

Fish Spoilage by Amine Forming Bacteria in Commercial Fish Samples

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Abstract: Scombroid fish poisoning is food borne chemical intoxication, found to be associated with consumption of scombroid fish containing unusually high levels of biogenic amines histamine, cadaverine and putrescine. Fish belonging to non scombroid group such as mahi-mahi, anchovies and sardines have also been implicated in such food poisoning. In this study, biogenic amines forming bacteria were screened and investigated from fish samples of Thane District. Among 54 strains isolated, 23 strains were histamine formers, 12 were cadaverine formers and 17 were putrescine formers. The study showed that all the fish samples tested had histamine levels much above the defect action level of FDA. Cadaverine and putrescine forming bacteria were isolated from both fresh and salted fish samples which indicate that cadaverine and putrescine formed in these samples would act to decrease threshold dose of histamine to provoke an adverse reaction in humans and thus contribute to scombroid toxicity by acting as potentiators of histamine.

Keywords: Scromboid poisoning, Histamine, Cadaverine, Putrescine, Fish spoilage

I. INTRODUCTION

Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin [1]. Scombroid fish poisoning results from eating the spoiled fishes of family Scombroidae. These fish contain characteristically high level of free histidine in their muscle tissue, which will be converted to histamine under conditions favorable to bacterial growth and synthesis of histidine decarboxylase [2], [3].

The tropical climate of India with an average temperature ranging between 25-40°C is suitable for proliferation of histamine, Cadaverine and putrescine forming bacteria in fish and fish products. Various stages of fish handling (harvest, procurement, retail marketing) and processing (drying, salting, freezing) have profound effect on histamine formation. Majority of fishes are salted and dried without removing gut which harbor large number of bacteria that possess the decarboxylase enzyme [4].

Histamine is potentiated by some other component or components in toxic fish [1], [5]. Such potentiators would act to decrease the threshold dose of histamine needed to provoke an adverse reaction in humans challenged orally [5], [6]. Doses of pure histamine required to produce mild reactions

were several times higher than the doses producing more severe symptoms when consumed with spoiled fish. Even after allowing for variability in human susceptibility and variable histamine content in different parts of fish, it seems that there is a difference between the relative lack of toxicity of pure histamine and the (often) apparent toxicity of histamine in spoiled fish [7].

The absorption, metabolism, and/or potency of one biogenic amine might be modified in the presence of a second amine [8], [9], [10]. The biogenic amines putrescine and cadaverine occur in appreciable quantities in toxic fish and at low levels in non-toxic fish [11]. When given in higher ratios relative to histamine than those that usually occur in toxic fish, these amines potentiate the biological activity of histamine in laboratory animals. Cadaverine and putrescine competitively inhibited mucosal DAO and potentiated histamine-induced contractions in guinea pig ileum [12]. The purpose of this study was to isolate and detect Hitsamine, Cadaverine and Putrescine forming bacteria, quantify histamine produced from fresh and salted fish samples and compare incidence of cadaverine and putrescine forming bacteria which could contribute to scombroid toxicity by acting as potentiators of histamine.

II. MATERIALS AND METHOD

Indian mackerel (*Rastrelliger kanagurta*) and sardines (*Sardinella gibbosa*) both fresh as well as salted samples were purchased from the retail fish shops in Thane district. They were subjected to microbiological and colorimetric analysis within an hour.

Muscle tissue (5 g) was obtained from fish from each of three locations (head, belly and tail) and transferred to 50 ml of 0.85% NaCl solution. The sample was homogenized for 2 min using high speed blender and centrifuged at 4000 rpm for 10 min. The supernatant was then made up to 25 ml with saline. The muscle extract was used immediately for histamine analysis.

A composite tissue sample was prepared by aseptically sampling 5 g from each location, diluting 1:10 (w/v) with sterile 0.1 % peptone water, homogenized using high speed blender. These samples were immediately used for microbiological analysis.

