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Identification of Histamine producing enterobacteriaceae in Indian mackerel (*Rastrelliger kanagurta*) using Polymerase Chain Reaction

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ABSTRACT: Fish is vital source of protein consumed by the world's population. Fresh fish flesh is the most common source of high protein food and an important source of protein in human nutrition in Sri Lanka. Not only as a food but also it has a substantial social and economic importance. The major unavoidable problem facing by the sea food industries in Sri Lanka is the rapid spoilage of fish before delivering it to consumers. Fish may harbor a number of biohazards as well as chemical contaminations such as biogenic amines and pathogenic microorganisms.. Histamine is formed by free histidine available in large amount in the fish belonging to the scombridae family with the action of histidine decarboxylase enzyme. Gram negative enterobacteriaceae family microorganisms involve in histamine spoilage of fish and fish products. Rapid detection of histamine forming microorganisms is essential to ensure the product safety and minimize the product loss in the market. In this study of detection of histamine producing enterobacteriaceae family microorganisms, Indian mackerel *(Rastrelliger kanagurta)* collected from local markets were diluted in Maximum Recovery Diluent (MRD) and colonies were grown on Violet red Bile Glucose Agar (VRBG). Various sizes of colonies were used in Polymerase Chain Reaction (PCR) and positive results for the *hdc* gene which is responsible for the histamine formation was obtained in 36 isolates out of 163 tested. From PCR, Gram staining and biochemical test *Enterobacter spp , Klebsiella spp ,Proteus spp* and *Morganella morgini* were identified as histamine producing enterobacteriaceae in Indian mackerel.

Key words: enterobacteriaceae, fish, histamine, *hdc*, polymerase chain reaction

Introduction

Fish is a nutritious animal based article, which makes a vital contribution to the survival health of a significant portion of world's population. Fish, which is referred as "Rich food for poor people" provides essential nourishment especially quality proteins, essential fatty acids, vitamins and minerals. It contains a good quantity of protein about 18-20% and contains all eight essential amino acids (Roos *et, al*., 2007). By having omega 3 fatty acids, consumption of fish involves in decrease in the incidence of coronary heart disease (Erkkila *et al.*, 2004), blood

pressure (Mozaffarian *et al.*, 2003) serum triglycerides (Chrysohoou *et al.*, 2007) and cancer (Augustsson *et al.*, 2003) .

Sri Lankans have a huge appetite of fish, as it is the most important source of animal protein intake of people in Sri Lanka. Though the island is off the Southern tip of India and surrounded by sea, with the coastline of over 1700 km on all sides the consumption is heavily dependent on sea foods to satisfy the varying consumer needs (Steffen *et al.*, 2008). The seafood industry plays a significant role in the economy of Sri Lanka by providing livelihood for more than 2.5 million coastal communities as well as fulfils more than 50% of animal protein requirement of people in the country (Subasinge,2005)

The importance of fish as a protein based article is well known, however it is more susceptible to spoilage as it is perishable food stuff. Raw fish and seafood are a highly perishable compared to other fresh meat commodities and have short lifetimes even at refrigeration temperature (Jinadasa *et al.*, 2014). Avoidance of contamination and unnecessary quality loses are essential. Spoilage is typically accompanied by change in physical characteristics. Change in odour, texture, colour of eyes, colour of gills and softness of the muscle are some of the characteristics observed in spoiled fish (Gopakumar, 2000;342). Contamination may occur due to the presence of pathogenic bacteria. Spoilage of food products is due to enzymatic, chemical, or microbial activities accounts for one-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone (Ghaly *et al.*, 2010). Microbiological contamination has been noted as the main cause of fish deterioration. The number and nature of the pathogenic bacteria on fish are influenced by water pollution, method of harvest, post harvest applications, storage temperature and handling practices (Jayasinghe; and Rajakaruna, 2005). Spoilage of fish can occur rapidly after catch which starts within three hours of catch in high ambient temperature in tropical countries (Mahmoud *et al.*, 2006).

