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Occurrence of Biogenic Amine Forming Bacteria in Cured Fishery Products of Thoothukkudi Region of Tamil Nadu, India

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Abstract

The occurrence of biogenic amine forming bacteria in cured fishery products produced and marketed in Thoothukkudi region of Tamil Nadu, India was investigated. Twelve varieties of commercially important salted, dried and smoked fishery products were examined. The total bacterial load in the samples of cured fishery products ranged from 10^2 to 10^5 cfu/g. The occurrence of histamine formers ranged from 0 to 75%, while the cadaverine formers varied from 1 to 66% and putrescine formers from 1 to 51%. Histamine formers were found to be very high in salted Indian ilisha. Salted seer fish recorded a high content of cadaverine formers, whereas dried anchovies (spratts) had highest putrescine formers. The moisture content and water activity (a_w) of the cured fishery products analyzed ranged from 2.35% to 47.91% and 0.41 to 0.98, respectively. The salt content varied from 2.22% to 20.26% in the cured samples examined. Histamine formers and putrescine formers were found to be predominant in most of the cured fishery products. Cured fishery products may pose a health hazard, if not prepared hygienically.

Introduction

In India, about 17% of fish catch is utilized for curing and in Tamil Nadu, it is still higher, with about 25% of fish catch being utilized for curing (Lakshmanan et al. 2002). Significant quantity of fish is subjected to curing in the Thoothukkudi region. Cured fish like dried anchovies are also exported from India, which brings in foreign exchange to a tune of 70 million USD. The cured fish export from Tamil Nadu was about 32% of the total Indian cured fish exports and the contribution from the Thoothukkudi region to Tamil Nadu was about 20% (Anon 2002).

Food poisoning due to high histamine in cured fish was not considered until 1982 when Murray et al. (1982) described a case caused by consumption of Spanish semi-preserved anchovies. Even though histamine is the most important compound in scomberotoxism, histamine toxicity is increased by other amines such as cadaverine and putrescine (Taylor and Sumner 1986). Histamine is responsible for the toxic processes of food poisoning due to consumption of fish species of the Scomberesocidae and Scombridae families (Frank et al. 1981) and fishes of the Clupeidae and Engraulidae families (Veciana-Nogues et al. 1990), which have naturally high quantities of free histidine in their muscular tissue.

The formation of high levels of histamine in fish products was directly correlated with the level of microorganisms present in the product, due to bacterial histidine decarboxylase action on histidine (Ababouch et al. 1991). The formation of histamine depends on the number of microorganisms rather than on the environment in which they grow (Yoshinaga and Frank 1982). Cured fishery products, which are not subjected to thermal treatment, could be the cause of some histamine outbreaks (Veciana-Nogues et al. 1990). High histamine concentrations in cured fish like salted fish could be due to poor quality of raw material, improper handling or other changes during storage (Rodrigues-Jerez et al. 1994). Histamine poisoning occurs worldwide with Japan, the US and UK reporting most incidents, whereas in other countries such as Canada, New Zealand, France, Germany, Norway, Sweden, The Netherlands, Australia, Sri Lanka, India, Indonesia and Egypt, the disease frequency is much less, which may be due to under-reporting by these countries (Ababouch 1991). Other amines produced during fish spoilage are believed to potentiate the effects of histamine and give rise to symptoms of scombroid poisoning (Liston 1990). Large amounts of histamine have often been detected in commercial fishery products of India, including salt-dried products (Nagendra et al. 1988; Chakrabarti 1991, 1993; Shakila et al. 2002). The presence of other amines, viz. cadaverine and putrescine, in commercial salted and dried fishery products was also reported and their concentrations were found to be higher than histamine (Wood and Bostoc 1988; Wootton et al. 1989). Against this background, the present work is aimed at finding out the occurrence of biogenic amine forming bacteria in cured fishery products, which will indicate the incidence of histamine poisoning, if any, in cured fishes.

