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RESEARCH ARTICLE

ESTIMATION OF AMINES AND AMINE FORMING BACTERIAS IN EDIBLE MARINE FISH *SARDINELLA LONGICEPS* AND ITS PRODUCT

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ABSTRACT

Biogenic amines are formed as a result of amino acid decarboxylation and are linked to food deterioration. Analysis of these metabolites may be great importance to determine food quality. The aim of the study is to estimate the biogenic amines and amine forming bacterias in edible marine fish *Sardinella longiceps* and its product. The fish samples were collected from landing center in Tuticorin coastal area, south east India and from local fish market, Ukkadam, Coimbatore. The dry fish were procured from retail shop in Coimbatore. From the result obtained the samples collected from the market showed the highest total viable count 76×10^5 cfu/g, then landing center and dry product which showed the lowest total viable count 1.0×10^5 cfu/g, 0.7×10^5 cfu/g respectively, which is below the permissible limit of 7×10^5 cfu/g. In this study the amine forming bacteria were isolated from the sample collected from the local market. Different bacterias were isolated from the market sample and the isolated bacterias are *E.coli*, *Pseudomonas*, *Klebsiella*, *Proteus mirabilis*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Salmonella typhimurium*, and *Enterobacter aerogens*. In dry product *E.coli* and *Staphylococcus aureus* were isolated. In the present study the concentration of four biogenic amines; Histamine, putrescine, tyramine and cadaverine in samples collected from market fish was determined, from the revealed results the concentration of histamine (11.004mg/ kg) was higher than the other three biogenic amines, cadaverine, tyramine, putrescine (7.586, 7.120, and 3.495mg/kg) respectively.

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INTRODUCTION

Fish plays an important role in the diet of human beings since it is a good source of animal protein. Fish is one of the most perishable of all stable commodities, and in the tropical climate of most developing countries it will become unfit for human consumption within about one day of capture, unless it is subjected to some form of processing.

Fish spoilage is accompanied by various physical and chemical changes in the gills, eyes, skin tissues and slime. Microbial activities accounts for major spoilage in fish. Once spoilage set, the odour/flavour, texture, colour and sometimes the chemical composition also changes. As soon as fish dies spoilage begins to set in. Upon death, the defence mechanisms of the fish no longer inhibit bacterial growth in the muscle tissue, and biogenic amine-forming bacteria may start to grow, resulting in the production of biogenic amines (FAD, 2011).

Biogenic amines (BA) are organic, basic, nitrogenous compounds of low molecular weight, mainly formed by the decarboxylation of amino acids and with biological activity. The presence of biogenic amines in fish are important from health and toxicological perspective since the consumption of fish or other products containing amines has been associated with some case of food poisoning. Biogenic amines may be hazardous to human health if their levels in foods or beverages reach a critical threshold (Ladero *et al*., 2010). The main source of exogenous amines is dietary, through the uptake of foods or beverages containing high concentrations of these compounds. Most important biogenic amines found in food are histamine, tyramine, putrescine and cadaverine, which are products of the decarboxylation of histidine, tyrosine, ornithine, lysine and phenylalanine respectively. Putrescine can also be formed through determination of agmatine. Microorganisms possessing the enzyme decarboxylases, which convert amino acids to amines, are responsible for the formation of biogenic amines in foods. Depending on the severity of the symptoms, the effects of biogenic amines are described as a reaction intolerance or intoxication or poisoning. Reaction symptoms include nausea, sweating, races, slight variations in blood pressure and mild headache.

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The ability to produce histamine has been found in both gram-negative and in gram-positive bacteria. Many gram-negative bacteria which commonly contaminate food are able to produce histamine.

The strongest histamine producers *Hafnia alvei, Morganella morganii, Klebsiella pneumonia* and more recently, *Morganella morganii, Photobacterium phosphoreum, Photobacterium psychrotolerans* and have been isolated from fish incriminated of scombroid poisoning incidents. The main tyramine producers in fish and fermented sausages are gram-positive bacteria within the genera *Enterococcus* (*Enterococcus faecalis* and *Enterococcus faecium*), *Lactobacillus* (*Lactobacillus curvatus and Lactobacillus brevis*), *Leuconostoc* and *Lactococcus* and *Carnobacterium* species. *Staphylococcus* may also have a role in the production of tyramin.