Fish naturally has enzyme and bacteria which do not cause any changes when it is alive. Upon death the bacteria start to invade the muscle and the proteolytic enzymes found in the gut area decompose the tissue and make undesirable changes and leads to enzymatic spoilage of fish. During the chemical spoilage of fish the polyunsaturated fatty acids found in fish undergo oxidative changes and results rancid flavour and odour as well as discolouration (Zhai *et al.*, 2012).

Post harvest handling plays a major role in introduction of microorganisms in fish. Improper storage temperature and packing, poor sanitary conditions of vessels, storage area and wash water used during degutting lead to cross contamination

and multiplication of microorganisms and consequently poor quality of fish are delivered to the consumers. Soon after death microorganisms on the surface, gills and gut of the fish utilize the nutrients specially protein and leads to nutritional loss. Activity of microorganisms causes undesirable changes in fish such as off flavours, texture and appearance. It is generally accepted that fish with microbial load of $>10^{6}$ cfu/ml is likely to be at the stage being unacceptable from the microbiological point of view and inappropriate for human consumption (Wogu and Maduakor, 2010).

Biogenic amines are low molecular weight, non volatile, aliphatic, heterocyclic organic bases which are formed in fish by microbial decarboxylation of amino acids (Al - Bulushi *et al.,* 2009). Histidine, tyrosine, tryptophan, ornithine and lysine are the most important free amino acids present in fish and converted into the biogenic amines histamine, tyramine, tryptamine, putrescine and cadaveride respectively (Biji *et al.,* 2016).

Histidine is an amino acid present in fish, which can help grow and repair body tissues and to maintain the myelin sheaths that protect nerve cells. It also aids in manufacturing blood cells and to protect the body from heavy metal toxicity in human beings. The bacteria, which produce histamine in fish commonly found in salt water environment. They naturally present in gills, surface and gut of live with no harm to the fish. The defence mechanisms of fish no longer inhibit the growth of bacteria in the muscle tissue upon the death of the fish. Histamine forming bacteria begins to grow resulting with the production of histamine (Taylor *et al.,* 2008).

Some species of fish have higher amount of naturally occurring histidine in their flesh. They represent the *Scombridae* family includes tuna, skipjack and mackerel. Histidine also presents in non scrombridae fish such as sardines, herrings and marlin. Fresh *Scombridae* fish does not contain histamine in their body tissue, however they have high level of free histidine in their muscle tissue, and those can be converted into histamine by histamine formers (Morrow *et al.,* 1991).

Histidine is converted into histamine by the microorganisms through the action of the enzyme histidine decarboxylase .There are two classes of histidine decarboxylase enzyme found in the microorganisms and cells with the difference of coenzymes associated with them. Gram negative bacteria and eukaryotic cells can be in grouped in one class where the second class is found in gram positive bacteria. Generally gram negative bacteria are associated with the histamine spoilage in fish while gram positive histamine forming bacteria are found in fermented products like wine, cheese, salami and sauerkraut (Coton and Coton ,

2004). Histamine forming bacteria have the capability of growing and producing histamine over a wide range of temperature. The optimum microbial activity found rapid between 32 - 37 ° C (FDA, 2001). Once the enzyme histidine decarboxylase is formed, it continuously acts on histidine and results in the formation of histamine even if the bacteria are inactive. The enzyme and bacteria can be inactivated by cooking. However once the histamine is formed it is difficult to eliminate it by the preservation methods like freezing, cooking and smoking (Koohdar *et al.*, 2011).

The Gram negative bacteria associated with the histamine spoilage are mainly comes from the species of Enterobactericiae. Members of the Enterobactericiae are gram negative rods, some of which are motile. Most species grow well at 37 ° C although some species grow better at 25 -30 ° C. They are distributed worldwide and can be found in soil, water, plants and animals (Bjornsdottir *et al.*, 2009). *Morganella morgini, Klebsiella pneumoniae, Proteus, Hafnia alvei, Salmonella, Escherichia coli* and *Enterobacter* are the major histamine formers in fish (Kim *et al.*, 2000).

