



Quality and Freshness of Fish available in Super markets of Cochin, India based on Biogenic Amine content

S. J. Laly^{1*}, T. K. Anupama², K. Ashok Kumar² and T. V. Sankar³

¹Mumbai Research Center of ICAR-Central Institute of Fisheries Technology, Navi Mumbai - 400 703, India

²ICAR-Central Institute of Fisheries Technology, P.O. Matsyapuri, Cochin - 682 029, India

³Kerala University of Fisheries and Ocean Studies, Cochin

Abstract

Quality and freshness of thirty nine samples of fresh and frozen fish sold in the super markets of Cochin were determined in terms of biogenic amine content, biochemical and microbial parameters. The contents of putrescine, cadaverin, histamine, agmatine, tyramine, spermine and spermidine were analysed and it showed a wide variation with higher content of spermine and cadaverine. Histamine content was less than 5 mg kg⁻¹ (Range: Nd - 2.19±0.6 mg kg⁻¹). Putrescine, cadaverine, agmatine, tyramine, spermidine and spermine were in the range of Nd - 7.72±1.7 mg kg⁻¹, Nd - 52.8 mg kg⁻¹, Nd - 6.22 mg kg⁻¹, Nd - 4.34 mg kg⁻¹, Nd - 51.8 mg kg⁻¹ and 0.21- 43.95 mg kg⁻¹ respectively. Levels of putrescine and cadaverine were highest in *Rastrelliger kanagurta* and *Lethrinus nebulosus*. In the present study 10.3% of samples exceeded Biogenic amine index (BAI) value of 20 mg kg⁻¹. Around 7.7% of samples were having TVBN content more than 30 mg% and 10.3% of samples were having APC greater than or equal to 7 log cfu g⁻¹. As indices based on biogenic amines are not highly correlated with other quality indices, assessment of biogenic amine indices along with other quality indices for fresh fish can be recommended.

Keywords: Biogenic amines, histamine, quality indices, freshness, fresh fish

Introduction

Food industry, of late is mainly focused towards health, nutrition and convenience. Seafood is a

healthy source of high-quality proteins, essential vitamins, minerals and beneficial polyunsaturated fatty acids and has created a considerable attention as food among people (Ashie et al., 1996) and the consumption is increasing worldwide. Susceptibility of fish to spoilage is very high as it is highly nutritious and the spoilage of fish is controlled by many extrinsic and intrinsic factors. Freshness quality must be maintained properly by proper preservation method as the spoilage process starts immediately after catch. Raw material quality, cooling methods, processing, packaging, transporting and storage conditions should be maintained properly to increase the freshness and shelf life of fish (Lauzon et al., 2010). It will be a major challenge to assess the traceability of seafood marketing channels and time - temperature control during distribution of raw material specifically in the domestic market. There is a huge difference in freshness and appearance of fish coming from traditional country crafts to multi day trawlers. In case of traditional local market retailers, the inadequacy of hygiene facilities in market can affect the keeping quality of raw material. While in the case of super markets, availability of adequate hygienic facilities including cold storage makes the environment more convenient in maintaining the supreme quality of freshness.

Biogenic amines are formed in fish and other food by microbial decarboxylation of amino acids. They are of concern in relation to food hygiene and public health, since they can be the result of activity of contaminating micro flora. Fish muscle can support various bacterial groups responsible for the production of a wide variety of amine compounds from respective amino acids. Biogenic amines are non-volatile aliphatic, alicyclic, and heterocyclic organic bases of low molecular weight. The formation of biogenic amine can depend upon amino acid

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*E-mail: lalyjawahar@gmail.com

content of fish, presence of bacterial biogenic amine decarboxylases and favourable environmental conditions (Brink et al., 1990).

