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Biogenic Amines in Fresh, Canned and Salt-dried Fishery Products of India

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Biogenic amines viz., putrescine, cadaverine, histamine and tyramine, present in fresh, canned and salt-dried fishery products of Indian origin were analysed by means of TLC – densitometric method. Fresh fish and canned fishery products did not contain appreciable amounts of amines. On the other hand, salt-dried fishery products contained very high levels of biogenic amines. The histamine content in about 17% of the samples was above the maximum allowable limit of 20 mg%. Presence of potentiating amines, viz., putrescine and cadaverine in higher concentrations reflects that poor quality raw material was used for curing process.

Key words: Biogenic amines, histamine, TLC – densitometry, canned fish, salt dried fish

Biogenic amines are formed in foods as a result of bacterial decarboxylation of amino acids. They are of importance from the point of view of food poisoning and also as chemical indicators of spoilage. They occur in a wide variety of foods. Large amounts have been reported in aged, fermented and spoiled fish, cheese and meat products (Lakritz *et al.*, 1975; Maga, 1978). Of these, amines present in fish, especially histamine, have been extensively studied.

Earlier, only scombroid fishes were mainly implicated in histamine toxicity. Later studies revealed that non-scombroid fish also could cause histamine poisoning. Other biogenic amines such as cadaverine and putrescine are also equally important as histamine, as they are known as potentiators of histamine toxicity (Taylor & Lieber, 1979; Hui & Taylor, 1983).

Adequate statistics on the incidence of food poisoning caused by histamine and other amines are not available in India due to lack of surveillance programmes. There are a few published reports detailing the histamine content of some fresh and processed fishery products of India (Subburaj *et al.*, 1984; Gopakumar *et al.*, 1988; Chakraborti, 1991, 1993; Vijayan *et al.*, 1994). The above studies have utilized the fluorimetric method of analysis, which is specific only for histamine. No work has been carried out so far on the occurrence of amines other than histamine in the fish and fishery products of India. Therefore, the present study was undertaken to examine the occurrence of biogenic amines in fresh, canned and salt-dried fish of Indian origin.

Materials and Methods

Fresh fish of commercial importance viz., mackerel, sardine, shrimp, tuna, seerfish and shark were purchased from the retail markets of Mysore. Canned fishery products were obtained from the retail shops of Mysore and Mangalore. Salt-dried fish was procured from Mangalore. All samples were subjected to biogenic amine analysis in triplicate.

Biogenic amine analysis was performed by the TLC-densitometric method following the procedure of Fleischer (1979) with slight modification. Extraction and derivatization

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of amines were similar to the method reported by Rosier & Petegham (1988). Fresh or canned fish (10 g) or salt-dried fish (5 g) were taken and homogenized with 30 ml of 5% hot (80-90°C) trichloroacetic acid(TCA) solution for 2 min and centrifuged at 3000 rpm for 10 min. The supernatant was filtered through Whatman No. 41 filter paper and used for derivatization. Pure biogenic amines viz., putrescine (Put), cadaverine (Cad), histamine (His) and tyramine (Tyr) were obtained from Sigma Chemicals Co., USA. Stock solution was prepared by dissolving accurately 0.20-0.25 g of each amine in 10 ml of 5% TCA. A ten fold dilution was used as the working standard, which was used for derivatization.

One ml of the TCA extract or working standard was taken in a screw cap test tube, to which 1.0 ml of phosphate buffer (pH 9.0, undiluted), a drop of 4 N sodium hydroxide solution and 2.0 ml of dansyl reagent (50 mg of dansyl chloride in 10 ml of acetone) were added. After mixing, the tubes were covered with aluminum foil and left in the incubator at 55°C for 1 h. The tubes were then cooled and stored at 5°C until further analysis.

For fractionation, 10 µl of the dansylated samples were applied and separated on a precoated silica gel GF 254 TLC plate, along with the standard amine mixture. Plates were developed using chloroform: triethylamine (100:25) mixture and the fluorescence was enhanced by spraying with isopropanol:triethanolamine (8:2) mixture. The concentration of the fluorescent amines was measured by the TLC scanning densitometer (Model CS-930, Shimadzu Corporation) under the fluorescence mode (365 nm).

Results and Discussion

The concentrations of biogenic amines present in fresh fish are given in Table 1. The levels were relatively low in the fresh fish. Fresh fish are reported to have only negligible amounts of biogenic amines (Yamanaka *et al.*, 1986; Gouygou *et al.*, 1992).