Early detection of biogenic amine producing bacteria is important in the seafood industry as it could be a cause of food poisoning. Therefore the use of methods for the early and rapid detection of these bacteria is important for preventing biogenic amine accumulation in food products. Molecular methods for detection of food borne bacteria are becoming an alternative for traditional methods (Landate *et al.*, 2007).

Fast detection of gram negative histamine producers is important for preventing microbial contamination and high histamine levels during processing of fishery products. As histamine is the decarboxylation product of histidine catalyzed by the enzyme histidine decarboxylase, it is practicable to develop a molecular based technique to find out the gene (*hdc*) which is responsible for the production of this enzyme (Takahashi *et al.*, 2003).

Indian mackerel (*Rastrelliger kanagurta*) is one of the most commercially important small pelagic fish in the tropical region providing protein to the diet (Jamaluddin *et al.*, 2010). In the present study the presence histamine producing bacteria in Indian mackerel was identified using polymerase chain reaction, biochemical tests and Gram staining.

Methodology

Location

The study was carried out at the Quality control laboratory of Institute of Post Harvest Technology, National Aquatic Resources Research and Development Agency (NARA), Colombo, Sri Lanka.

Sampling

Forty five Indian mackerel (*Rastrelliger kanagurta*) fish samples were collected into sterile bags from local fish markets at Mattakuliya, Colombo 15 area over a period of four months. The samples were kept at chilled condition until subjected for the analysis.

Sample preparation

Muscle part surrounding the gut area was used for the analysis. 90 mL of Maximum Recovery Diluent (MRD) (Oxoid, England) was added to 10 g muscle of each fish and blended well in order to obtain a homogenized mixture. A series of tenfold dilution of each sample was made with MRD as diluents and blended sample as inoculums. 1.0 mL of each dilution was plated with 15 mL of Violet Red Bile Glucose (VRBG) Agar (Oxoid, England). The inoculated plates were incubated at 37 $\pm$ 1 °C for 48 hours.

Purification and conservation of isolates

For the identification of colonies various types of colonies were selected according their size and morphology and streaked on Nutrient agar (NA) (Oxoid, England). After incubating at 37 $\pm$ 1 °C for 24 hours total 163 isolates were streaked on NA slants and incubated at 37 $\pm$ 1 °C for 48 hours. They were used in Polymerase Chain Reaction (PCR), biochemical test and gram staining.

Extraction of DNA

The colonies of bacterial isolates from NA were mixed with 250 µL of sterile de ionized distilled water and well mixed by vortex mixer (Clifton cyclone, UK). The suspension was heated in a dry block heater (LabTec, Quebec) at 95 ° C for 10 minutes and immediate cooling was done on ice. Cell debris of cell lysates were pelleted by centrifugation (Tomos, UK) at 13 000 rpm for 3 minutes and supernatants were used as DNA templates for PCR assay for the detection of *hdc* gene for suspected isolates.

PCR analysis

The supernatant was used as DNA template and PCR was performed according to the protocol mentioned in Table 1

Table 1 : Protocol for PCR analysis

Components	Volume (µL)
DNA template	4.0
PCR water	12.25
5X buffer	1.75
2.5 mM dNTP	1.0
25 mM $MgCl_2$	5.0
Taq DNA polymerase	0.5
Forward primer	0.25
Reverse primer	0.25
Total volume	25.0

hdc forward primer – TCH ATY ARY AAC TGY GGT GAC TGG RG
hdc reverse primer – CCC ACA KCA TBA RWG GDG TRT GRC C

21 µL of PCR master mixture (excluding DNA template) was aliquot into each PCR tubes containing 4 µL DNA templates. The cycling conditions were initial denaturation at 94 ° C for 4 minutes, denaturation at 94 ° C for 1 minute, annealing at 58 ° C for 1 minute, extension at 72 ° C for 1 minute with the final extension at 72 ° C for 4 minutes at the end of 35 cycles.