Biogenic amines formed in fish mainly include putrescine, cadaverine, histamine, tyramine, agmatine, spermine and spermidine. Putrescine, cadaverine, spermidine and spermine have an aliphatic structure, histamine and tryptamine have a heterocyclic structure and tyramine and phenylethylamine have an aromatic structure. The primary reason for amine determination in foods is due to their potential toxicity and significance as food quality indicators. Amines like histamine, cadaverine, putrescine and tyramine are very important from food safety point of view besides being chemical indicators of fish spoilage (Lehane & Olley, 2000; Kim et al., 2009). Biogenic amines at low concentrations are essential for many physiological functions, while ingestion of large amounts may result in health problems like amine toxicity which depend mainly on the individual efficiency of detoxification (Shalaby, 1996). Toxicological effects to consumers include hypertension, headache, diarrhea, rash, and localized inflammation when ingested in extreme amounts, cardiac palpitation and even death in very severe cases (Rawles et al., 1996). Biogenic amines can be considered as precursors of carcinogenic amines such as N-nitrosamines, and they are also used as indicators of food decomposition (Mietz & Karmas, 1978). The detoxification system in the body can degrade biogenic amines to less active form by the action of diamine oxidases and monoamine oxidases in the system. Whenever there will be a high intake of biogenic amines it can result deactivation of the detoxification system. In situations of insufficient enzyme activity due to disease, secondary drug or medicinal effect even low amounts of biogenic amines cannot be metabolized efficiently (Bodmer et al., 1999).

The most important and frequent intoxication caused by biogenic amines in seafood involves histamine produced by decarboxylation of amino acid histidine. It can elicit allergy-like symptoms and is usually related to the consumption of scombroid fish such as tuna or mackerel having high levels of free histidine in their muscle tissue. Although cadaverine and putrescine have been found as potentiators of histamine toxicity, fish safety has been assessed purely from histamine content in most of the studies. Putrescine and cadaverine can potentiate the toxicity by inhibiting

histamine metabolizing enzymes (Stratton et al., 1991). Production of histamine is greater at high abusive temperatures (21°C or higher) and particularly rapid at 32.2°C (FDA, 2011). Toxic effects of histamine poisoning can happen after consumption of histamine higher than 500 mg kg⁻¹ and the allowable level in fish maximum is 50 mg kg⁻¹ (FDA, 2011).

The unique nutritional composition of seafood makes a path for faster decomposition of seafood. The various factors coming under microbial, biochemical and physical are contributing towards it. The speed of loss of freshness will vary with the species, physiological condition of fish, level of microbial load and temperature of storage. Among the biochemical quality indicators, the most commonly evaluated indicator for freshness is TVBN (Total volatile base nitrogen).

In view of the toxicological effects of biogenic amines the information on its contents in fresh and frozen fish is important for sensitive individuals. In this context, the present study was conducted to determine the content of biogenic amines, biochemical and microbial quality indicators in the fresh and frozen fish available in the super markets of Cochin.

Materials and Methods

Chemical standards were purchased from Sigma-Aldrich (USA) and HPLC ultra gradient solvent Acetonitrile for use in LCMSMS was purchased from J. T. Baker, USA. Water was filtered using Cascada LS water, Lab water technology (Pall Corporation). A total of thirty nine samples of fish sold in fresh and frozen condition from five different super markets of Cochin were collected and transported to the laboratory. Each sampling included five fish species in fresh condition and three fish species in frozen condition, with a total of 39 fresh and frozen fish samples. Fresh fish samples include *Lethrinus nebulosus*, *Nemipterus japonicus*, *Rastrelliger kanagurta*, *Lutjanus gibbus* and *Gerres filamentosus*. The frozen fish samples include *Pangasianodon hypophthalmus*, *Rastrelliger kanagurta* and *Stolephorus indicus*. The fresh samples collected were immediately iced in 1:1 (fish:ice) and transported in an insulated box. Frozen samples collected were transported in insulated boxes and were allowed to thaw before evaluation.

The samples were analysed in triplicate. pH of the samples was determined as per (AOAC, 1990) using

digital pH meter (Hanna instruments, HI 2221 pH/OHP meter). Total volatile base nitrogen (TVB-N) was determined by the micro diffusion method (Conway, 1950).

