International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume: 3 | Issue: 3 | Mar-Apr 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 - 6470

Quantitative Analysis of Histamine Production in Idli Batter

Rubasri Gunasekaran[1], Murugan A[1], Prathapkumar Shetty[2]

[1]Department of Microbiology, Periyar University, PeriyarPalkalai Nagar, Salem, Tamilnadu, India
[2]Department of Food Science and Technology, Pondicherry University, Pondicherry, India

How to cite this paper: Rubasri Gunasekaran | Murugan A | Prathapkumar Shetty "Quantitative Analysis of Histamine Production in Idli Batter" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-3 , April 2019, pp.1190-1193, URL: https://www.ijtsrd.com/papers/ijtsrd22942.pdf

IJTSRD22942

ABSTRACT

Biogenic amine formed from protein rich foods is menace throughout the world. Fermented food products are major concern due to the presence of histamine, leading to histamine poisoning. Related in human, Histamine consumption may leads to severe health concerns at chronic levels due to effects on the restless, headache, vomiting, hypotension, flushing. Dried fish are known to be one of the most popular fish products consumed in the Indian subcontinent, often prepared under unhygienic conditions. The present study was aimed to determine the occurrence of histamine and histamine-forming bacteria in dried fish and idli batter procured from Pondicherry markets by high-performance liquid chromatography.

KEYWORDS: *Histamine, High Performance Liquid Chromatography, dried fish and idli batter.*

INTRODUCTION:

Idli is one of the very widely used fermented foods of India, particularly in the South. It is prepared from rice and black gram mungo (*Phaseolusmungo*), a legume. Biogenic amines (BAs) are toxic substances, formed in food products as a result of microbial action either with fermentation or at storage (**Shalaby, 1996; Santos, 1996**).The most important BAs found in human food are putrescine, cadaverine, β-phenylethylamine, tyramine, spermine, histamine, spermidine, tryptamine and agmatine. BAs cause food poisoning by stimulating the nerves and blood vessels in humans and animals. Microbial spoilage of food may be accompanied by the increased production of histamine. The presence of biogenic amines can be used as an indicator of food spoilage. Therefore, it is important to monitor biogenic amines levels in foods during the fermentation and ageing. The toxicological level of BAs is very difficult to establish because it depends on individual characteristics and the presence of other amines. However, a maximum total BAs level of 750–900 mg/kg has been proposed (**Ladero *et al.*, 2010**). The microbiological complexity of seafood is linked to the specific as well as non-specific microbial contaminants originating from the natural environment or being acquired during processing. The wide range of environmental habitats,(freshwater to saltwater, tropical waters to arctic waters, pelagic swimmers to bottom dwellers, and degree of pollution) (**Gram and Huss, 1996**). Some technological processes such as salting, ripening, fermentation ormarinating can increase the possibility of formation of BAs. A low pH (4.0–5.5), which can be achieved in salted anchovies, for instance, is favorable for enhanced amino acid decarboxylase activity. Moreover, important proteolysis is observed during ripening of salted anchovies, resulting in the liberation of peptides and free amino acids including histidine (**Hernández-Herrero *et al.*, 2002**). Biogenic amines, including histamine, are formed in food by the action of histidine decarboxylases on histamine amino acid. Possibility of alternative biosynthetic routes and presence of different types of microorganisms in the fermenting medium as well as the microbial interactions makes it more complicated to determine the exact responsibility of amine biogenesis. Recently, a number of techniques based on HPLC, capillary electrophoresis, etc. have been developed for the detection of BAs (**Cinquina *et al.* 2004**). These methods can identify the different types in food samples and accurately quantify them. Having notice of the above points the present study was aimed to quantify the amount of histamines present in the dried fish and idly batter purchased from local markets of Pondicherry using high performance liquid chromatography.

MATERIALS AND METHODS

Sample Collection

Batter (Idli) samples were collected from 0-26 hours of incubation, in sterile containers and transferred to

laboratory for further analysis. Dry fish containing concentration of histamine was used on positive control throughout studies.

