

Formation of Histamine and Other Biogenic Amines During Storage of Freshwater Fish Chunks

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Abstract

Biogenic amine formation in the freshwater fish chunks viz. Catla (*Catla catla*) and Rohu (*Labeo rohita*) was evaluated during its storage at 30°C and 5°C along with the sensory and microbiological quality characteristics. Sensorially, the fish remained in acceptable condition for 6 to 10 h at 30°C and 3 to 5 days at 5°C. At this stage, the total bacterial load reached a level of 10^7 cfu·g from the initial load of 10^5 to 10^6 cfu·g. Fresh fish contained negligible amounts of biogenic amines. Upon storage, significant increases in the concentrations of amines were noticed especially between 6 and 12 h of storage at 30°C, and between 3 and 5 days of storage at 5°C. Formation of histamine was noticed and its level exceeded the maximum limit of acceptability of 20 mg% after 18 h of storage at 30°C and 5 days of storage at 5°C. When the concentrations of cadaverine were 10mg% or below; the fish remained in acceptable condition and it can be considered as a good indicator of quality than histamine. Therefore, storage of freshwater fish beyond 12 h at 30°C and 5 days at 5°C can only lead to the accumulation of histamine and cause toxicity.

Introduction

Biogenic amines such as histamine, cadaverine, putrescine and tyramine are formed in foods as a result of decarboxylation of amino acids bacteria (Maga 1978). The amines are important as agents of food intoxication and indicators of fish spoilage. Putrescine and cadaverine are good indices of spoilage of marine fish (Meitz and Karmas 1978; Yamanaka et al. 1986, Taylor and Sumner 1987). Histamine has been implicated in the toxicity of scombroid fishes and even non-scombroids like mahi-mahi, herring, sardines and blue fish (Taylor 1986). Information is available on the formation of histamine and other biogenic amines in the fishes of marine origin such as tuna (Wendakoon et al. 1990, Mendes 1999), sardine (Yamanaka et al. 1986, Marrakchi et al. 1990), horse mackerel (Okuzumi et al. 1990) and anchovy (Veciana-Nogues et al. 1990), while no reports is available regarding their occurrence in the freshwater fish. Further, the universal index of fish spoilage is trimethylamine (TMA), a volatile amine. But TMA or TMAO could not be taken as a freshness index, because it is absent in the muscles of freshwater fish muscle (Huss 1988). The present study was undertaken to determine whether non-volatile biogenic amines appear in freshwater fishes, catla and rohu during storage, and to assess the suitability of these different amines as freshness index.

Materials and Methods

Fish samples

The freshwater fishes, catla (*Catla catla*) and rohu (*Labeo rohita*) were obtained from the fish retail shop in Mysore, India. They were brought immediately to the laboratory, eviscerated, washed in potable water, made into chunks of 100 g each and packed in polythene bags. The chunks were then divided into two lots. One lot was stored at ambient temperature ($30 \pm 2^\circ\text{C}$) and the other was kept in the refrigerator (5°C). Samples from each lot were drawn at regular intervals and analyzed in triplicate for sensory quality, total bacterial counts and biogenic amine concentrations.

Sensory and microbiological examination

Fish were graded according to their appearance, color, odor and texture on a 5-point scale by a six member trained panel. The scores given were 5 – Excellent, 4 – Good, 3 – Acceptable, 2 – Dubious and 1 – Unacceptable. Samples with scores of 3.0 and above were considered acceptable for human consumption.

Ten grams of fish samples were homogenized in 90 ml of sterile peptone water and multiple dilutions up to 10^5 were made with the same diluent. Two sets of serially diluted homogenates were prepared and inoculated into agar plates. One set was incubated at 37°C and the other set at 5°C . The total bacterial count (TBC) was made according to standard methods (Speck 1976).

Analysis of biogenic amines

Fish muscle (10 g) was homogenized in 30 ml of hot (80 to 90°C) 5% trichloroacetic acid (TCA) and the amines were derivatized to dansylamines by adding dansyl chloride (Rosier and Petegham 1988). Standard solutions of histamine, cadaverine, putrescine and tyramine (Sigma Chemicals, Co, USA) were prepared at a concentration of 20 mg in 1 ml of 5% TCA. Working standards were made by 10-fold dilution. The amines were derivatized by taking 1.0 ml of sample or standard in a screw cap test tube and adding 1.0 ml phosphate buffer (pH 9.0), 2.0 ml of dansyl chloride (50 mg in 10 ml acetone) and a drop of 4N sodium hydroxide. The test tubes were covered with aluminum foil and incubated at 55°C for 1 h.