Putrescine and cadaverine production has mainly been related to gram-negative bacteria, especially in the family's *Enterobacteriaceae, Pseudomonaceae* and *Shewanellaceae*, generally associated with spoilage. Enterobacteria genera *Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella* and *Shigella* are associated with production of considerable amounts of putrescine and cadaverine in food (Kim *et al*., 2009).

Jeyasekaran and Shakila (2003) reported that the occurrence of biogenic amine forming bacteria in cured fishery products of Thoothukkudi region. Huang *et al*., (2010) analysed the histamine level and histamine forming bacteria in dried fish products sold in Penghu Island of Taiwan.

Estimation of formatting biogenic amines concentration in fresh and processed sardine fish products during different storage conditions was tested by Manar and Farag (2013). Sumitha *et al*., (2014) identified two bacterial strains as histamine producing bacteria in dried fish samples.

The reasons for amine determination in fish are twofold. The first is potential toxicity; the second is the possibility of using them as food quality indicators. Therefore, the present investigation was designed to study the biogenic amines and biogenic amine forming bacteria's in fish and fish products.

MATERIALS AND METHODS

Samples collection

Fresh raw sardine (*Sardinella longiceps*), were collected from landing center in Tuticorin, Tamil Nadu and from local fish market, Ukkadam, Coimbatore. The dry fish of *Sardinella longiceps* were procured from retail shop in Coimbatore, Tamilnadu.

Sample preparation

The collected samples were immediately placed in ice box containing crushed ice, kept cold and transported to the laboratory. The samples were beheaded, gutted, washed and filleted. Then the known weights of muscle samples were homogenate and filter and then used for further analysis. Similarly the muscle samples were prepared from dry fish.

Bacteriological Examination

Total viable count

One gram (1g) of fish sample was dissolved in sterile deionized water and serially diluted. One millilitre (1ml) of appropriate dilutions was seeded on plate count agar using spread plate method, and the medium was then incubated at 37^0 C for 24 hours. The plate count agar was examined and colonies present were counted and recorded after incubation at 37^0 C for 24 hours to get the total colony count in cfu $g.^{-1}$

Isolation of microorganism

One gram (1g) of fish sample was serially diluted, 1 ml of an appropriate dilution was inoculated on nutrient agar plates and the plates were incubated for 24 hours at 30^0 C. After 24 hours, sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar plates, then it was incubated for 24 hours at 30^0 C in order to get pure cultures. The pure cultures were then stored in a refrigerator at 4^0 C. The isolates were identified using their macroscopic, cultural and biochemical characteristics.

Biogenic amine analysis

Preparation

For biogenic amine determinations, a rapid HPLC (High performance liquid chromatography) method was used. Five grams of fish muscles were taken and transferred to a 250 ml centrifuge tube. Then, the sample was homogenised with 20 ml of 6% TCA for 30 minutes in mortar and pestle, and then it is subjected to centrifuge at 10,000 rpm for 10 minutes at 4° C and filtered through Whatman No.1 filter paper. The aliquot was brought to 50 ml with distilled water and stored in freezer (-18°C) until derivatisation. Before injection into the HPLC, 2ml of fish aliquot were mixed with benzoyl chloride.

Reagents

Biogenic amines standards (Putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride and tyramine dihydrochloride) were purchased from Jayam Scientific Company. For HPLC grade, Acetonitrile was used as solvent A and deionized ultrapure millipore water was used as solvent B.

Preparation of standard amine solution

Putrescine dihydrochloride (182.9mg), cadaverine dihydrochloride (171.4mg), histamine dihydrochloride (165.7mg) and tyramine dihydrochloride (126.7mg) were dissolved separately in 10 ml HPLC grade water. A composite standard comprising all the above biogenic amines were also used. The final concentration of free base for each amine was 10mg/ ml solution.

Analytical method

Derivatisation procedure, chromatographic conditions and quantification of biogenic amines were done according to the method of Ozogul (2002).

Apparatus and Columns

HPLC analyses were performed with Merck- Hitachi Model D-6500 (Merck- Hitachi L- 4500) and an intelligent pump (Merck- Hitachi L- 6200A). The column was a Waters Spherisorb ODS-2 C_{18} (125 × 4.60 mm, particle diameter 5 µm).