Materials and Methods

Twelve varieties of cured fishery products such as salted fish, dried fish and smoked fish were obtained from the dry fish market, fish curing yards and dry fish godowns of Thoothukkudi region of Tamil Nadu, India. The cured fish samples of each species from three different batches were tested for the occurrence of biogenic amine forming bacteria such as histamine formers, cadaverine formers and putrescine formers using Moeller's decarboxylase medium supplemented with the amino acids, histidine, lysine and ornithine. They were also examined for the general bacteriological quality by analyzing total bacterial load. About 25g of cured fish samples was homogenized in 225 ml of sterile 0.85% physiological saline and decimal serial dilutions were made in the same diluent and finally they were pour plated on Trypticase soy agar (TSA) (Hi-Media, Mumbai, India). The plates were then incubated at 37^0C for 24 – 48 h. After incubation, the number of colonies developed on the plates were counted for the total bacterial load and expressed as cfu/g (APHA 1976). For the enumeration of total bacterial load from the salted fishery products, 7.5% sodium chloride was added with the plating medium.

Representative colonies were picked up from the TSA plates, purified and checked for the decarboxylase activity using Moeller's decarboxylase broth base (Hi-Media, Mumbai, India) supplemented with the amino acids viz., L-histidine hydrochloride, L-lysine hydrochloride and L-ornithine hydrochloride at 0.5% level (w/v). Medium without the added amino acid served as control. Purified bacterial isolates were inoculated into the three media and incubated at 37^0C for 72 – 96 h. The isolates, which showed positive reaction for decarboxylase test, were considered as amine forming bacteria. Besides examination for bacteriological quality, the samples were tested for the moisture and salt contents by following the standard techniques prescribed in AOAC (1990). Moisture was determined by drying the fish samples at 100^0C for 6-8 h in hot air oven. Salt was estimated by titrating the fish sample extract against standard silver nitrate solution. Water activity (a_w) of the samples was determined by the calculation method involving the values of moisture, salt and fat contents of fish sample, as described by Doe et al. (1982).

Results

The general bacterial quality of commercially important cured fishery products examined in this study is shown in Fig.1. The total bacterial load ranged from 10^2 to 10^5 cfu/g. Smoked tuna (masmin) recorded the lowest bacterial count of 1.20 x 10^2 cfu/g, whereas salted sciaenids had a highest bacterial load (9.40 x 10^5 cfu/g). About 33% of the cured fishery products had

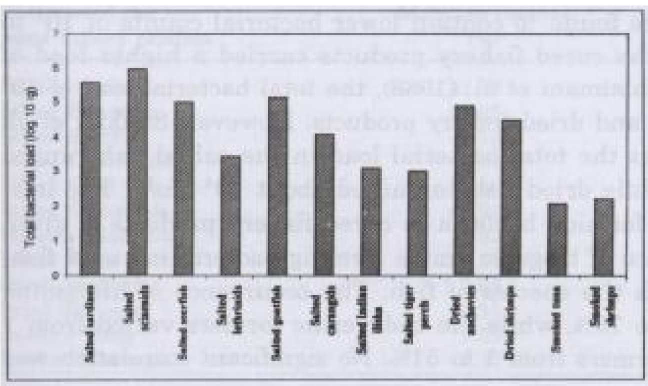

Fig. 1. Total bacterial load of commercially important cured fishery products

a bacterial load of 10^4 cfu/g. Only 25% of the cured fishery products carried a higher load of 10^5 cfu/g. Remaining cured fishery products (42%) were found to contain lower bacterial counts of 10^2 and 10^3 cfu/g. The incidence of biogenic amine forming bacteria in the commercially important cured fishery products is given in Table 1. Among the cured fishery products tested, histamine forming bacteria was found to be high in salted Indian ilisha (75%), salted Tiger perch (70%), salted lethrinids (33%), and salted seer fish (24%). Cadaverine formers were high in salted seer fish (66%), dried anchovies (48%), salted Indian ilisha (42%), salted Tiger perch (40%) and salted lethrinids (29%). Dried anchovies (spratts), salted Tiger perch, salted seer fish, salted Indian ilisha and salted lethrinids were found to contain high putrescine forming bacteria at the levels of 51%, 50%, 44%, 33% and 30%, respectively. Among the cured fishery products examined, histamine formers were not detected in smoked shrimps and salted goat fish.