Agarose gel electrophoresis

The PCR products were run on 2% of agarose gel (Sigma) in 1 X TAE buffer. The gel was stained with ethidium bromide. 10 µL of PCR products were loaded into sample wells and 90 V was used for 30 minutes. The gel was visualized and photographed under trans-illuminator.

Bio chemical test

The isolates were undergone for oxidase test, indole test, citrate test, urease test Vogeskar Prousker (VP) test, Triple Sugar Iron (TSI) reaction. The biochemical key shown in Table 2 was used to identify the species.

Table 2. Biochemical key for the identification of Enterobacteriaceae

Microorganism	Oxidaset est	Indole test	Citrate test	Urase test	VP test	TSI slant	TSI butt	H_2S production	Gas roduction
Proteus spp	-	-/+	-	+	-/+	R/Y	Y	+	+
Salmonella	-	-	+	-	-	R	Y	+	+
E coli	-	+	-	-	-	Y	Y	+	+
Klebsiella spp	-	-	+	+	+	Y	Y	-	+
Enterobacter spp	-	-	+	-/+	+	Y	Y	-	+
Morganella morgini	-	+	-	+	-	R	Y	-	+
Hafnia alvei	-	-	-	-	d	Y	Y	-	+

(+, 90 – 100% strains are positive, d, 26 -75% strains are positive, - , 0-10% strains are positive, R- Red, Y- Yellow)

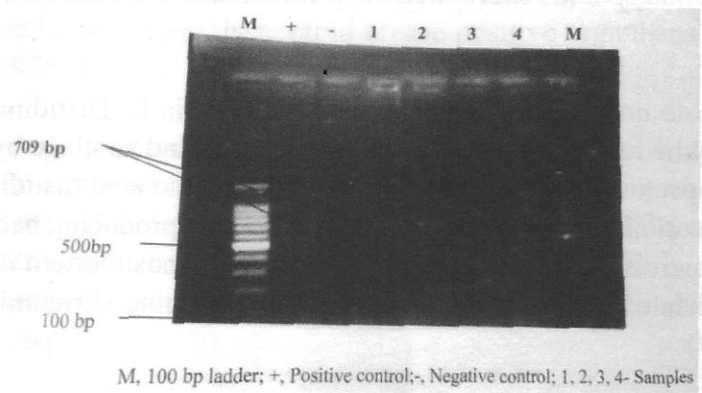
Gram staining was carried out for the isolates.

Results

The VRBG plates showed four different sizes of colonies. They were classified in as very small, small, medium and large colonies.

Polymerase Chain Reaction

hdc gene (709 bp) which is responsible for the synthesis of histidine decarboxylase enzyme was successfully amplified from 36 isolates out of 163 isolates checked. They were obtained from the small and very small colonies on VRBG plates.



M, 100 bp ladder; +, Positive control;-, Negative control; 1, 2, 3, 4- Samples

Fig 1. PCR assay detects the *hdc* gene (709bp)

Gram staining

Pink colour was obtained from Gram staining at *40 magnification and the colonies were confirmed as gram negative bacteria

In the present study from PCR analysis, biochemical test and gram staining following micro organisms were detected as the histamine producing enteric bacterial families in Indian mackerel.

Table 3. Biochemical identification of Enterobacteriaceae in Indian mackerel

Microorganism	Oxidase test	Indole test	Citrate test	Urase test	VP test	TSI slant	TSI butt	H_2S production	Gas production
Proteus spp	-	+	-	+	+	Y	Y	+	+
Klebsiella spp	-	-	+	+	+	Y	Y	-	+
Enterobacter spp	-	-	+	-	+	Y	Y	-	+
Morganella morgini	-	+	-	+	-	R	Y	-	+

(+, positive, -, negative, R-red, Y- Yellow)

Proteus spp and *Morganella morgini* were obtained from very small colonies while *Klebsiella spp* and *Enterobacter spp* from small colonies on VRBG plates.