HPLC Chromatographic conditions

An elution gradient was used as follows: 0-6.00 min, K600; 6.01-25.00 min, K563; 25.01-27.00, K130 and27.01-30.00, K600. Flow rate was 0.8 mL/min. A calibration curve (1, 2.5, 5, 10, and 25 nmol/ml) was established daily. Retention times were standardized for tyramine, histamine, putrescine, cadaverine, respectively. Detection limits were 50 p mol/mL for tyramine, histamine, putrescine, and cadaverine. Tissue samples contained large quantities of free amino acids which were detected and eluted primarily in the solvent front. It was necessary, therefore, to alter the excitation and emission wavelengths to 430 and 600 nm respectively from 1.00 to 2.60 minutes after injection. They were subsequently returned to 330 and 466 nm, respectively.

RESULTS AND DISCUSSION

For the present research the selected fish *Sardinella longiceps* were collected from landing centre and market. The dry samples were collected from local market. All the samples were cleaned and skinned out aseptically, then subjected to various analyses.

The total viable count of fresh sardine sample collected from landing centre showed 1.0×10^5 cfu/g and the market sample showed 76×10^5 cfu/g which is far exceed than the permissible limit of food and drug administration(7×10^5 cfu/g) (table 1).

In this present study the sample collected from the market has the highest bacterial count then compared to the landing centre. This is may be due to, the 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 this gives opportunity for amine producing bacteria to proliferate (Joshi *et al*., 2011).The present study agreed with the findings of Faith *et al* (2013), that microbial load increases with handling, duration of storage and temperatures.

Total viable count of the dry sample was recorded as 0.7 cfu/g, which is below the acceptable level (7×10^5 cfu/g). The present result was in agreement with Mansure (1989), who determined that the total bacterial count of *Puntius* species ranged from 1.0×10^5 to 1.5×10^5 cfu/g. Comparison of the total bacterial count of the fresh and dried samples showed that fresh (landing center) bacterial count was 1.0×10^5 cfu/g and dried sardine bacterial count was 0.7×10^5 cfu/g, which was lower than the fresh sample. This indicates that drying reduced the microbial load of the samples. The present study agreed with the findings of Oladipo and Bankole (2013), on microbial quality of fresh and dried *Clarias gariepinus* and *Oreochromis niloticus*, revealed that the total viable count of fresh samples were 2.0 $\times10^5$ cfu/g and which was higher than the dried *Clarias gariepinus*(1.8×10^5cfu/g).

The distribution of the bacteria species present in the sample (landing and market) is shown in table (2). A total of 9 organisms were isolated from the market sample and the isolated organisms were *E.coli, Pseudomonas, Klebsiella, Proteus mirabilis, Shigella dysenteriae, Staphylococcus aureus, Streptococcus faecalis, Salmonella typhimurium,* and *Enterobacter aerogens.* The microorganisms of fish intended for human consumption depend on the environmental conditions of its natural habitat. The isolated bacteria include species of the genera *Pseudomonas* and *Enterobacter*, it is clearly indicates that bacteria present in fishes are normally associated with those found in their natural environment and proportion of the initial population can easily be changed after harvesting but this depends on the ability of those bacteria to adapt to the new conditions.

A total of 2 organisms were isolated from the dry sample and the isolated organisms were *E.coli* and *Staphylococcus aureus* (Table-2). This conforms to the report of Ozyurt *et al*., (2009), that heat treatment during drying of fish would destroy or inactive most pathogens found in fish. *Staphylococcus aureus* has also been detected during the process of drying and subsequent smoking of eels in Alaska (Eklund *et al*., 2004).

In the present study, the concentration of four biogenic amines: Histamine, putrescine, tyramine and cadaverine in samples collected from market fish was determined (Table 3).The result revealed that the concentration of histamine (11.004mg/kg) was higher than the other three biogenic amines, cadaverine, tyramine, putrescine (7.586, 7.120, and 3.495mg/kg) respectively. In sardine, the quantity of histamine was always higher compared to other biogenic amines, because the free histidine was the most abundant amino acid. Histamine production is associated with the growth of bacteria that possess the enzyme histidine decarboxylase. In fish, several histamine-producing bacteria have been implicated as primary contributors to histamine formation. They are *Morganella morganii, Klebsiella pneumonia, Enterobacter aerogens, Proteus vulgaris, Hafnia alvei* and *Escherichia coli*. These bacteria are capable of producing hazardous amounts of histamine in a very short period of time when the fish are kept at elevated temperatures. Food and Drug Administration established a defect and hazard action level of histamine in fish at 500mg/ kg (FDA, 1996) as a permissible limit for histamine content. Histamine content was significantly correlated to *Enterobacteriaceae* and *Pseudomonas* counts.