The moisture and salt contents and water activity (a_w) of the cured fishery products investigated in this study is given in the Table 2. Among the salted fishery products tested, salted sardines recorded very low moisture content of 11.78%, while salted carangids had a highest moisture level of 47.91%. Smoked tuna (masmin) were to found to contain the lowest moisture content of 2.35%. Dried anchovies (spratts) had a slightly higher moisture level of 9.69%. Among the salted fishery products examined, salted Tiger perch recorded very low salt content of 7.28%, while salted Indian ilisha had a highest salt level of 20.26%. Dried anchovies were to found to contain the lowest salt content of 2.22%. Dried shrimps had a slightly higher salt level of 2.63%. The salt content in smoked tuna was 5.85%. Among the cured fishery products examined, dried anchovies recorded a lowest a_w of 0.41, while salted seer fish had a highest a_w of 0.98. Salted lethrinids had a slightly lower a_w of 0.88. Salted sardines were to found to contain the a_w of 0.56. Both the smoked tuna and smoked shrimps contained the same levels of a_w (0.44).

Discussion

The total bacterial load in the commercially important cured fishery products examined in this study ranged from 10^2 to 10^5 cfu/g (Fig.1). About 42% of the products were found to contain lower bacterial counts of 10^2 to 10^3 cfu/g. Only 25% of the cured fishery products carried a higher load of 10^5 cfu/g. According to Kalaimani et al. (1988), the total bacterial load of 10^3 cfu/g is normal in salted and dried fishery products. However, Shakila et al. (2002) have reported that the total bacterial load in the salted fish ranged from 10^4 to 10^5 cfu/g, while dried fish contained about 10^4 cfu/g. The incidence of biogenic amine forming bacteria in cured fishery products is given in Table 1. The occurrence of biogenic amine forming bacteria in cured fishery products varied with the species of fish. The occurrence of histamine formers ranged from 0 to 75%, while the cadaverine formers varied from 1 to 66% and putrescine formers from 1 to 51%. No significant correlation was observed between the total bacterial load and biogenic amine forming bacteria in cured fishery products. Amine forming bacteria was high in salted Tiger perch, salted Indian ilisha, salted seer fish, dried anchovies and salted lethrinids. But, Shakila et al. (2002) have reported that the amine forming bacteria was high in salted sardines followed by salted lethrinids, whereas in dried products, the amine formers were high in dried anchovies than in dried shrimps. Among the smoked products tested in this study, smoked tuna carried a high level of histamine formers. Histamine forming bacteria was also found to be high in salted sardines, salted lethrinids, salted Indian ilisha and salted Tiger perch, besides smoked tuna, as Taylor (1986) has reported that sardines, anchovies and tuna were more frequently associated with the histamine poisoning outbreaks. Histamine formers were not detected in smoked shrimps and salted goat fish, which indicated that the histidine decarboxylating bacteria may be absent in those products or such products may not contain high levels of histidine.

Table 1. Incidence of biogenic amine forming bacteria in commercially important cured fishery products.

Name of the cured fishery product	Incidence of biogenic amine forming bacteria		
	Histamine formers (%)	Cadaverine formers (%)	Putrescine formers (%)
Salted Sardines	11	1	1
Salted Sciaenids	4	3	7
Salted Seerfish	24	66	44
Salted Lethrinids	33	29	30
Salted Goat fish	0	16	5
Salted Carangids	13	14	22
Salted Indian ilisha	75	42	33
Salted Tiger perch	70	40	50
Dried Anchovies	10	48	51
Dried Shrimps	14	5	20
Smoked Tuna	14	8	4
Smoked Shrimps	0	4	12

Table 2. Moisture and salt contents and water activity (a_w) of commercially important cured fishery products.