Discussion

Fresh fish do not contain free histamine but contain L- Histidine. Additional histidine may be released during the decomposition and spoilage by proteolysis whereby the protein structure was degraded and amino acid histidine liberated. The contamination of fish after caught with histamine producing bacteria and the releases of microbial contents of the intestine due to postmortem disintegration results in muscle tissue and accumulation of histamine (Eitenmiller, and De Souza, 2009).

The family Enterobacteriaceae is primarily responsible for the decomposition of the scombroid fish. Among different enteric bacteria, *Morganella morgini, Klebsiella pneumoniae, Hafnia alvei Proteus vulgaris, Proteus mirabilis, Enterobacter aerogenes, Enterobacter cloacae, Serratia planticola, Serratia liquefaciens and*

Citrobacter freundii have been identified in past studies as histamine formers in fish (Tembhurne *et al.,* 2013).

From the present study enteric bacterial species in Indian mackerel were isolated on VRBG medium. This simultaneous presence of crystal violet and bile salts can inhibit the growth of gram positive bacteria. The degradation of glucose to acid is shown by the red colour of the pH indicator, natural red (Mossel *et al.,* 1993). For the future confirmation the colonies were identified by PCR, biochemical test and Gram staining.

The indole positive bacteria such as *Proteus, E coli* and *Morganella morgini* can produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethyl amino benzaldehyde) is added to a broth with indole in it, a dark pink colour develops. The indole test must be read by 24 hours of incubation as the indole can be further degraded if prolonged incubation occurs. The acidic pH produced by microorganisms limits their growth (Kornacki *et al.,* 2015; 670).

Urease broth is a differential medium that tests the stability of an organism to produce an exoenzyme, called urease that hydrolyses urea to ammonia and carbon dioxide. The broth contains two pH buffers, urea , a very small amount of nutrients for the bacteria and the pH indicator phenol red. Phenol red turns yellow in an acidic environment and fuchsia in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created the media turns in to pink in colour. Many enteric bacteria can hydrolyze urea. However only a few bacteria can degrade urea rapidly and Members of *Proteus* are included among these rapid urease positive organisms (Blazevic D and Ederer, 1975; 129).

Certain bacteria can produce stable acid end products when cultivated in specific media. After fermentation of glucose particular enteric bacteria metabolize pyruvic acid to acetyl methyl carbonyl. This end product reacts with alpha napthol in the presence of 40% KOH to produce a red colour complex. This is an important biochemical property used for the identification of *Klebsiella* and *Enterobacter* (Janda and Abbot, 2006; 110).

The TSI reactions are used for the fermentation of membrane of enteric bacteria based on their fermentation of lactose, sucrose, glucose and their production of H_2S. Many of the enteric organisms will ferment glucose with the production of acids which will change the colour of the medium in the butt and along the

slant from red to yellow because of a reduction in the pH with in the first few hours. However as the glucose is present in small amounts, the supply is soon exhausted and the organisms growing on the surface of the slant in the presence of oxygen are forced to catabolize peptones and amino acids for their energy supply. Alkaline end products are produced from these substances, which revert the pH of the slant to an alkaline pH and thus change the colour of the agar slant back to red after 18-24 hours.

Organisms such as *Salmonella spp* and other organisms, which attack glucose but do not ferment lactose or sucrose, will produce an alkaline slant and acid butt in TSI slants in 18 to 24 hours. Since metabolism is progressing at a slower rate in the butt, this reversion does not usually take place in the butt until 48 hours or longer. If the glucose is metabolize to CO_2, the gas will be seen as bubbles or cracks in the agar butt. If hydrogen sulphide is formed during growth, a gray or black streak of iron sulphide is seen originating where the inoculating needle entered and throughout the agar butt (Murray *et al.,* 2005; 258).