Five grams of fish sample after homogenization was extracted for one minute with 25 ml of 6% trichloroacetic acid (TCA). The homogenate was given a centrifugation at 8000 rpm for 10 min at 4°C. Centrifuged sample was then filtered through Whatman no.1 filter paper and 0.45 µ filter. Then the filtrate was diluted with methanol water (1:1). Samples were stored at -20°C until further analysis. Analysis of biogenic amines without derivatisation was carried out using LC MS MS with some modification to the method given by Sagratini et al. (2012). LC separation was achieved by passing the sample (1 µl) through a column (Water acquity BEH column RM 8) using a mobile phase 0.05% trifluoroacetic acid (TFA) containing water (A) and 0.05% TFA containing acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient program was: 0 min 30% B, 0–15 min 90% B, 15–20 min 30% B, 20–25 min 30% B. Analysis was done using LC MS MS (API 4000 Q Trap of AB Sciex, Canada). Instrument control, data acquisition and evaluation were done with the Analyst software (v. 1.5.2).

Calculated Quality index (QI) and biogenic amines index (BAI) as per the procedures described by Mielitz & Karmas (1977), Veciana-Nogues et al. (1997a), respectively. The indices were calculated as

$$\text{Quality index} = (\text{histamine} + \text{putrescine} + \text{cadaverine}) / (1 + \text{spermidine} + \text{spermine})$$

$$\text{Biogenic amines index} = (\text{histamine} + \text{cadaverine} + \text{putrescine} + \text{tyramine})$$

A 25 g of fish was cut, weighed aseptically and transferred to a stomacher bag. Added 225 ml of sterile physiological saline into the bag and the whole suspension was taken for homogenization for 60 seconds in a stomacher (Lab Blender 400; Seward Medical, London, UK). Then the homogenized sample was subjected to serial dilution using 9 ml sterile saline solution. Determined Aerobic plate count (APC) using plate count agar by the spread plate method (AOAC, 2002). Coliform counts were determined by petrifilm method at dilutions of ten and hundred (AOAC, 2005).

Results and Discussion

The biochemical quality of fresh and frozen fish available in supermarkets was analyzed in terms of pH and TVBN and it is give in Table 1. pH of live fish muscle is near to 7.0 and it can vary during post-mortem from 6.0 to 7.1 depending on season, species and other factors. The variations in pH can happen depending upon degradation of nitrogenous components. The highest average pH was found in case of frozen *P. hypophthalmus* (8.52) and lowest in case of *R. kanagurta* (6.24). The generation of basic compounds such as ammonia and trimethylamine is resulted through protein decomposition, deamina-

Table 1. pH and TVBN content of fresh and frozen fish from supermarkets

Samples	n	pH		TVBN (mg%)		
		Range	Avg	Range	Avg	% exceeded 30 mg%
<i>R. kanagurta</i>	5	5.95±0.0-6.43±0.02	6.24±0.2	3.5±0.5-14±1.1	6.5±1.9	Nil
<i>L. nebulosus</i>	6	6.31±0.01-7.17±0	6.68±0.33	2.45±0.5-44.8±0	15.52±6.5	20
<i>N. japonicus</i>	5	6.65±0-7.14±0	6.83±0.2	2.1±0.2-30.1±0.7	11.34±5.66	16.7%
<i>L. gibbus</i>	5	6.52±0.02-7.26±0	6.78±0.42	2.1±0.01-14.7±0.7	8.84±3.6	Nil
<i>G. filamentosus</i>	5	6.72±0.01-7.2±0.01	6.89±0.24	8.4±0.8-35±0.5	18.66±8.2	20
Frozen <i>P. hypophthalmus</i>	4	8.2±0.21-8.83±0.01	8.52±0.3	2.8±0.3-7±0.2	4.03±1	Nil
Frozen <i>S. indicus</i>	4	6.72±0.02-7.02±0.02	6.86±0.13	4.9±0.7-12.6±0.92	8.23±1.8	Nil
Frozen <i>R. kanagurta</i>	5	5.94±0-6.37±0	6.27±0.22	8.4±0.1-12.6±0	10.2±1.1	Nil
Total	39					

tion by the action of endogenous enzymes and microbes (Finne, 1982). As per (Zang & Deng, 2012), post mortem pH limit of acceptability for fish is 6.8–7. Frozen *P. hypophthalmus* samples showed higher levels of pH in all the cases.