Name of the cured fishery product	Moisture content (%)	Salt (%)	Water activity (a_w)
Salted Sardines	11.78	10.78	0.56
Salted Sciaenids	38.25	15.43	0.75
Salted Seerfish	45.14	17.73	0.98
Salted Lethrinids	45.80	12.62	0.88
Salted Goat fish	24.16	11.52	0.73
Salted Carangids	47.91	14.21	0.83
Salted Indian ilisha	16.43	20.26	0.69
Salted Tiger perch	23.68	7.28	0.78
Dried Anchovies	9.68	2.22	0.41
Dried Shrimps	16.53	2.63	0.79
Smoked Tuna	2.35	5.85	0.44
Smoked Shrimps	8.32	6.08	0.44

Cadaverine formers were high in salted seer fish and salted goat fish and they were very low in salted sardines and dried shrimps (Table 1). All the cured fishery products tested in this study were found to contain cadaverine forming bacteria, though at low proportions. Salted sciaenids, salted carangids, dried anchovies (spratts), dried shrimps and smoked shrimps were found to contain high putrescine forming bacteria (Table 1). The incidence of putrescine formers was high in the cured fishery products examined in this study, except in salted sardines. It is quite interesting to note that putrescine formers were recorded in highest numbers in both the shrimp products tested. This is due to the fact that the shrimp muscle contains high levels of ornithine and arginine, which on bacterial decarboxylation yield putrescine. Further, putrescine has also been identified as a chemical index of decomposition for the shrimps and lobsters (Meitz and Karmas 1977; Shakila et al. 1995). Shakila et al. (2002) have also reported that the putrescine forming bacteria were isolated in high numbers in salted fish than the other amine forming bacteria. The proportion of cadaverine and putrescine forming bacteria was quite high and histamine-forming bacteria was recorded in low numbers in dried anchovies (Table 1). Anchovies, particularly semi-preserved product, have earlier been reported to be involved in the outbreaks of histamine poisoning (Veciana-Nogues et al., 1990; Rodriguez-Jerez et al., 1994). The higher incidence of histamine formers and putrescine formers in such cured fishery products may lead to histamine toxicity (Huss 1995).

The moisture content of the cured fishery products analyzed ranged from 2.35% to 47.91% (Table 2). Gopakumar (1997) has reported that the moisture content of most of the cured fish products varies from 25 to 40% depending upon the type of products and species of fish used. Kalaimani et al. (1988) supported the present findings of very low moisture content in the smoked tuna. The salt content varied from 2.22% to 20.26% in the cured fishery products examined (Table 2). However, Kalaimani et al. (1988) have reported that the salt content in most of the cured fish products ranged from 5 to 30%. The pH of the product, temperature of storage, microbial load, salt content and a_w are factors generally considered to influence storage life of cured fish products (Gopakumar 1997). For traditionally dried or cured products stored under normal conditions in a tropical climate, a_w can be considered the best index to determine how quickly microorganisms will grow in them. The water activity (a_w) of the cured fishery products analyzed ranged from 0.41 to 0.98 (Table 2). Of the four cured fish products tested for water activity, Kalaimani et al. (1988) found that white baits (dried anchovies) had a very low a_w of 0.14. According to Poulter et al. (1982), salted sun dried fish having a_w of 0.65 above could have a predicted mould-free shelf life between 100 and 450 days. For brined fish with moisture content of 25% or salted dried fish having the moisture content of 25 to 45% (a_w = 0.70), a shelf life of 100 days is expected. A quality dried fish product with an expected shelf life of around 9 – 10 months should have moisture content of below 20%. The higher a_w levels observed in the cured fishery products examined indicated that they were more prone for the growth of bacteria including the amine formers. Wide variation in the moisture and salt contents and water activity levels observed in this study could be attributed to the differences in the species, size, method of salting and drying processes and storage conditions such as temperature and relative humidity. Salted fishery products tested consisted of both the eviscerated and uneviscerated. Similar variations in moisture and salt contents in cured fish were also reported by Chakrabarti (1991).

Conclusion

It can be concluded that histamine formers and putrescine formers were found to be predominant in most of the cured fishery products examined. Their presence in these products was mainly because there exists a group of bacteria, which can survive at low moisture content, high salt contents, low a_w levels and ability to decarboxylase amino acids. The commercially important cured fishery products of Thoothukkudi region of Tamil Nadu, India contained considerable numbers of biogenic amine forming bacteria, which on proliferation under suitable conditions may contribute to the accumulation of toxic amines and lead to public health hazard. Hence, proper sanitary and hygienic practices should be followed along with the use of quality fish raw material to produce the safe and quality cured fishery products.

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