Rapid detection of pathogens and other microbial contaminants in food is critical for ensuring the safety of consumers. Traditional methods to identify food borne micro organisms often rely on time consuming cultivation in diverse type of culture media followed by a considerable amount biochemical testing. Recent advances in methodology make detection and identification faster, more convenient, more sensitive and more specific than conventional assays. The new rapid methods include antibody based assays, DNA probes, modification of conventional kits and automated identification system.

The new molecular tool applied in this study was able to be easily determined whether *hdc* genes were present or not in the bacterial isolates from Indian mackerel. It provides valuable information on their potential to form histamine. This fast and reliable test, allowing for the simultaneous detection of the genes involved in the main biogenic amine production directly on colonies. The food producing industries are in a potential to microbiologically and chemically check their raw materials before processing. PCR method can be used for the rapid identification of these pathogens. The end products also can be checked in same manner. In raw food items Gram negative bacteria are the main histamine formers while Gram positive bacteria are responsible for histamine production in fermented food items (Bremer *et al.,* 2003).

The fish is mainly spoiled due to the poor post harvest handling. Evisceration and removal of the gills after catch can reduce the number of histamine for bacteria but not eliminate the whole (Viscian *et al.*, 2012). In a study Thadhani *et al.*, 2001 studied the effect of Garcinia (*Garcinia cambogia)* extract in histamine reduction and found that the extract can inhibit the formation on histamine but not destruct the formed histamine. With the use of extracts of garcinia, tamarind and bilimbi Gunaratna *et al.*, 1997 found that the destruction of histamine by fruit extracts or fruit pieces in water is probably associated with the acidic nature of the fruits or specific anions of the acids in fruits which may interact chemically with the histamine.

Maintaining the proper storage temperature, placing the fish under refrigerated temperature as soon as it is caught, thawing the fish in refrigerated temperature if the fish is frozen, hygienic transport, aseptic packing, usage of hygienic vessels and wash water are some of the best practices followed to prevent the histamine spoilage in fish.

Sri Lankan fishery sector needs 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 (Ariyawansa *et al.*, 2016).

Conclusion

The Violet Red Bile Glucose Agar (VRBG) can be successfully used to isolate the enteric bacteria. The small colonies in the VRBG plates were identified as *Enterobacter spp* and *Klebsiella spp* while the very small colonies were identified as *Proteus spp* and *Morganella morgini.* From the polymerase chain reaction (PCR), biochemical test and Gram staining *Enterobacter spp, Klebsiella spp, Proteus spp and Morganella morgini* were identified as histamine producing enterobacteriaceae in Indian mackerel. Furthermore the results concluded that PCR is a suitable method to detect histamine producing enterobacteriaceae as it is efficient, reliable and faster compared to traditional methods.