Fish composite samples were serially diluted in sterile 0.1% peptone water and 0.1 ml were spread plated in duplicate on tryptic soya agar (TSA) (HiMedia, Mumbai, India). For salted fish samples, tryptic soya agar with 7.5% NaCl was used and incubated at 37°C for 48 hours. Representative isolates were selected from TSA plate. Purification of isolates was done by sequential streaking on TSA plates and incubation at 37°C for 48 hours.

The pure cultures were transferred to TSA slants containing 2% NaCl and incubated at 37°C for 24 hours. Each isolate taken from each slant was plated on Niven's agar medium [13], Moeller's decarboxylase broth base supplemented with 0.5% L-lysine hydrochloride and Moeller's decarboxylase broth base supplemented with 0.5% L-ornithine hydrochloride and incubated at 37°C for 48 hours to screen for histamine, cadaverine and putrescine production respectively.

Histamine was quantified using colorimetric method reported by Patange *et al.* [14].

III. RESULTS AND DISCUSSION

The Total bacterial counts of fresh fish were about 10^7 cfu/g and salted fish were about 10^6 cfu/g. Though the tropical fish normally contain high bacterial population [4], in this study, the total bacterial load of fresh and salted fishes indicate that fish samples may be mishandled or temperature abused prior to sampling as these values were well above 5×10^5 cfu/g set by ICMSF standard as a measure of a good quality product [15].

The total bacterial counts of fresh and salted fishes obtained in this study were higher than the bacterial counts reported by Shakila *et al.*, Ababouch *et al.* and Lakshmanan *et al.* [4], [16], [17].

Bacterial load of salted fish was found to be about one log lower i.e. 10^6 cfu/g. This may be due to the additional salt which may reduce the water content of fish which in turn retards proliferation of microorganisms [4].

In case of fresh sardines, counts of histamine forming bacteria obtained were slightly higher than fresh mackerels while in case of salted mackerels and salted sardines counts were slightly higher in mackerel than in sardine. A slightly higher incidence was noticed in the fresh sardines which belong to the family Clupeidae. Sardines are normally implicated in histamine poisoning [16]. The counts of histamine forming isolates obtained in this study (Fig. 1) were higher (only 0.3-0.5 log lower than the total bacterial load) as compared to other studies reported by Shakila *et al.* and Jeyasekaran *et al.* in which histamine forming bacterial counts were 2-3 log lower than total bacterial load [4], [18].

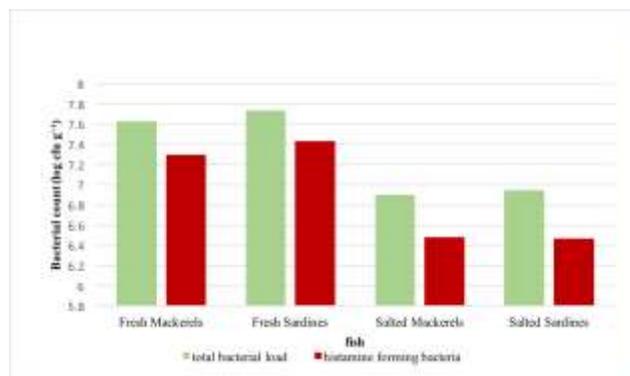


Fig. 1 Total bacterial load and histamine forming bacteria in fish samples

Among 54 isolates, 23 were Niven's positive isolates, 12 were cadaverine formers and 17 were putrescine formers. Niven's positive isolates were tested to verify their ability to produce histamine, only 8 were confirmed as histamine producer as determined by colorimetric assay of histamine.

Incidence of amine forming bacteria in fresh and salted fish was found to vary with species and product. In fresh fish, incidence of histamine forming bacteria were higher (46%) in mackerels than in sardines (38%). cadaverine forming bacteria were higher (23%) in sardines than in mackerel (20%) while putrescine forming bacteria were higher (33%) in mackerel than in sardines (30%). In salted fish, incidence of histamine forming bacteria were higher in mackerels (50%) than in sardines (33%), cadaverine forming bacteria were higher (28%) in mackerels than in sardines (16%) while putrescine forming bacteria were higher (41%) in salted sardines than in mackerels (21%) (Fig. 2).