Isolation of Histamine Forming Bacteria

To isolate histamine-forming bacteria one ml of sample was taken from the homogenized dry fish samples & idly batter samples are serially diluted using phosphate buffer saline (PBS) and 0.1 ml aliquots of the diluents (10^{-3},10^{-5},10^{-7}) was spread on histamine-forming bacterium isolation agar, (HBI agar) fortified with L-histidine. Three plates were used for each dilution and incubated at 37 °C for 6 to 7 days. The colony formed was counted and the bacterial counts were expressed in colony forming units (Cfu/g). Colonies with blue or purple color on the plates were picked and further inoculated. Niven's medium are used for the confirmation of histamine producing bacteria

Histamine Determination

Samples were inoculated in Niven's broth at 35^0C for 48 – 72 hours incubated and the histamine content was measured. The reaction color was measured by an adsorption spectrophotometer at 580 nm. The histamine values were evaluated as the ratio of concentration to growth of bacteria (OD_{580}).

Histamine Analysis

Standard Preparation

Histamine dihydrochloride was dissolved in 50 mL of 0.1 M HCl and used as the standard stock solutions.

Sample Preparation

5 g idli batter sample was ground and transferred to 50 mL centrifuge tubes. The sample was homogenized for 3 min with 20 mL of 6% trichloroacetic acid (TCA), centrifuged (10,000 g, 10 min, 4 0C) and filtered through Whatman No. 2 filter paper. TCA was added to the filtrates to bring a final volume of 50 mL. Standard histamine solutions and 2 mL aliquots of the food sample extracts were derivatizedwith benzoyl chloride according to **Hwang et al. (1997)** method. The benzoyl derivatives were dissolved in 1mL of acetonitrile and 20 µL aliquots were injected for HPLC analysis.

Determination of Optimal Reaction Temperature and Time for Amine Benzoylation

To 2ml of mixed amine solution containing 0.1mg of each amine, 12 ml of 2 M sodium hydroxide was added, followed by 10 µl of benzoyl chloride. The solution was mixed by using a vortex mixer and was allowed to stand at 20, 30, 40 and 60 0C for 20, 40 and 60 min. The benzoylation was stopped by adding 2 ml of saturated NaCl solution, and the solution was extracted with 3 ml of diethyl ether. After centrifugation, the upper organic layer was transferred into a tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of acetonitrile and 20-µl aliquots were injected for HPLC analysis procedure. The optimal reaction temperature and the time for amine benzoylation were obtained by the responding peak heights of amine and remaining benzoyl chloride in HPLC analysis.

Determination of the Calibration Curve of Standard Amine

Preparation of standard amine solution (histamine dihydrochloride [82.8 mg])

To 2 ml of mixed standard amine solutions containing 0-0.5mg of each amine, 1 ml of 2 M sodium hydroxide was added, followed by 10µl of benzoyl chloride .The reaction temperature and the time were set 300c for 40 min, respectively. After benzoylation, the standard sample was extracted and determined by HPLC analysis as described.

HPLC Conditions

The contents of the histamine in the test samples were determined according to (**Hwang et al., 1997**) method with slight modification. The detection of histamine was performed using Prominence Ultra-Fast Liquid Chromatographic (UFLC) system (Shimadzu, Japan). Model LC20AD with PDA Detector (set at 233 nm) C18 column (250×4.6 mm) was used for separation. The gradient elution program began with 50:50 (v/v) acetonitrile: water at a flow rate of 1 mL/min for 0.5 min, followed by a linear increase to 85:15 aectonitrile: water during the next 6.5 min. the acetonitrile : water mix held constant at 85:15 for 5 min and then decrease to 50:50 (0.8 mL/min) during the next 2 min. Histamine standard was analyzed with test samples to check the chromatographic consistency. The samples were injected twice. Peak heights of the histamine standard were used to prepare the standard curves for determination of histamine concentration in the test sample.

RESULTS AND DISCUSSION

Microbiological analysis of idli batter and dry fish

Histamine forming bacterial count in idli batter sample during the natural fermentation hours (0, 2, 4, 6, 8, 10 and upto 26) were enumerated using Niven's medium spread plate method. At 0 hr histamine forming bacterial Counts were 1.4 x103 CFU/ml. At 2nd hour the bacterial counts were 4.6x103 CFU/ml. At 4th hour the bacterial count was 1.5x103 CFU/ml. At 26th hr histamine forming bacterial counts was 12.6x105 CFU/ml respectively. histamine forming bacteria in dried ribbon fish, tuna and yellow tail king were 8.36×107 CFU/ml, 6.23×107 CFU/ml and 2.4×107 CFU/ml . Bacterial count gradually increases from 0 hour to 26 hour. Histamine forming bacterial count found in idli batter sample during natural fermentation was less compared to dried fish sample. During natural fermentation diverse group of microorganism and pH play a major role in amine forming bacteria. It has been reported that decaroxylase activity increased with pH ranged between 4-5.6 and the amine concentration also found to depend on the growth of the bacteria.