References

1. Al - Bulushi, I., Poole, S., Deeth, H.C and Dykes, D.A. (2009). Biogenic amines in fish: Roles inintoxication, spoilage and nitrosamine formation : A Review, *Critical Reviews in Food Science and Nutrition*, 49, 369-377.
2. Ariyawansa, S., Ginigaddarage, P., Jinadasa, K., Chandrika, J.M., Arachchi, G.G and Ariyaratne, S. (2016). Assessment of Microbiological and biochemical quality of fish in a supply chain in Negombo, Sri Lanka, *Procedia Food Science*, 6, 246-252.
3. Augustsson, K., Michaud, D.S., Rimm, E.B., Leitzmann, M.F., Stampfer, M.J and Willett, W.C. (2003). A prospective study of intake of fish and marine fatty acids and prostate cancer. *Cancer Epidemiology Biomarkers and Prevention,* 12 (1), 64-67.
4. Biji, K.B., Ravishangar, C. N., Venkateswarlu, R., Mohan, C.O and Gopal, T.K.S. (2016). Biogenic amines in Sea food: A review, *Journal of Food Science and Technology*,53 (5), 2310-2318.
5. Bjornsdottir, K., Bolton, G.E., Green, P.D.M., Jaykus, L,A and Green D.P. (2009). Detection of gram negative histamine producing bacteria in fish : A comparative study, *Journal of Food Protection*, 72 (9), 1987-1991.
6. Blazevic, D and Ederer, C. (1975). *Principles of biochemical tests in diagnostic Microbiology,* New York: Willey.Bremer, P.J., Fletcher,G.C and Osborne, C (2003). *Listeria monocytogenes* in seafood, New Zealand Institute for Crop and Food Research Limited. 1- 13.
7. Chrysohoou, C., Panagiotakos, D.B., Pitsavos, C., Skoumas, J., Krinos, X and Chloptsios, Y. (2007). Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region: the ATTICA study. *American Journal of Clinical Nutrition*, 85(5),1385-91.
8. Coton, E and Coton , M. (2004). Multiplex PCR for colony direct detection of Gram-positive histamine- and tyramine-producing bacteria, *Journal of Microbiological Methods*, 63 (3), 296–304.
9. Eitenmiller, R.R and De Souza, S.C. (2009). Enzymatic Mechanisms for Amine Formation in Fish, *Sea food toxins*, 36, 431-442.
10. Erkkila, A.T., Lichtenstein A.H, Mozaffarian D and Herrington D.M. (2004). Fish intake is associated with a reduced progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease, *American Journal of Clinical Nutrition*, 80 (3), 626-32.
11. FDA. (2001). Scombrotoxin (histamine) formation. Ch. 7. In Fish and Fishery Products Hazards and Controls Guidance. 3rd ed., p. 83-102. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

12. Ghaly, A.E., Dave, D., Budge, S and Brooks, M.S. (2010). Fish Spoilage Mechanisms and Preservation Techniques: Review , *American Journal of Applied Sciences*, 7 (7), 859-877.
13. Gopakumar, K. (2000). Enzymes and Enzyme products as Quality Indices. Seafood Enzymes. New York, Basel, U.S.A.
14. Gunaratne,S., Samarajeewa,U . , Fonseka, T.S.G and Ranjani, I,V. (1997). The Effect of Cooking Ingredients on Histamine in Fish, *Tropical Agricultural Research* ,9,69-76.
15. Jamaluddin ,J.A.F, Ahmad ,A.T, Basir, S and Rahim, M.A. (2010). Rastrelliger systematics inferred from mitochondrial cytochrome b sequences, *African Journal of Biotechnology*, 9,3063-3067.
16. Janda, J. M and Abbot, S,L. (2006). The Enterobacteria, Washington : ASM Publishers.
17. Jayasinghe, P.S and Rajakaruna, R.M.A.G. (2005). Bacterial contamination of fish sold in fish markets in the central province of Sri Lanka, *Journal of National Science Foundation Sri Lanka*, 33 (3), 219-221.
18. Jinadasa, B.K.K.K., Ginigaddarage, P.H and Ariyawansa, S. (2014). A Comparative Quality Assessment of Five Types of Selected Fishes Collected from Retail Market in Sri Lanka, *American Journal of Food Science and Technology*, 2 (1), 21-27.
19. Kim, S. H.,Gigrey, B.B., Velauez, B.J., Price, R.J and An, H. (2000). Histamine and biogenic amine production by *Morganella morgini* isolated from temperature-abused albacore, *Journal of Food Protection,* 63 (2), 244-251.
20. Koohdar, V.A., Razavilar, V., Motalebi, A.A., Mosakhani, F and Valinassab, T. (2011). Isolation and Identification of Histamine-forming bacteria in frozen Skipjack tuna (Katsuwonus pelamis), *Iranian Journal of Fisheries Sciences*, 10 (4), 678-688.
21. Kornacki,*J.L.,* Gurtler, *J,B and* Stawick,*B.A.* (2015). *Compendium of Methods for The Microbiological Examination of Foods,* American Public Health Association.
22. Landete, J.M., De Las Riivas, B., Marcobal, A and Munoz, R. (2007). Molecular methods for the detection of biogenic amine producing bacteria on foods, *International Journal of Food Microbiology,* 117, 258-269.
23. Mahmoud, , B.S.M., Yamazaki, K., Miyashita, K., Shin, I.I and Suzuki, T. (2006). A new technology of fish preservation by combined treatment with electrolyzed NaCl solutions and essential oil compounds, *Journal of Food Chemistry*, 99, 656-662.
24. Morrow, J.D.M.D., Margolies,G.R.M.D., Rowlands, J.B.S and Roberts, L.J. (1991). Evidence that histamine is the causative toxin of scombroid fish poisoning, *The New England Journal of Medicine*, 716-720.