Separation of amines was made on precoated silica gel GF₂₅₄ plate, 20x20 (E.Merck, India) with a chloroform:triethylamine (100:25) mixture (Fleischer 1979). Spot fluorescence was enhanced by spraying with isopropanol: triethanolamine (80:20) mixture. Detection of spots were made under UV light (365 nm) and identities were established by comparison with the standards. Quantification was made with a computerized UV-vis spectrodensitometer (Shimadzu Corporation, Japan) operated in fluorescence mode at 365 nm.

Statistical analysis

All determinations were done in triplicates. Date pertaining to the changes in the biogenic amine contents with respect to storage period was tested for significance by two-way analysis of variance (Snedacor and Cochran 1980).

Results

Sensory and microbiological quality during storage

There was no difference between catla and rohu, in terms of sensory and microbiological qualities (Table 1). Fish obtained from the market were of good quality with a score above 4.5. Upon storage, the quality of fish deteriorated, and a putrid smell or off-odor became noticeable after 12 h at 30°C and 5 d at 5°C. The initial TBC of the fish were 10⁶ cfu·g and increased gradually and reached 10⁸ cfu·g after 12 h at 30°C and 5 d at 5°C.

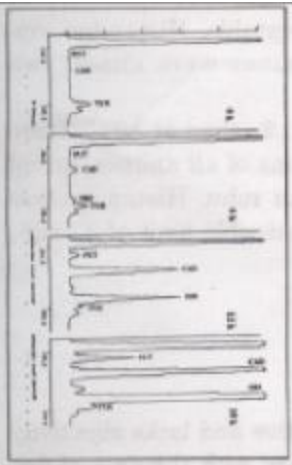


Fig. 1. Densitometric scanning pattern of the biogenic amines formed during storage of catla at 30°C. PUT–Putrescine, CAD– Cadaverine, HIS– Histamine, TYR – Tyramine h – hours of storage.

Biogenic amines during storage

Histamine, cadaverine, putrescine and tyramine were resolved well on the precoated silica gel TLC plate. Figure 1 shows the densitograms of biogenic amines formed during storage of catla at 30°C. The concentrations of the amines clearly increased during storage.

Fresh catla and rohu contained negligible amounts of cadaverine and putrescine but with slightly higher amounts of tyramine (Fig 2). These

Table 1. Sensory and microbiological quality of catla and rohu chunks stored at 30 and 5°C.

Temp. (°C)	Duration of storage (h·d)	Catla		Rohu	
		Sensory quality	Total bacterial count (g ⁻¹)	Sensory quality	Total bacterial count (g ⁻¹)
30	0	4.50	1.05 x 10 ⁶	4.75	1.11 x 10 ⁶
	6	3.50	2.73 x 10 ⁷	3.75	1.64 x 10 ⁷
	12	2.00	4.14 x 10 ⁸	2.50	3.75 x 10 ⁸
	18	1.00	8.40 x 10 ⁸	1.00	8.71 x 10 ⁸
	24	1.00	9.01 x 10 ⁸	1.00	8.33 x 10 ⁸
5	3	3.25	1.32 x 10 ⁷	3.50	1.24 x 10 ⁷
	5	2.00	1.41 x 10 ⁸	2.00	1.94 x 10 ⁸
	7	1.00	3.47 x 10 ⁸	1.00	2.45 x 10 ⁸

Scores for sensory quality: 5–Excellent, 4–Good, 3–Acceptable, 2–Dubious, 1–Unacceptable

amines increased and histamine appeared only after 6 h of storage. A significant increase ($P<0.01$) in the concentrations of cadaverine and histamine were observed during storage; but not with putrescine and tyramine. The concentration of cadaverine was higher than those of the other amines. The histamine concentration stayed below the maximum permissible limit of 20 mg% prescribed by European Economic Community (Ababouch 1991) up to 12 h of storage, and exceeded the limit only after 18 h, at which time the fish was already judged unacceptable. Histamine concentrations were the same, but those of other amines were almost two times higher in catla than rohu.