The production of histamine in idli batter during fermentation periods.

Dry fish sample served control. Histamine production in idli batter increased 0.0726 mg/100g, 8.744mg/100g and 1.0713mg/100g. Histamine production gradually decreased from 6th hr 0.4526mg/100 g to 18 thhr 0.2057mg/100g, at the end of the fermentation period 26th hr 28.6733 mg/100g. Histamine production in an idli batter showed a gradual increase and decrease in the histamine content during the fermentation periods which was due to the microflora such as lactic acid bacteria present in the fermented batter sample, shift in Ph from acid to alkaline, protein content in the sample and the fermentation time. Dried fish sample showed high histamine content compared to idli batter. Due to long storage time and unhygienic conditions dried fish was found to contain high histamine. Dry fish sample compare was higher than the acceptable limit (5mg/100g) as per guidelines of USFDA.

Graph 1.Evaluation of histamine production in the idli batter using HPLC

Previous report shows the presence of free amino acids in fermented idli batter. As the fermentation time increases, more free amino acids are released due to protein hydrolysis in black gram dhal and by the bacteria involved in fermentation. Histamine & biogenic amines are produced by enzymatic reaction their level increases in the chain, even under chilling condition. So it is to perform the analysis very quickly after the sampling or when it is possible and depending on the methods to prepare the acidic extract that can be kept about one week at 4 °C. Almost all foods has bacteria on it right from the start. This is because most food comes from the natural environment where bacteria are always present when meat is stored in the temperature danger zone of between 50° & 60°C. Even for short periods, the number of bacteria present can multiply very quickly. Fish are a particular concern when it comes to maintaining temperature of less than 50c.This is because if the fish contain an aminoacid called histidine the bacteria can turn this onto histamine which is a physiological amine responsible for many allergic reactions. Histamine is formed it is not destroyed by freezing, cooking, smoking, curing or canning. This is the same histamine that causes problem for some people when high levels are produced in cheese and wine. Food comparability is dose dependent (cumulative effects of biogenic amines like histamine, histamine liberators &enzyme inhibitors)comparability varies individually, some persons are moves susceptible to histamine than to liberators or the other way around. In the first phase of 4-6 weeks, leave away all ingredients labelled as incompartible until all symptoms have disappeared permanently. Later on slightly incompatible foods can be triedout one after the other to see if there is a reaction identify your individual torlerancelevel. Regarding liberators it can last several days until they have added up to an incompartible dose. In many foods like wine cheese, fish, meat products etc, A wide &unpredictable variation of histamine levels can be observed.

CONCLUSION

Natural fermented idly batter contain histamine and histamine producing bacteria. Dry fish is a part of the meal in the coastal regions in various Asian countries including India. Results in this study shows that, presence potential histamine producers in the dry fish and idly batter. Level of histamine in dry fish sample analysed were relatively low indicating the low risk. The samples analysed in this study were storing from market with proper handling method. However, in case idli batter which is prepared under unhygienic conditions and low quality, there may be risk of potential poisoning. More samples from wider geographical locations and various quality conditions needs to be analysed to properly understand the risk associated with the fermented idly batter with respect to histamine poisoning.

Acknowledgements

Authors would like to thank Pondicherry University for the facilities provided for this project and Masters of philosophy to the third author.

REFERENCES

[1] SHALABY, A. R. 1996. Significance of biogenic amines to food safety and human health. *Food research international*, 29(7), 675–690. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/S0963-9969(96)00066-X".org/ HYPERLINK "http://dx.doi.org/10.1016/S0963-9969(96)00066-X"10.1016/S0963- HYPERLINK "http://dx.doi.org/10.1016/S0963-9969(96)00066-X"9969(HYPERLINK "http://dx.doi.org/10.1016/S0963-9969(96)00066-X"96)00066-X

[2] SILLA SANTOS, M. H. 1996. Biogenic amines: their importance in foods. *International journal of food microbiology,* 29, 213–231. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/0168-1605(95)00032-1".org/ HYPERLINK "http://dx.doi.org/10.1016/0168-1605(95)00032-1"10.1016/0168- HYPERLINK "http://dx.doi.org/10.1016/0168-1605(95)00032-1"1605(HYPERLINK "http://dx.doi.org/10.1016/0168-1605(95)00032-1"95)00032-1