Table 1. Biogenic amine concentration in fresh fish sold in the retail market

Species	Amines mg.100 g^{-1}			
	PUT	CAD	HIS	TYR
Mackerel (*Rastrelliger kanagurta*)	ND	0.31	ND	0.52
"	ND	0.57	ND	0.74
"	ND	1.35	ND	1.23
"	0.81	3.11	ND	1.90
"	0.95	2.80	0.54	2.58
"	1.25	9.42	2.34	3.04
Sardine (*Sardinella gibbosa*)	ND	0.49	ND	0.29
"	0.33	2.74	ND	1.62
"	1.67	2.27	1.68	1.71
Shrimp (*Parapenaeopsis stylifera*)	1.14	ND	ND	0.88
"	4.71	0.64	ND	2.87
Tuna (*Thunnus* sp.)	0.44	1.82	ND	1.16
Seerfish (*Scomberomorus* sp.)	1.42	1.77	ND	0.94
Shark (*Scoliodon* sp.)	ND	0.48	ND	0.28
Squid (*Loligo* sp.)	1.82	ND	ND	ND

ND - Not detected; All values are average of three determinations

Cadaverine and tyramine were detected in more than 90% of the samples. No histamine was detected in 80% of the samples. Even in the samples where histamine was detected, the level was far below the maximum allowable limit of 20 mg%, prescribed by the EEC (Ababouch, 1991). Earlier, there are reports that histamine contents were more than 20 mg% in the fishes of Indian origin (Subburaj *et al.*, 1984; Nagendra *et al.*, 1988). However, Gopakumar *et al.* (1988) have detected only negligible amounts of histamine (<1 mg%) in the fresh fish which is comparable to the present observation. Therefore, the levels of biogenic amines including histamines in the fresh fish sold in the market were very insignificant and unlikely to cause any toxicity to the consumers.

Biogenic amine concentration in commercial canned fishery products is presented in Table 2. In the canned products, the amines such as putrescine, cadaverine,

Table 2. Biogenic amine concentration in commercial canned fishery products

Product	Amines mg.100 g^{-1}			
	PUT	CAD	HIS	TYR
Mackerel in brine	ND	0.58	ND	ND
"	0.53	1.30	ND	ND
"	4.43	5.40	0.78	0.13
Mackerel in brine and oil	0.14	2.95	ND	ND
Mackerel in curry	ND	0.14	ND	ND
Sardines natural pack	0.21	2.48	ND	0.29
Sardines in oil	ND	0.17	ND	ND
Sardines in brine	0.70	0.94	ND	ND
"	0.65	0.60	ND	ND
Sardines in brine and oil	2.24	3.22	0.54	0.15
Yellow fin tuna in oil	ND	0.12	ND	ND
Little tuna in oil	ND	0.18	ND	ND
Light meat tuna	0.86	1.56	ND	ND
Seer fish in oil	ND	0.42	ND	ND
Prawns in curry	1.71	0.21	ND	0.34
Chank meat in oil	ND	0.11	ND	ND

ND - Not detected; All values are average of three determinations

histamine and tyramine were detected in negligible amounts. Out of the 16 samples, histamine was detected only in two samples and its concentration was very insignificant (<1 mg%). The concentration of other amines viz., putrescine and cadaverine were slightly higher in the samples where histamine was detected. Canned products analysed did not seem to pose any toxicity problems. Earlier workers have reported high histamine levels in canned fish (Taylor *et al.*, 1978; Kim & Bjeldanes, 1979; Ababouch *et al.*, 1988) but none of the samples analysed during this study had such high levels of histamine.

Data on distribution of the amines in commercial salt-dried fish are presented in Table 3. The values are expressed on fresh weight basis in order to compare the amine levels with the limits prescribed by the regulatory agencies. The salt-dried fish analysed belonged to three classes viz., finfish (Class I), elasmobranchs (Class II) and shellfish (Class III). Under the Class I, two batches of mackerel, sardines and catfish had histamine content ranging from 32 to 53 mg%, which was above the hazard action