Results in table (3) showed that cadaverine content was (13.007mg/kg). It is worthy to mention that cadaverine formation unlike histamine occurs in a wide range of fish species, which could be explained by readily available free lysine in most fish species. Cadaverine formation begins early increase steadily and correlates well with histamine, muscle

alterations, microbial activity, therefore it can be considered a good indicator of incipient and late spoilage of fish. Cadaverine content in sardine was significantly correlated to coli forms count. Lakshmanan *et al*., (2002) observed that the bacteria that produce cadaverine and putrescine survive and multiply rapidly between 9 and 12 days, and contribute to the formation of amines after the ice storage of emperor fish and of shrimp. Rodrigus *et al*., (2013) suggest that the presence of cadaverine may serve as an indicator of muscle change, which is caused by increased activity of microorganism. Halasz *et al*., (1994) observed that the bacteria of the family *Enterobacteriaceae* are usually implicated in the formation of cadaverine.

From the result the tyramine (7.120mg/g) levels are lower than the histamine (11.004mg/g) and cadaverine (7.586mg/g).Tyramine has been identified as the major mutagen precursor. In our study, tyramine was detected in examined fish; it was in the range of 7.120mg/kg. This level of contamination is far below the allowable level of tyramine in fish. Nout (1994) pointed out that maximum allowable level of tyramine in foods showed be in the range of 100-800 mg/kg.
When analyzing the results of biogenic amines the putrescine have no adverse health effect, but they may react with nitrite to form carcinogenic nitrosamines and also can be proposed as indicators of spoilage. Furthermore higher levels of putrescine have been recognized as potentiators of histamine or tyramine toxicity, but no recommendations about level have been suggested. In our investigation putrescine level was 3.0mg/g. The bacteria of the genus *Pseudomonas* are responsible for the formation of putrescine in fishes.

Table 1 Total viable colony count of sample collected from different sources

S.No	Parameters	Landing center	Market	Dry sample
1.	Total viable count @ 37^0 C	1.0×10^5 cfu/g	76×10^5 cfu/g	0.7×10^5 cfu/g

Table 2 Species identification of *Sardinella longiceps* Collected from different sources

S.No	Microorganisms	Landing center	Market	Dry sample
1	*Escherichia coli*	-	+	+
2	*Proteus mirabilis*	-	+	-
3	*Shigella dysenteriae*	-	+	-
4	*Staphylococcus aureus*	-	+	+
5	*Pseudomonas aeruginosa*	-	+	-
6	*Streptococcus faecalis*	-	+	-
7	*Salmonella typhimurium*	-	+	-
8	*Klebsiella pneumonia*	-	+	-
9	*Enterobacter aerogens*	-	+	-

Table3 Biogenic amine analysis of *Sardinella longiceps* collected from market

S.No	Biogenic Amines	Content in Market Sample (mg/kg)
1	Histamine	11.004
2	Cadaverine	7.586
3	Tyramine	7.120
4	Putrescine	3.495

CONCLUSION

Fish are rich source of high quality proteins which represents a risk in the decomposition process. The disintegration of protein yields peptides and amino acids, which are susceptible to further decay, resulting in biogenic amines. Biogenic amines accumulation in fish results mainly from decarboxylation activity of bacteria toward free amino acids.

Histamine is identified as the major chemical hazard of seafood. It is the causative agent of scombroid poisoning and it is formed by time or temperature abuse of fish muscle. Since histamine is a heat resistant it can remain intact in fish. Histamine is heat resistant, it can remain integral in canned or other processed fish products. It is also know that once histamine is formed, it cannot be eliminated by heat treatments therefore, recontamination of the fish with the enzyme-forming bacteria is required for extra histamine to form (FDA, 2001). Cadaverine and putrescine are considered to be histamine potentiators. Tyramine act as a catecholamine releasing agent, resulting in increased blood pressure.

Whatever the fish is making a late frost and improper the conditions of its transporting and keeping in ice, so auto digestion processes and biogenic amines decarboxylased bacteria act faster. The amount of histamine in this investigation is lower than standard limit by the FDA,2011 (Food and Drug Administration).