Volatile bases will be formed during decomposition of proteins and non protein nitrogen compounds will increase along with the increase of storage period. TVBN is a most commonly used index of spoilage and it includes trimethylamine, dimethylamine and ammonia. The average TVBN value of fresh and frozen fish collected from super markets is ranging between 4.03 to 18.66 mg%. 10.3% of total samplings exceeded 30 mg% of TVBN. Around 16.67% of *L. nebulosus* samples exceeded the acceptability limit of TVBN ranging from 30 to 35 mg% as per Lakshmanan et al. (1990). 20% of *N. japonicus* and *G. filamentosus* were having TVBN content of 30 mg%. As these volatile compounds can leach out along with ice melt water, a lower TVBN value (Oehlenschläger, 1997) can be observed although the fish is in poor sensory and microbial quality. In case of freshly caught fish TVBN will be ranging between 5 and 20 mg% (Connel, 1995).

Biogenic amine contents in fresh and frozen fish available from super markets of Cochin showed a wide variation and are given in Fig. 1 and 2. Histamine, the most toxic amine in food in all the samples analyzed was very much below the hazard level and this could be due to the fact that the species of fish analyzed were non scombroids except Indian mackerel, *R. kanagurta*. In the case of Indian mackerel the maximum histamine level found was $0.943 \pm 0.2 \text{ mg kg}^{-1}$ as the samples were in fresh condition and in frozen mackerel it was 0.07 ± 0 . Among the samples evaluated maximum level of histamine was detected in *L. nebulosus* ($2.19 \pm 0.6 \text{ mg kg}^{-1}$) followed by *N. japonicus* ($1.34 \pm 0.4 \text{ mg kg}^{-1}$). In the case of *L. gibbus* and frozen *P. hypophthalmus* samples presence of histamine was not detected. Jeyashakila et al. (2003) reported large variations in the formation of histamine among fish species and further reported on histamine formation in *N. japonicus* and *Lethrinus miniatus* after 15 and 18 h of storage in ambient condition. There are reports indicating the absence of histamine in fresh condition of sardines, tuna and mackerel (Shakila et al., 2001; Viciena-Nogues et al., 1997a; Yatsunami & Echigo, 1993) while few others reported up to 5 ppm in fresh fish (Gopakumar et al., 1985; Vijayan et al., 1994). Frozen temperature (-18°C or below) can stop

the growth of histamine forming bacteria and prevent any preformed histidine decarboxylase from producing histamine. Ben-Gigirey et al. (1998) reported a non significant increase of histamine during nine month frozen storage of Albacore tuna (*Thunnus alalunga*). Histamine levels detected in the present study are very insignificant to cause any histamine poisoning to the consumers.

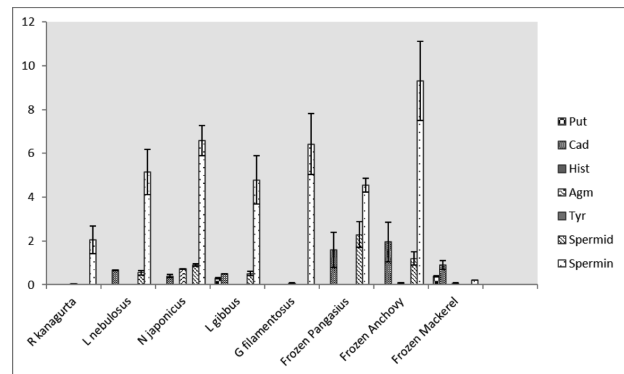


Fig. 1. Biogenic amine content (minimum) of fresh and frozen fish from super markets

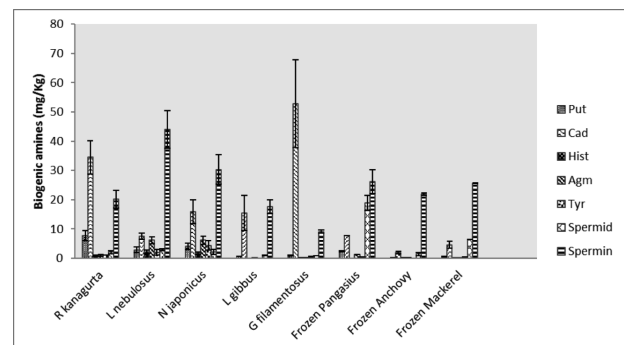


Fig. 2. Biogenic amine content (maximum) of fresh and frozen fish from super markets

