Determination of Quality of Marine Fishes Based on Total Volatile Base Nitrogen test (TVB-N)

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Abstract: This study was carried out to evaluate the quality of five marine fishes yellowfin tuna (*Thunnus albacores,* Bonnaterre, 1788), sailfish (*Istiophorus platypterus,* Shaw, 1792), slaya/sardine (*Sardinella gibbosa*, Bleeker, 1849), squid (*Loligo duvaucelii,* D' Orbigny, 1835) and shrimp (*Fenneropenaeus indicus*, H. Milne Edwards, 1837) based on total volatile base nitrogen test (TVB-N). The studied fish was collected from 19 commercial retail outlets in Sri Lanka in 2012, kept under ice and immediately transferred to laboratory for further analysis. The total volatile basic nitrogen (TVB-N) test was performed to assess the quality status of fresh fish. The limits of TVB-N for fishery products in Commission Regulation (EC) NO 2074/2005, which was ranged between 25 to 35 milligrams of nitrogen/ 100grams of fish flesh. The results of the present study revealed that base the total volatile base nitrogen (TVB-N) content, 5% of yellowfin tuna, 8% of sailfish, 12% of sardine, 100% of squids and 42% shrimp were not fit for the human consumption.
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Key words: quality, total volatile base nitrogen (TVB-N), retail fish market

1. Introduction:

A well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children's proper growth and development. Fish are a great source of protein, vitamins, and minerals and omega-3 fatty acids, a key nutrient for brain development (Spencer et al., 1971, Jaclyn et al., 2010)

Fisheries is an important role of international trades and food sector, currently capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 and with a total value of US$217.5 billion, of which about 128 million tonnes was utilized as food for people. With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period 1961–2009, outpacing the increase of 1.7 percent per year in the world's population (FAO, 2012). Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits and action of autolysis enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002).

Sri Lanka is an island in Indian Ocean, which has good opportunity for fisheries. Its exclusive Economic Zone area is about 517,000 km^2 , and the length of coastal line is about 1817 km with a continental shelf of about 30,000 km^2 (width from 22-40 km). Sri Lankan fisheries sector is mainly depended on the marine capture fishery, followed with aquaculture. In 2012 our total marine capture fishery production was 417,220 MT, about 257,540 coming from the coastal fisheries and the rest (about 159,680 MT) from the deep sea fisheries. The value of aquaculture production in same year was 68,950 MT (MOFAR, 2013).

The quality of fish landings in the country is generally poor and wastage is high, especially in catches of multi-day boats. About 25–30 % of the catch landed by these boats is of poor quality, as the fish holds of these boats are not refrigerated. These boats aim at quantity rather than quality, and sell the poorer quality or spoiled fish to dried fish processors at a low price. Lack of knowledge regarding improved fish handling and post-harvest practices has contributed to the poor quality of fish and fishery products (FAO, 2013). Thus, fishery products have also been recognized as carriers of health hazards such as disease causing microorganisms Salmonella sp. and *Vibrio* sp., in addition to parasites, natural toxins, heavy metals and other pollutants (Venugopal, 2002). The increasing demand in the commodity as well as the need to protect the consumers against food-borne diseases have made regulatory authorities such as the United States Food and Drug Administration and the European Union stipulate stringent guidelines with respect to the quality of processed fish items traded by exporting countries. Hence, many developing countries attempt to set up standards for their fishery product in order to comply with requirement of international trade and markets (Sulieman *et al*., 2012).

Fish quality is a complex concept involving a whole range of factors, which for the consumer include for example: safety, nutritional quality, availability, convenience and integrity, freshness, eating quality and the obvious physical attributes of the species, size and product type. Information about handling, processing and storage techniques, including time/temperature histories that can affect the freshness and quality of the products is very important for the partners in the chain (Abbas *et al*., 2008) . The quality of fish can be estimated by sensory tests, microbial methods or by chemical methods such as measuring volatile compounds, lipid oxidation, determination of ATP breakdown products and the formation of biogenic amines (Gulsun *et al*., 2009). Total volatile basic nitrogen (TVB-N) is important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage (Amegovu *et al*., 2012, Wu and Bechtel, 2008). A European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt as to the freshness of the fish, inspectors must use TVBN as a chemical check (Castro *et al.*, 2006).

Total volatile bases (TVB) is a group of biogenic amines formed in non-fermented food products during storage (Horsfall *et al*., 2006). The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish (Wu and Bechtel, 2008). The increase in the amount of TVB parallel with the increase in TMA during spoilage. As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA (Yusuf *et al.*, 2010). The source of DMA and TMA in fresh and processed fishery products is trimethylamine oxide (TMAO). They are believed to use it to increase osmotic concentration and thus depress the freezing point of body fluids (Castro *et al.*, 2006). Post-mortem degradation of TMAO and the subsequent accumulation of volatile amines play a large role in the quality loss of the fish products due to the objectionable odors associated with the degradation products. The formation of TMA from the reduction of TMAO is cause by bacterial degradation. Dimethylamine formation is produced during frozen storage and does not have a pungent odor compared to TMA and ammonia, and is not linked to bacteria degradation (Wu and Bechtel, 2008).