Reference

Abu Zar Faith, Abde Rahman Pooladgar, Shamsedin Ghaem Maghami and Rahman Ioni. 2013. The changes evaluation of biogenic amines (histamine) by HPLC, in shanak yellow fin fish (*Acanthopagrus latus*) within 18 days of ice storage. Indian *Journal of Science Research and Technology*, 1 (1):29-34**.**

Eklund, M.V., Peterson, M.E., Poysky, F.T., Paranjpye, R.N., Pelroly, G.A. 2004. Control of bacterial pathogens during processing of cold- smoked and dried salmon strips. *Journal of Food Protect*, 67: 347-351**.**

Food and Drug Administration (FAD). 2011. Fish and fishery products hazards and controls guidance, 4th Edn. Washington, DC: Department of Health and Human Services**.** Food and Drug Administration Center for Food Safety and Applied Nutrition.

Food and Drug Administration (FDA), 1996. Decomposition and histamine in raw, frozen tuna and mahi, canned tuna and related species**.** Food and Drug Administration, Center for Food Safety and Applied Nutrition**.**

Frenandes, C.F., Flick, G.J., Thomas, T.B. 1998. Growth of inoculated psychotropic pathogens on refrigerated fillets of aquacultured rainbow trout and channel catfish. *Journal of Food Protection*, 61: 313-317.

Halasz, A., Barath, A., Simon-Sarkadi, L., Holzapfel, W. 1994. Biogenic amines and their production by microorganisms in food. Trends Food Science Technol. 5:-42-130.

Huang, Y.R., Liu, K.J., and Tasi, Y.H. 2010. Histamine and histamine forming bacteria in dried fish products sold in Penghu Island of Taiwan. Food Control 21:1234-1239.

ICMSF (International Commission on Microbiological Specifications for Foods) 1986. Sampling plans for fish and shellfish, In: Microorganisms in Foods. Sampling for microbiological analysis: Principles and Scientific Applications, 2: 181-196.

Jeyasekaran, G. and Shakila, J. 2003. Occurrence of biogenic amine formation bacteria in cured Fishery products of Thoothukkudi region of Tamil Nadu, India. Asian Fisheries Science; 16:195-202.

Joshi, PA, Bhoir, VS. 2011. Study of histamine forming bacteria in commercial fish samples of kalayan city. *Indian Journal of Current Science Research*, 1(2):39-42.

Kim, MK, Mah, J.H. and Hwang, H.J. 2009. Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Journal of Food Chemistry*, 116, 87–95.

Ladero, V., Callea-Enriquez, M., Fernandez. M., Alvarez.M. 2010. Toxicological effects of dietary biogenic amines. In Current Nutrition and Food Science, 6:145-156.

Lakshman, R., Shakila, R.J., and Jeyasekaran, G. 2002. Survival of amine forming bacteria after the ice storage of fish and shrimp. *Journal of Food Microbiology*, 19, 617-625.

Manar and Farag, M.A. 2013. Estimation of formatting biogenic amines concentration in fresh and processed sardine fish products during different storage conditions. *World Journal of Fish and Marine Science* 5(6):628-636.

Mansur, M.A., Gheyasuddin, S. and Bhuiya, A.K.M.A. 1989. Preparation of a new ready to-use dried semifermented fish product of increased shelf- life from *Puntius* species. *Bangladesh Journal of Fish*, 19(1): 27-32.

Nout, M.J.R., 1994. Fermented foods and food safety. Food Research International, 27:291-298.

Oladipo, I.C. and Bankole, S.O. 2013. Nutritional and microbial quality of fresh and dried *Clarias gariepinus* and *Oreochromis niloticus*. *International Journal of Applied Microbiology and Biotechnology research*, 1: 1-6.

Ozogul, F., Taylor, K.D.A., Quantick,P and Ozogul,Y. 2002. Biogenic amines formation in Atlantic herring (*Clupeaharengus*) stored under modified atmosphere packing using a rapid HPLC method. *International Journal of Food Science and Technology*; 37, 515-522.

Ozyurt,G.,. Kulley, E., Ozkutuk, S. and Ozogul, F. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goat fish (*Upenus molluccensis*) during storage in ice. Food control, 114:505-510.

Rodrigues B.L, Alvares.T.S.Costa M.P, Sampaio.G.S.L, Marsico E.T. and Conte.C.A. 2013. Concentration of biogenic amines in Rainbow Trout (O*ncrohynchus mykiss*) preserved in ice and its relationship with physicochemical parameters of quality. *Journal of Aquacatic Research Development*, 4(3).

Sumitha, D., Sundar, K. and Haladyshetty, P. 2014. Occurrence of histamine forming bacteria and histamine in dried fish. *Advanced Journal of Food Science and Technology*, 6(4):428-432.

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