Amines were formed at a slower rate at 5°C (Fig 3), than at 30°C. There was a significant increase ($P<0.05$) in the concentrations of all amines, except tyramine between the 3rd and 5th day of storage in rohu. Histamine was higher than the other amines and it exceeded the permissible limit of 20 mg% after 5 days of storage of rohu, but not catla.

Discussion

Sensory evaluation is reported to be highly subjective and lack objectivity (Connell 1975). Microbial counts are also known to vary with the area of fish capture and waters, and hence it cannot be a reliable index of quality. The sensory and microbiological qualities of catla and rohu did not show any remarkable difference during the storage.

The amines resolved well on the precoated silica gel plates with distinct Rf values. TLC-densitometric method is reported to be simple, fast and inexpensive (Taylor 1986; Chin and Kohler 1983) for the separation and quantification of amines.

The quantity of amines formed in the fish at 30°C was quite higher than that at 5°C. Variation in the formation of different amines at different tem-

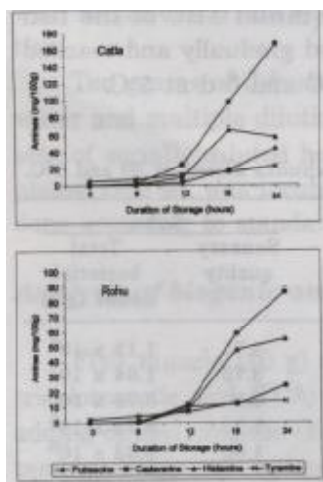


Fig. 2. Biogenic amine changes in the freshwater fish viz. catla and rohu chunks stored at 30°C.

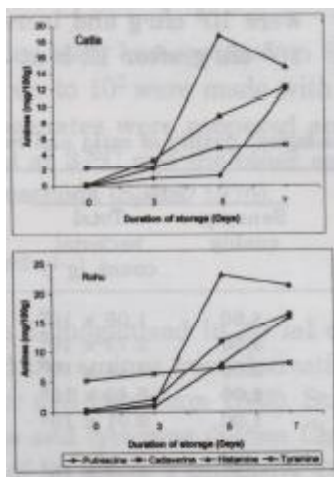


Fig. 3. Biogenic amine changes in the freshwater fish viz. catla and rohu chunks stored at 5°C.

peratures was probably due to the effect of temperature on the amine forming bacteria. Frank et al (1985) reported that the biogenic amine formation in fish is due to the activity of mesophilic more than psychrophilic bacteria. In addition, the dominant amines formed were cadaverine and histamine at

30°C and, histamine at 5°C. The growth and activity of the amine forming bacteria vary at different temperatures and according to the availability of the amino acid substrates (Yamanaka et al. 1986, Okuzumi et al. 1990). Variation was also observed in the formation of amines within the species. Earlier reports state that the histamine to cadaverine ratio varies among species (Klausen and Lund 1986; Middle brooks et al. 1988).

Cadaverine as chemical indicator of quality

Cadaverine increased at the onset of fish spoilage and may be taken as a chemical indicator of fish quality. The concentration of cadaverine increased remarkably between 6 and 12 h of storage at 30°C and between the 3rd and 5th days of storage at 5°C. In rohu held at 5°C, the putrescine also increased significantly between the 3rd and 5th days of storage. At this stage, the sensory quality fell and TBC increased. Yamanaka et al. (1986) have suggested cadaverine as a good indicator of spoilage and recommended the limit as 15 mg%. In the present study, it is observed, at the time of visual spoilage (i.e., 12 h) at 30°C, the cadaverine concentration was 24 mg% in catla and 12 mg% in rohu; whereas at 5°C (i.e. on the 5th day), it was 9 mg% in catla and 12 mg% in rohu, respectively. Hence, a cadaverine concentration of 10 mg% would be more appropriate to be taken as an indication of spoilage in freshwater fish.

Histamine toxicity

High levels of histamine were formed in catla and rohu during storage at 30°C and 5°C, and exceeded 20 mg% but only after the fish was organoleptically graded as unacceptable. However, histamine toxicity could be enhanced by other amines such as a cadaverine and putrescine (Taylor 1986). Therefore, to avoid incidence of histamine toxicity, freshwater fish must be consumed within 12 h if fish are handled at ambient temperature, and within 5 days if stored in the refrigerator.

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