Putrescine content, a good indicator of late spoilage requires acidic pH and high concentration of ornithine. Cadaverine is a volatile amine, associated to spoiled food. Cadaverine and putrescine can be used as freshness indices for fish and shellfish as the occurrence of putrescine and cadaverine is usually related to the hygiene conditions and could point out to the presence of contaminating spoilage microflora, especially gram negative strains of family Enterobacteriaceae and genus *Pseudomonas* (Rawles et al., 1996; Zhai et al., 2012). In the present study maximum putrescine content was found in the case of Indian mackerel ($7.72 \pm 1.7 \text{ mg kg}^{-1}$) followed by Japanese threadfin bream ($4.14 \pm 1.1 \text{ mg kg}^{-1}$). Some of the samples of *L. nebulosus*,

R. kanagurta, *N. japonicus*, frozen *P. hypophthalmus* and frozen *S. indicus* were found with the absence of putrescine, indicating the fresh condition. About 73.33% of samples had a putrescine content of less than 10 mg kg⁻¹. Cadaverine was present in 93.33% of the samples analyzed. In 83.33% of samples, high cadaverine was noticed (Fig. 2) with higher values noticed for *G. filamentosus* (52.8±15 mg kg⁻¹), *L. nebulosus* (37.5 mg kg⁻¹) and *R. kanagurta* (34.5±5.7 mg kg⁻¹). Putrescine and cadaverine can act as potentiators of toxicological effects of histamine and tyramine (Halasz et al., 1994; Prester, 2011). No maximum levels for putrescine and cadaverine in food have been proposed yet. Certain fish spoilage studies indicated production of cadaverine in the early part of storage (Rossi et al., 2002; Pons Sanchez-Cascado et al., 2005). Putrescine values lower than 1 mg 100 g⁻¹ were found to be for good quality carp meat, values between 1 and 2 mg 100 g⁻¹ for acceptable quality and values over 2 mg 100 g⁻¹ for poor quality carp meat based on sensory scores (Krizek et al., 2002). Yamanaka et al. (1989) suggested that cadaverine (upper acceptable limit of 10 mg 100 g⁻¹) may be used as an indicator of freshness in salmonoids. Hence in the present study 20% of samples with high content of cadaverine (>10 mg kg⁻¹) can be considered to be of poor quality.

Spermidine and spermine are polyamines and are essential components of living cells. They are important in regulating nucleic acid function, protein synthesis, as well as in membrane stabilization (Greif et al., 2006). They are formed naturally in food materials and are not as a consequence of

bacterial decomposition. Veciana-Nogues et al. (1997b) indicated higher contents of spermidine than spermine in foods of animal origin. In the present study maximum content of spermine was found in *L. nebulosus* (43.95±6.5 mg kg⁻¹) followed by *N. japonicas* (30.35±5.02 mg kg⁻¹). Maximum content of spermidine was noticed in *R. kanagurta* (51.8±1.5 mg kg⁻¹) followed by Frozen *Pangasius* (18.9±2.5 mg kg⁻¹). Spermine, spermidine and putrescine, can accelerate the development of tumors, as they are found in tissues with high growing rate and so their ingestion is forbidden to cancer patients (Kalac et al., 2009) and has potential to be considered as chemical hazard of concern. Spermine and spermidine can also increase the histamine transport across the gastrointestinal wall (Chu & Bjeldanes, 1981).

Tyramine is a monoamine compound formed from the amino acid tyrosine. Fresh fish contains little or no tyramine, but a large amount can be found in spoiled or fermented fish (Prester, 2011). It can also potentiate histamine toxicity by inhibiting histamine metabolizing enzymes. Presence of tyramine was detected in very small quantity in some of the samples analyzed with a maximum of 4.34±1.6 mg kg⁻¹ in *N. japonicus*. Maximum content of tyramine was 0.52 mg kg⁻¹ in case of *R. kanagurta*. Tyramine in the fish samples evaluated was very much below 100 mg kg⁻¹ that is the recommendation level of Nout (1994). Importance of tyramine in foods is due to its toxicological implications. Agmatine derives from the amino acid arginine and acts as an antidepressant. Agmatine along with cadaverine have been associated with autolytic changes respon-

Table 2. Quality index and Biogenic amine index of fresh and frozen fish from super markets