[3] LADERA, V., CALLES ENRÍQUEZ, M., FERNÁNDEZ, M., ALVAREZ, M. A. 2010. Toxicological effects of dietary biogenic amines. *Current nutrition and food science*, 6, 145–156. http://dx.DOI.org/10.2174/157340110791233256

[4] GRAM L., AND HUSS H. H. 1996. Microbiological spoilage of fish and fish products. *Int. J. Food microbiol*. 33, 121–13710.1016/0168-1605(96)01134-8. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/0168-1605(96)01134-8".org/ HYPERLINK "http://dx.doi.org/10.1016/0168-1605(96)01134-8"10.1016/0168- HYPERLINK "http://dx.doi.org/10.1016/0168-1605(96)01134-8"1605(HYPERLINK "http://dx.doi.org/10.1016/0168-1605(96)01134-8"96)01134-8

[5] HERNÁNDEZ-HERRERO M. M., ROIG-SAGUÉS A. X., LOPEZ-SABATER E. I., RODRÍGUEZ-JEREZ j. J., MORA-VENTURA M. T. 2002. Influence of raw fish quality on some physicochemical and microbial characteristics as related to ripening of salted anchovies (*engraulisencrasicholusl*). *Food chem. Toxicol*, 67, 2631–2640. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08790.x

[6] REDDY NR, SATHESK, PIERSON MD, SALUNKHE DK. 1982. 'idli', an indian fermented food: *a review.j food qual*, 5(2), 89–101. http://dx.Doi.org/ 10.1111/j.1745-4557.1982.tb00736.

[7] NISHA P, LAXMI A, REKHA S. 2005. Effect of stabilizers on stabilization of 'idli' (traditional south indian food) batter during storage. *Food hydrocoll*, 19:179–186. http://dx.Doi.org/10.1016/j.foodhyd.2004.03.007

[8] HEMALATHASP, SRINIVASAN K. 2007. Influence of germination and natural fermentation on bioaccessibility of zinc and iron from food grains. *Eur j clinnutr*, 61(3), 342–348. http://dx.doi.org/10.1038/sj.ejcn.1602524

[9] ANNAN NT, PLAHARWA, POLL L, JAKOBSEN M. 2005. Effect of soybean fortification on ghanaian fermented maize dough aroma. *Int j food scinutr*, 56(5), 315–326. http://dx.doi.org/10.1080/09637480512331390655.

[10] RAJALAKSHMI R, VANAJA K. 1967. Chemical and biological evolution of the effects of natural fermentation on the nutritive value of foods prepared

from rice and gram. *Br j nutr*, 21, 467–473. http://dx.doi.org/10.1079/bjn19670048

[11] TSANGALIS D, ASHTON JE, MCGILLAEJ, SHAH NP. 2002. Enzymic transformation of isoflavone phytoestrogens in soymilk by β–glucosidase producing bifidobacteria. *J food sci*, 67, 3104–13. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08866.x

[12] ROSS, R. P., MORGAN, S., HILL, C. 2002. Preservation and fermentation: past, present and future. *International journal of food microbiology* 79, 3–16. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/S0168-1605(02)00174-5".org/ HYPERLINK "http://dx.doi.org/10.1016/S0168-1605(02)00174-5"10.1016/S0168- HYPERLINK "http://dx.doi.org/10.1016/S0168-1605(02)00174-5"1605(HYPERLINK "http://dx.doi.org/10.1016/S0168-1605(02)00174-5"02)00174-5

[13] CINQUINA, A. L., CALÍ, A., LONGO, F., DESANTIS, L., SEVERONI, A., AND ABALLE, F. 2004. Determination of biogenic amines in fish tissues by ion- exchange chromatography with conductivity detection. *J. Chromatogr.* 1032, 73–77. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/j.chroma.2004.01.013".org/ HYPERLINK "http://dx.doi.org/10.1016/j.chroma.2004.01.013"10.1016/j.chroma.2004.01.013

[14] HOLZAPFEL, W. H., GEISEN, R., SCHILLINGER, U. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International journal of food microbiology* 24, 343–362. http://dx.doi HYPERLINK "http://dx.doi.org/10.1016/0168-1605(94)00036-6".org/ HYPERLINK "http://dx.doi.org/10.1016/0168-1605(94)00036-6"10.1016/0168- HYPERLINK "http://dx.doi.org/10.1016/0168-1605(94)00036-6"1605(HYPERLINK "http://dx.doi.org/10.1016/0168-1605(94)00036-6"94)00036-6