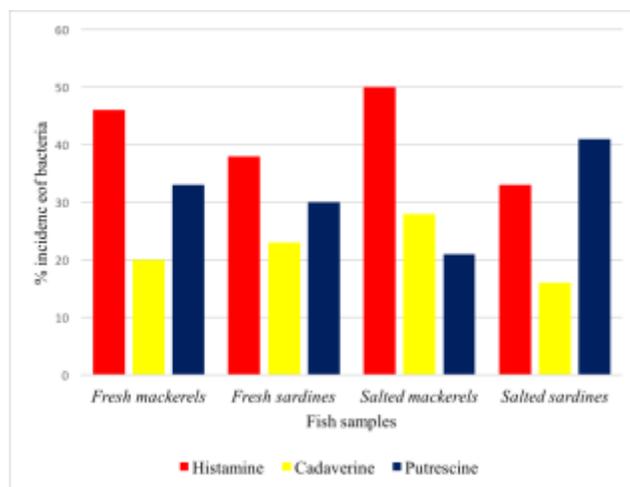


Fig. 2 Incidence of amine forming bacteria in fish samples

FDA identified growth of histamine forming bacteria as the primary cause of histamine production [7]. Presence of cadaverine and putrescine forming bacteria may lead to the

formation of cadaverine and putrescine which functions as potentiators of histamine poisoning [19]. These amines are also regarded as quality indicators in fish and fishery due to their early appearance and heat stable nature [4].

In a total, 40 samples of fresh and salted fish samples were analyzed. 2 fresh mackerels, 2 fresh sardines, 1 salted mackerel and 3 salted sardines samples showed histamine concentration ≥ 20 mg/100 g. 7 fresh mackerels, 7 fresh sardines, 8 salted mackerels and 6 salted sardines samples showed histamine concentration in between 10-20 mg/100 g. Mackerel show quite marked fish-to-fish and seasonal variations in chemical composition and susceptibility to spoilage by various microbiological, enzymic and auto-oxidative processes. Therefore only certain fish in an apparently homogenous batch will develop all factors necessary to induce HFP [20]. Toxic levels of histamine have been found in dried and/or smoked products of mackerel, horse mackerel and sardines [1]. The study showed that all the fish samples tested had histamine levels much above the defect action level of FDA and revealed the incidence of histamine as well as cadaverine and putrescine formers in fish samples.

Post catching contamination with amine forming bacteria may occur at several levels- aboard the fishing vessel, in the distribution system and at the level of the user [1]. In the market environment, histamine, cadaverine and putrescine forming bacteria are widely distributed and sources of contamination are carrying baskets, ice, the market floor and water used for wetting fish [21]. If fish are stored ungutted, the gut itself may be a source of contamination, particularly if chilling is delayed [7]. According to FDA, factors affecting growth of histamine producing bacteria include type and size of fish, handling techniques and cooling methods [7]. In this study, most of the fishes in the markets were kept outside the ice vessels for sell for considerable amount of time which results in ambient temperature abuse of fish and such conditions gives opportunity for histamine, cadaverine and putrescine forming bacteria to proliferate and produce histamine, cadaverine and putrescine.

IV. CONCLUSION

In all fish samples, average concentration of histamine formed was 15 mg/100 g, which was well above the defect action level (5 mg/100 g) but below the toxicity level (50 mg/100 g) as per the guidelines of FDA. These findings suggest that commercial fish samples had been mishandled and ambient temperature abused and also these samples had considerable incidence of histamine forming bacteria which on proliferation under suitable condition may lead to toxic histamine accumulation. The Cadaverine and putrescine forming bacteria were isolated from both fresh and salted fish samples indicate that cadaverine and putrescine formed in these samples would act to decrease threshold dose of histamine to provoke an adverse reaction in vivo and thus

contribute to histamine toxicity by acting as potentiators of histamine.

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