Table 3. Biogenic amine concentration in commercial salt-dried fishes

Product	Amines mg.100 g^{-1}			
	PUT	CAD	HIS	TYR
I. FIN FISHES				
Group A (His >20 mg%)				
1. Mackerel (*Rastrelliger kanagurta*)				
a. Batch 1	26.44	95.59	32.10	39.84
b. Batch 2	18.96	67.81	46.03	24.16
2. Oil sardine (*Sardinella longiceps*)				
a. Batch 1	18.07	132.44	53.06	14.69
b. Batch 2	10.03	77.13	35.26	9.09
3. Catfish (*Arius arius*)	12.70	90.58	26.34	9.65
Group B (His >10 mg%)				
1. Mackerel (*Rastrelliger kanagurta*)	7.61	43.90	15.58	8.58
2. Clupeids (*Thryssa* sp.)				
a. Batch 1	30.06	243.88	14.21	16.09
b. Batch 2	30.18	226.37	16.17	11.38
3. Anchovy (*Stolephorus* sp.)				
a. Batch 1	46.18	89.39	10.14	9.85
b. Batch 2	56.08	172.22	11.59	21.37
4. Lesser sardine (*Sardinopsis* sp.)	10.91	85.66	12.58	13.23
Group C (His >1 mg%)				
1. Carangid (*Caranx* sp.)				
a. Batch 1	56.95	351.18	0.72	152.16
b. Batch 2	26.56	206.01	0.34	39.77
2. Ribbon fish (*Trichurus* sp.)	3.37	1.33	0.41	1.55
Group D (His - not detectable)				
1. Silver bellies (*Leiognathus* sp.)				
a. Batch 1	24.18	73.95	ND	11.49
b. Batch 2	21.16	90.49	ND	14.77
2. Flat fish (*Cynoglossus* sp.)				
a. Batch 1	5.97	23.64	ND	4.80
b. Batch 2	8.85	29.17	ND	4.83
3. Seer fish (*Scombeormorus* sp.)	54.68	112.80	ND	15.42
4. Threadfin bream (*Nemipterus* sp.)	10.04	38.87	ND	20.17
5. Perch (*Epinephelus* sp.)	15.31	94.62	ND	26.02
6. Pipe fish (*Sigarmus* sp.)	13.25	51.77	ND	17.79
7. Anchovy (*Stolephorus* sp.)	2.29	3.93	ND	0.80
II. ELASMOBRANCHS				
1. Shark (*Scoliodon* sp.)				
a. Batch 1	2.46	8.95	ND	4.57
b. Batch 2	2.75	15.10	ND	7.84
c. Batch 3	12.45	33.57	ND	14.97
2. Ray (*Raja* sp.)	11.42	62.60	ND	9.34
III. SHELLFISHES				
1. Shrimp (*Penaeus* sp.) (Whole)				
a. Batch 1	112.71	52.80	ND	70.47
b. Batch 2	102.55	45.32	ND	75.32
2. Shrimp (Peeled deveined)	8.83	2.45	ND	5.19
3. Small shrimp (*Acetes* sp.)	12.77	0.58	ND	8.97

ND - Not detected; All values are average of three determinations

level of 20 mg% prescribed by the EEC. High levels of cadaverine was also detected in these samples and the cadaverine to histamine ratio was very high (1.5-3.5:1), which is similar to the observation of Klausen & Lund (1986) who have reported that mackerel contains 2-5 times more cadaverine than histamine. Putrescine concentration was found to be lower than histamine. The fishes of clupeids group, lesser sardines, two batches of anchovies and one batch of mackerel contained histamine in the range of 10-17 mg%, which was above permissible level (10 mg%). Among these, the clupeids and anchovies had comparatively higher cadaverine and putrescine concentration than histamine. The fishes that contained histamine, except catfish, mostly belonged to the family Scombridae and Clupeidae, which are often known to be involved in histamine toxicity problems (Taylor, 1986).

Most of the non-scombroid fishes contained no histamine except carangids and ribbon fish, in which negligible amounts of histamine were detected. However, the concentrations of other amines such as putrescine, cadaverine and tyramine were found to be quite high. Wide variations in the concentrations of the different amines were observed among the species and this could be probably due to the differences in the availability of the substrate (amino acids). However, variations observed within the species viz., mackerel and anchovy, perhaps reflect the differences in the initial freshness of the raw material.

In elasmobranch and shellfish (Class II and III), histamine was totally absent, whereas other biogenic amines were detected. Shark and ray had comparatively lower levels of putrescine, cadaverine and tyramine. Shellfish had high levels of putrescine and tyramine than cadaverine. Similar observations have been reported by Meitz & Karmas (1978) and Shakila *et al.* (1996). Presence of lower levels of amines in peeled and deveined shrimps compared to whole shrimps could be partially due to the effect of beheading and deveining process.

Earlier studies reveal that 50% of the salt-dried fish of Indonesian origin had histamine content above 20 mg% (Hanson *et al.*, 1985). However, in India, 20% of the fish was reported to contain histamine exceeding this limit (Chakraborti, 1991). In the present study, only about 17% of the samples contained histamine above hazard action level. Histamine content of salt-dried fish analysed by Chakraborti (1991) varied from 4.1 to 80.6 mg%, which was comparable with the present observations. Biogenic amine profiles of South Asian salt-dried fish showed that the cadaverine content ranged from 66-333 mg%, putrescine from 0-218 mg% and histamine from 0-80 mg% (Wootten *et al.*, 1989), which is more or less similar to the values obtained in the present study.

Therefore, it can be inferred that some of the salt-cured fish sold in Indian markets do have high levels of histamine (>10 mg%) and are likely to cause toxicity to the consumers. The concentrations of other amines such as putrescine and cadaverine were also found to be relatively high in salt-dried products, although histamine levels were very low. This perhaps reflects the poor quality raw material employed for curing process. On the contrary, the fresh and canned fish available in the retail markets contain insignificant levels of amines including histamine and they do not pose any toxicity problems to the consumers.

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