Samples	n	Quality index	Biogenic amine index (mg kg ⁻¹)		
		Range	Avg	Range	Avg
<i>R. kanagurta</i>	5	0.09-2.22	0.47	1.08-40.88	12.2
<i>L. nebulosus</i>	6	0.031-4.42	0.55	0.663-46.69	7.55
<i>N. japonicus</i>	5	0.052-5.97	1.15	0.651-25.6	6.19
<i>L. gibbus</i>	5	0.05-9.57	2.32	0.65-21.72	6.41
<i>G. filamentosus</i>	5	0.29-3.2	0.81	3.89-53.97	15.56
Frozen <i>P. hypophthalmus</i>	4	0.05-0.41	0.99	0.92-10.67	1.47
Frozen <i>S. indicus</i>	4	0.14-1.72	0.43	0.4-2.64	1.78
Frozen <i>R. kanagurta</i>	5	0.19-4.95	1.59	4.95	2.92
Total	39				

sible for loss of freshness in fish muscle before onset of microbial spoilage (Ruiz capillus & Moral, 2001). In 93.33 % of samples agmatine was present below 5 mg kg⁻¹ and maximum content was present in case of *N. japonicus* (6.17±1.2 mg kg⁻¹) followed by *L. nebulosus* (6.22±1.4 mg kg⁻¹).

There are no guidelines for biogenic amine content in fresh fish except for histamine. Histamine content in the samples analysed were very much below US FDA guidance level (50 mg kg⁻¹) as per FDA, (2011) and the acceptable level as per EU Regulation No 2073/2005 (100 mg kg⁻¹). Samples with high content of cadaverine and putrescine can be considered to be of poor quality.

Use of more than a single biogenic amine is necessary to overcome the limitations arising due to

considering a single amine concentration alone in determining quality. There was no correlation between histamine production and organoleptic quality (Medes, 1999). Table 2 shows the quality and biogenic amine indices of fresh and frozen fish available in the super markets of Cochin. The maximum QI was noticed in case of *L. gibbus* (9.57) followed by *N. japonicus* (5.9) and frozen Mackerel (4.95). The highest value of average QI was found in case of *L. gibbus* (2.32). Mielitz & Karmas, (1977) proposed the value of 10 as the limit of fish acceptability for quality index (QI) of canned tuna. As per Mielitz & Karmas (1977), for canned tuna QI of 0–1 was for good (class 1) product and for borderline (class 2) the QI was 1–10. High correlation between storage time and quality index was reported in case of Barramundi slices stored at two temperatures (Bakar et al., 2010). QI index showed

Table 3. Range, average and samples exceeded the limit of APC of fresh and frozen fish from supermarkets

Samples	n	APC (log CFU g ⁻¹)			Coliforms (log CFU g ⁻¹)		
		Range	Avg	Percentage exceeded limit (%)	Range	Avg	Percentage exceeded limit (%)
<i>R. kanagurta</i>	5	4.75 - 5.52	5.20	Nil	1.6 - 3.86	2.72	60
<i>L. nebulosus</i>	6	5.69 - 7.52	6.45	20	Nd - 3.77	2.51	20
<i>N. japonicus</i>	5	5.49 - 7.18	6.52	16.7%	1.7 - 2.77	2.08	Nil
<i>L. gibbus</i>	5	5.43 - 6.56	6.11	Nil	2.91 - 3.58	3.23	20
<i>G. filamentosus</i>	5	6.08 - 7.24	6.29	20	1.3 - 3.66	2.48	20
Frozen <i>P. hypophthalmus</i>	4	3.6 - 5.29	4.70	Nil	Nil	Nil	Nil
Frozen <i>S. indicus</i>	4	4.61 - 4.89	4.73	Nil	Nil	Nil	Nil
Frozen <i>R. kanagurta</i>	5	3.72 - 5.87	4.75	Nil	Nil	Nil	Nil

Table 4. Correlation between quality indices of spoiled fish

pH	TVBN	APC	Putrescine	Cadaverine	BAI	QI	
pH	1						
TVBN	-0.87**	1					
APC	-0.91**	0.99**	1				
Putrescine	-0.86**	0.5	0.64	1			
Cadaverine	0.08	-0.56	-0.42	0.44	1		
BAI	0.06	-0.54	-0.39	0.46	1**	1	
QI	0.37	-0.78*	-0.66	0.16	0.96**	0.95**	1

**Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

variation mainly on fish species. The initial decomposition of rockfish was with an index value of 2 d⁻¹10, whereas in salmon the index value for initial decomposition was 0.8 d⁻¹8 (Mietz & Karmas, 1978).

