgN/100 g**	
	Average	**Unacceptable percentage (>35 mgN/100 g)**
Goldstripe sardinella (*Saalaya*)	23.0	0
Indian scad (*Linna*)	27.0	17
Cuttlefish (*Della*)	143.0	56
Sailfish (*Thalapath*)	23.5	0
Trevally (*Paraw*)	25.0	8
Herrings (*Hurulla*)	28.8	12.5
Tuna spp.	42.3	15
Prawns (*Issa*)	55.3	60
Indian mackerel (*Kumbalawa*)	26.0	0
Anchovy (*Haalmassa*)	24.0	0
Flyingfish (*Piyamassa*)	24.0	0
Rockfish (*Gal maalu*)	22.0	0

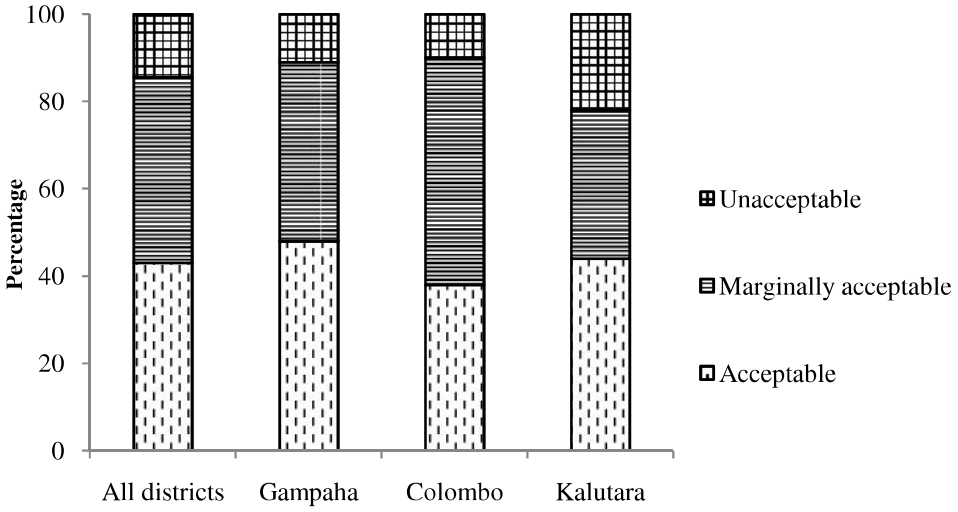

Fig. 5. Percentage of acceptable samples based on histamine and TVB-N levels

Discussion

The APC indicates that the level of microorganisms in a product (Maturine and Peeler, 1998) and does not relate to food safety hazards in fish and fishery products. However APC is useful to determine the product quality, shelf life and post heat processing contaminations. The results reveal the quality status of the fish.

E.coli can be found in aquatic environment which is recognized as an indicator organism of faecal contamination and is commonly isolated from seafood (Papadopoulou *et al.*, 2007). *E.coli* is the most common facultative anaerobic organism in the intestinal tract of humans and warm blooded animals. The main source of *E.coli* infections are contaminated water and contaminated food handlers. All *E.coli* strains are mesophilic with optimum growth at 37°C. They do not grow at cool temperatures and can be destroyed easily by heating (Huss *et al.*, 2004). Therefore, Coliform and *E.coli* contamination can be controlled by introducing good hygienic practices such as temperature control of seafood, good personal hygiene of fish handlers, usage of good quality water in washing fish and utensils etc. In a similar study conducted in Cochin, India *E.coli* was detected in 98.7% and 96.8% of fresh and frozen fish samples respectively (Nambiar and Surendran, 2003).

Salmonella are mesophilic bacteria present in gastrointestinal tract of humans and animals including birds. Environments such as water reservoirs contaminated with human or animal excreta may also harbour *Salmonella*. This organism is rarely detected in temperate waters but can occur in tropical waters and fish and shellfish from such waters (Huss *et al*., 2004). Since Sri Lanka is an island with tropical climate and surrounded by tropical waters, presence of *Salmonella* spp. in fish may be a result of cross contamination of polluted waters. Several studies had also reported the presence of *Salmonella* in fish sold in retail markets of Sri Lanka and India (Jinadasa *et al*., 2014; Nambiar and Iyer, 1990 ; Nambiar and Surendran, 2003) which shows that fish of tropical countries may contain *Salmonella.* In contrast studies conducted in Brazil, Greece and Spain reported that none of the samples contained *Salmonella* (Da Silva *et al*., 2010; Herrera *et al*., 2006; Papadopolou *et al*., 2006).

Although there wasn't any sample contaminated with *S. aureus* in this study, some studies have reported to isolate *S. aureus* at 2-10% in fish and bivalves but is much more commonly found in cooked, handled crustaceans at 24-52% levels (Da Silva *et al.*,2010). *S. aureus* is a part of normal human and animal microflora and may find in aquatic environment polluted with sewage. Presence of *S. aureus* in raw fish is less important since it cannot propagate in competition with natural microflora of fish and also it needs to occur in high concentrations to produce the disease causing amount of enterotoxin. Enterotoxigenic strains are most commonly transferred from food handlers with hand infections, cold or sore throat (Papadopolou *et al*., 2006 ; Huss *et al*., 2004).

According to quality classifications, TVB-N level of fish and fishery products containing up to 25 mgN/100 g can be considered as “high quality”, up to 30 mgN/100 g can be considered as “good quality”, up to 35 mgN/100 g can be considered as “limit of acceptability” and more than 35 mgN/100 g are considered as spoilt and TVB-N is produced mainly by bacterial decomposition of fish flesh and increases with storage time in unfrozen conditions (Ozyurt *et al*., 2009). In another study also of all the squid samples analysed, TVB-N content was more than the rejection limit (Jinadasa *et al*., 2014).

Formation of histamine is more rapid at high temperatures (21.1°C or higher) than at moderate abuse temperatures (7.2°C) (USFDA, 2011). In a previous study in Sri Lanka it has found that 8% of yellowfin tuna and 6% of sailfish had more than 100 mg/kg of histamine (Jinadasa *et al*., 2014). Even though fish were stored in moderate low temperatures, if the fish were subjected to high temperature abuse initially the histamine formation may become high. Once the histamine is formed it cannot be destroyed even the fish is stored in chill temperatures or after cooking.

Conclusions

Results of this study pertaining to microbiological quality show that 14.5% of the samples exceeded the acceptable limit of APC, 34% , 7% and 3% of samples exceeded the total coliform, faecal coliforms and *E.coli* acceptable levels respectively and 13% of the samples detected with *Salmonella* spp.. When considering the chemical parameters 14% of the samples were unacceptable with a high TVB-N content and 1% of the samples were unacceptable due to high histamine content. Only 21% of the analysed samples had acceptable values for all the parameters checked. Therefore it is quite clear that the quality of seafood should be improved. This can be done by adopting good handling practices and good chilling practices.

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