25. Mossel, D.D.A., Wisser, M and Cornelissen, A.M.R. (1993). The examination of foods for enterobatericeae using a test of the type generally adopted for the detection of *Salmonella*, *Journal of Applied Bacteriology*,24, 444-452.
26. Mozaffarian, D ., Lemaitre, R.N., Kuller, L.H., Burke, G.L., Tracy, R.P and Siscovick,D. S. (2003). Cardiac benefits of fish consumption may depend on the type of fish meal consumed the cardiovascular health study, *Circulation*, 107(10),1372-1377.
27. Murray, P.R., Rosenthal, K.S and Pfaller,M.A. (2005). Medical Microbiology, 6th Ed, Philadelphia: Elsevier Mosby.
28. Roos, N., Wahab, M.A., Chamnan, C and Thilsted, S.H. (2007). The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries, *Journal of Nutrition,* 137 (4), 1106-1109.
29. Steffen, L. M., Folsom, A.R., Cushman, M., Jacobs, D.R and Rosamond,W.D. (2008). Greater fish, fruit, and vegetable intakes are related to lower incidence of venous thromboembolism: the longitudinal investigation of thromboembolism etiology, Circulation, 115 (2), 188-95.
30. Subasinge, S. (2005). Tsunami redefining seafood consumption pattern in Sri Lanka, *Infofish*,1-3.
31. Takahashi, H., Kimura, B.,, Yoshikawa, M., and Fuji, T. (2003). Cloning and sequencing of the histidine decarboxylase genes of Gram negative histamine producing bacteria and their application in detection and identification of these organisms in fish, *Journal of Applied Environmental Biology,* 69, 2568-2579.
32. Taylor, S.L., Statton, J.E and Nordlee, J.A. (2008). Histamine Poisoning (Scombroid Fish Poisoning): An Allergy-Like Intoxication, *Journal of Toxicology,* 27, 225-240.
33. Tembhurne, M., Ghag, A., Sanathkumar, H and Nayak, B.B. (2013). Dominance of Enterobacteria among Histamine-Producing Bacteria Isolated from Indian Mackerel, *Advances in Microbiology*, 3(7), 537-542.
34. Thadhani, V.M., Jansz, E.R., Peiris, H. (2001). Destruction of histamine by cooking ingredients – An artifact of analysis, *Journal of Nation Science Foundation of Sri Lanka,* 29, 3&4, 129-135.
35. Viscian,P., Schirone, M., Tofalo, R and Suzzi, G. (2012). Biogenic amines in raw and processed sea food, *Frontiers in Microbiology,* 3, 188-197.
36. Wogu, M,D and Maduakor, C,C. (2010). Evaluation of Microbial Spoilage of Some Aquacultured Fresh Fish in Benin City Nigeria,*Ethiopian Journal of Environmental Studies and Management,* 3 (3), 18-22.
37. Zhai , H.,Yang,X.,Li,L.,Xia,G.,Cen, J.,Huang,H.,andHao,S.(2012). Biogenicamines in commercial fish and fish products sold in Southern China. *Journal of Food Control,* 25, 303–308.