Veciana-Nogués et al. (1997) proposed as a guiding BAI limit value of 50 $\mu\text{g g}^{-1}$ for acceptance of tuna and Baixas-Nogueras et al. (2005) proposed a BAI limit of acceptability in a range of 15–20 $\mu\text{g g}^{-1}$ for hake. BAI showed a maximum value in case of *G. filamentosus* (53.97) followed by *L. nebulosus* (46.65) and *R. kanagurta* (40.88). In the present study 10.3 % of samples exceeded the BAI value of 20 mg kg^{-1} . The maximum average value of BAI is 15.56 (*G. filamentosus*). Ozogul & Ozogul, (2006) observed that both indices increased as a function of storage time and showed good correlation with the sensory alterations of *Sardina pilchardus* kept at 4°C in the air, packed under modified atmosphere and under vacuum. Reports on increase in the two indices with storage time, indicate the usefulness of these indices in determining the spoilage of fish (Ozogul & Ozogul, 2006; Bakar et al., 2010).

In seafood specific spoilage organisms can produce ammonia, biogenic amines, organic acids and sulfur compounds from amino acids, hypoxanthine from ATP degradation products, and acetate from lactate. APC levels of samples were compared with the levels specified for fresh and frozen fish by International Commission on Microbiological Specifications for Food (ICMSF, 1986). The levels of APC and coliforms were given in Table 3. The rejection limit specified is 10^7 cfu g^{-1} and lower (marginal) level of acceptability is $5 \times 10^5 \text{ cfu g}^{-1}$. In the present study 20% of samples of *L. nebulosus* and *N. japonicus* have crossed the rejection limit and were found to be spoiled. Some samples of *L. gibbus* also reached near to the rejection limit. The results are in agreement with the high content of cadaverine and putrescine in spoiled samples and those were not fit the condition for human consumption. *L. nebulosus* samples were in higher microbial load with a minimum of 5.7 cfu g^{-1} . The lowest microbial load was seen in case of *R. kanagurta* (4.75 to $5.52 \text{ log cfu g}^{-1}$).

Coliforms were detected in many samples. Highest incidence was observed in case of *R. kanagurta*. It was also noticed in case of *L. nebulosus*, *L. gibbus* and *G. filamentosus*. Total coliforms is an indicator of sewage contamination. The higher incidence can be due to contamination from handling and storage.

The source of contamination can be the water used for washing and preparation of ice.

Correlation between quality indices such as pH, TVBN, APC, BAI, QI, Putrescine and cadaverine of spoiled fish were given in Table 4. Although TVBN and APC was highly correlated ($p < 0.01$), they didn't show any correlation with biogenic amine indices. BAI was highly correlated with QI and Cadaverine content ($p < 0.01$). Although biogenic amines can be produced through bacterial enzymic action, their type and level can depend upon the specificity of microbial flora and their count (Veciana-Nogues et al., 1997a). Along with biogenic amine indices other quality indicators can be evaluated to ascertain the status of freshness of fish. Veciana-Nogues et al. (1997a) reported that the type and level of formation of biogenic amines can depend upon the specificity of microbial flora and their count. Okozumi et al. (1990) reported that formation of cadaverine and putrescine was higher when the *Pseudomonads* were the dominant flora.

It can be concluded that biogenic amine levels showed a wide variation in the fresh and frozen samples collected from super markets of Cochin. Histamine content was very low in the samples to elicit any toxicological problems in the consumers and this is similar to the previous reports on fish in retail market. Although a high significant correlation was observed between TVBN and APC, but there was no significant correlation with biogenic amine indices. The biogenic amine index value calculated based on biogenic amines, exceeded 20 mg kg^{-1} in 23.33% of samples. So a system for monitoring the freshness of fish available in the domestic markets of the country is essential.

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