To date many analytical methods have been developed for quantitative measurements of TVB-N and TMA-N concentrations. They include steam distillation, colorimetric, photometry, high performance liquid chromatography, gas chromatography, capillary electrophoresis, semi-conducting metal oxides sensors, selective electrode as well as flow injection/gas di□usion (FIGD) with spectrophotometric or potentiometric detection. Among all these methods, steam distillation and the colorimetric method of Dyer (1945) are the most known and widely used procedures for TVB-N and TMA-N determinations (Abbas *et al.*, 2008, Wu and Bechtel, 2008, Capillas *et al*., 2001, Capillas *et al.*, 2000, Malle and Poumeyrol, 1989, Venugopal, 2002).

The aim of this study was to investigate the freshness quality of yellowfin tuna, sailfish, sardine, squid and shrimp from retail market of Sri Lanka, in terms of TVB-N values. It is also anticipated that the determination of the freshness quality of these species will serve as a base for further comparison of the quality of these fish handled under commercial conditions.

2. Material and method:

A total of 155 fresh fish samples (37 of yellowfin tuna, 36 of sailfish, 26 of sardine, 36 of shrimp and 20 of squid) were purchased in February to December 2012 from 19 places around local Sri Lanka (Fig. 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 polythene bags, recorded surface temperature using IR thermometer, stored in ample amount of ice in insulated boxes, and transported to Institute of Analytical Chemistry Laboratory, National Aquatic Resources Research and Development Agency. The fish samples were headed, eviscerated (if necessary), thoroughly washed to remove the blood and carefully filleted and homogenized using food homogenizer.

All chemicals/reagents used were of analytical-reagent grade and were purchased from Sigma chemicals (Switzerland). Distilled, deionized water was used throughout this investigation.

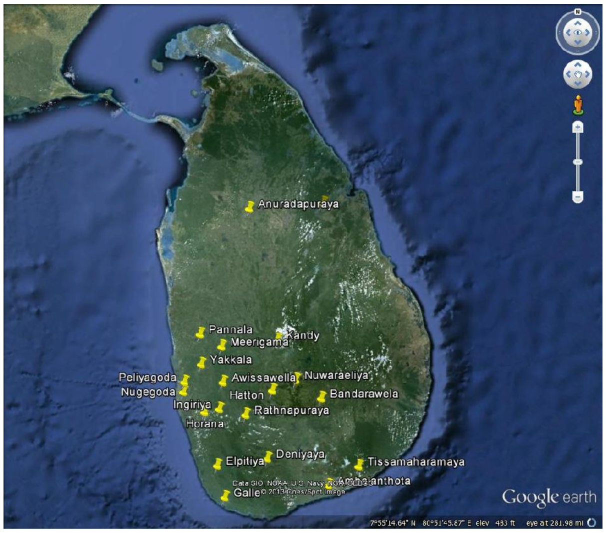

Fig. 1: Map of the sampling place

Determination of TVB-N by the o□cial method TVB-N was determined based on an adaptation of the current o□cial European steam-distillation method (O□cial Journal, 1995). The method is based on the extraction of TVB using alkaline solution and the titration of the recovered ammonia as follows: 100 g of flesh of homogenized fresh fish sample was weighed and blended with 200 ml of 7.5% tricholoroacetic acid in 2 mins. The blend was then filtered through Whatman no 1 filter paper to obtain clear extract. Then 25 ml of the extract was pipetted into the distillation tube and placed in the distillation flask in VELP mark semi auto distillation aparature (model UDK-6, Milan, Italy). Then program was started and 30 ml of 10% NaOH and 100 ml of distilled water were added, the apparatus 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 100 ml of distillate had been 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 of with 25 ml of distilled water sample. The recovery of method was calculated using the spiking of 4 hrs dried (105°C), $(NH_4)_2SO_4$ to recovery calculation.

$$\mathrm{TVB-N}\left(\frac{\mathrm{mg}}{100}\mathrm{g}\right) = \frac{14\,\frac{\mathrm{mg}}{\mathrm{mol}}\ \mathrm{x\ a\ x\ b\ x\ 300}}{25\ \mathrm{mL}}$$

a = mL of Sulfuric acids
b = Normality of Sulfuric acid

The five types of samples were extracted and analyzed in duplicate. Analysis values were averaged for each duplicate and the means were derived from an analysis of variance run on Statistical Package for Social Sciences (SPSS 16, Chicago, USA) and Microsoft Excel 2011 software. For tests of statistical significance between TVB-N values the data was subjected to Tukey's test for significant differences ($p < 0.05$).

3. Results and discussion:

Quality assurance procedures included the use of analytical grade reagents, method blanks and matrix spikes. The suitability of the analytical method was evaluated in terms of their respective limits of detection, precision and recoveries using spiked samples. The recoveries were maintained between 90-110%. The spiked results obtained from fish samples indicated that the methods used were suitable for the determination of metal concentrations in the samples investigated.

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: The surface temperature and percentage of fish samples displayed below 4°C

	Avg. Surface temperature, °C	% of 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

Temperature is the single most important factor affecting post-harvest quality of the products. It is often critical to reach the desired short-term storage temperature rapidly to maintain the highest visual quality, flavour, texture, and nutritional content of fresh fish. 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 and maintains essential quality. The proper storage temperature after receipt is critical to maintaining product safety and 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) . Proliferation of microorganisms requires appropriate high temperatures, while at lower temperatures close to 0°C; their activity is reduced, thereby extending the shelf life of fish products. Most microorganisms that cause food borne illness to typically grow best between 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.

Total volatile basic amines (TVB) are one of the most widely used measurements of seafood quality and appear as the most common chemical indicators of marine fish spoilage (Zhong-Yi et al., 2010). It is a general term, which includes the measurement of tri-methylamine (produced by spoilage bacteria), di-methylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the de-amination 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. The concentration of TVB-N in freshly caught fish is typically reported to vary between 5 and 20 mg/100 g (Muhammet and Sevim, 2007) . The TVB-N values increased according to time of storage (Gulsun et al., 2009) proposed that the quality classification of fish and fish products regarding TVB-N values would be ‘‘high quality” up to 25 mg/100 g, ‘‘good quality” up to 30 mg/100 g, ‘‘limit of acceptability” up to 35 mg/100 g, and ‘‘spoilt” above 35 mg/100 g (Amegovu et al., 2012, EU/EC, 2008, Gulsun et al., 2009, Huss, 1995). Meanwhile, (Kimura and Kiamukura, 1934) recommended TVB-N levels of 10 mg/100g or less for fresh fish, 20–30mg/100g for beginning of spoilage and over 30mg/100g for spoiled fish. In other hand some researchers suggested that limit of TVBN from 25 to 35 mg N per 100 g of muscles have also been proposed for rejecting commercial fresh whole fish and processed fish products (Connell, 1995, Dalgaars, 2000). (Reilly et al., 1985) stated that TVB-N is not reliable as indices of quality, but for most markets, TVBN is a determinant of quality of fresh fish because of its close relationship with sensory score and bacterial counts (Amegovu et al., 2012). (Carol et al., 2001), pointed out that the determination of TVB-N does not help to discriminate among the early stages of deterioration in fish however, in the latter stages of deterioration, they represent quite sensitive indices. TVB-N determinations could therefore be routinely used as standard methods for indicating the approach of shelf-life limits in wet fish. (Boee et al., 1982) working on the storage of shrimp also has observed on the storage of shrimp has observed that TVB-N increased evenly.

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. Since TVB-N is produced mainly by bacterial decomposition of fish flesh, thus higher values of could account for the higher TVB-N values of squids.

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 (Haaland and Njaa, 1988). Its amount increases with time of storage in the unfrozen state (Bechtel et al., 2010). Tri-methylamine originates from bacterial decomposition. The presence in fish is therefore, taken as an indication 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 shell-fishes spoil more rapidly compared with other fishes, mainly because they are small. Second, as their guts are not removed immediately after harvesting, shell-fishes are prone to early autolytic spoilage (Ashie *et al*., 1996).

According to the literature, the ventral part of fish had the highest TVB-N value, whereas the TVB-N value was the lowest in the dorsal part of fish (Fatih and Yesim, 2000). But, in the practical reason, unable to take whole fish from the large fish like yellowfin tuna and sailfish. The quality and storage life of fish may decrease if they have not been gutted. Fish contain many bacteria in the digestive system and strong digestive enzymes are produced during the feeding periods, which will be able to cause rapid autolysis post-mortem during the later stage of storage. This may give rise to a strong off-flavour, which might be related to the breakdown of protein and the production of nitrogenous volatile materials, especially in the ventral area, or even cause belly-burst (Huss, 1995).

In sum, TVB-N is insensitive to "freshness", which means that it cannot be used as a freshness indicator. However, it is sensitive in terms of "fitness" for human consumption, and it is also a good spoilage indicator (Fatih and Yesim, 2000). But traditionally, the evaluation of fish quality has been based on organoleptic tests. Chemical assessment of spoilage of fishery products dates back to about a century ago when the determination of the volatile nitrogenous bases (TVB-N) was published as standard method for the inspection of fish. Since then, TVB-N has been widely applied to assess the quality of fish and seafood (Maike and Bo, 2001, Carol *et al*., 2001).

Conclusions:

This study gives a clear perspective on the variation of TVB-N quality comparatively in five fishes collected from retail markets of Sri Lanka. Considering the analysed TVB-N, 5% of yellowfin tuna, 8% of sailfish, 12% of sardine, 100% of squids and 42% shrimp samples were not good for human consumption. Based on this conclusions and filed observations, this study recommended promotion of sanitation and hygiene campaign in all fresh fish outlets whether local or super markets including fresh fish vendors through regular visitations and training by quality regulators.

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