ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2011; 2(5):1493-1496

ORIGINAL RESEARCH ARTICLE

A Preliminary Investigation of Freeze Storage on the Production Trimethylamine Nitrogen in *Leiognathus splendens (*Family: Leiognathidae)

P.Inbasekar[1], A.Sivaraj[2], K.Sathiyaraj[2], S. Sundaresan[2], B.Senthil kumar[*2]

[1]*Jai Shree Venkatesha Arts & Science College, Dharmapuri – 636809. Tamilnadu, India.*
[2]*Department of Zoology, Thiruvalluvar University, Serkkadu,Vellore – 632106. Tamilnadu, India.*

Received 05 Jul 2011; Revised 08 Oct 2011; Accepted 17 Oct 2011

ABSTRACT

The aim of the present study is evaluating the effectiveness of TMA-N as parameter estimating the freshness of fish and the impact of storing fish with gut and without gut in the production of TMA-N. The increase in the amount of TMA-N in the fresh fish was highly significant ($p< 0.001$) from 2.72 ± 0.194 to 4.244 ± 0.276 after 4 hours of refrigerated storage and to 8.584± 0.921 after 24 hours of storage in refrigeration. TMA-N values were significantly elevated in both with gut and without gut when compared to fresh fish.

Key words: Ice storage, Total volatile basic nitrogen (TVB-N) Trimethylamine-nitrogen (TMA-N), Indicator, Quality assessment, Spoilage.

INTRODUCTION

Fish is a highly perishable commodity that needs early preservation. Fresh fish is normally preserved by cold storage or in ice. Attempts are also made to use organic acids to prolong the storage life of fish [1]. Total volatile bases (TVB) and trimethylamine (TMA) are a group of biogenic amines formed in non-fermented food products during storage [2]. Biogenic amines are toxic compounds found in fermented and non-fermented foods [3,4]. In non-fermented foods such as fish, meat and certain vegetables, they are formed as a result of undesirable microbial activities. The flesh of fish is very susceptible to microbial spoilage. Its preservation therefore involves prompt treatment by preservative methods. Most of the modern methods of freezing foods initially were developed for freezing fish [5]. Spoilage of fish is principally due to the activities of enzymes produced by *Pseudomonas patheromonas* strains [6]. These organisms invariably become predominant during prolonged storage of fish whether frozen or not. Freezing inactivates or kills some but not all of the microorganisms present and growth will take place after thawing if time permits [7,8]. Hence the present study was undertaken to find out the fitness of the fish that are available in the market for consumption and to find out the effect of storage at refrigeration condition for different durations on the production of TMA-N. It is therefore the intent of this study tom determine the rate at which the spoilage chemicals are produced in fresh fish stored at room temperature and at refrigeration temperatures.

MATERIALS AND METHODS

Sample collection:

Leiognathus splendens is commonly available fish in Pondicherry and Madras coast almost on regular basis. Some samples were bought from the market. As soon as the fresh samples were collected from the landing centers and after purchase from the market, they were stored or preserved at different temperatures like ambient temperature and refrigerator conditions. Samples collected from fish landing centers were preserved at refrigerator condition for different durations like 4 hours, 24 hours, and 72 hours. Some were preserved with gut and some preserved after removing the gut.

Sample preparation:

100g of minced or chopped samples were prepared. 200ml 7.5% trichloroacetic acid was added and blended. Centrifuged blended solution at 2000-3000rpm until supernatant is practically clear.

***Corresponding Author:** Dr. B. Senthil kumar**, Email:** senthil_cahc@yahoo.co.in, **Phone No:** +91- 9442378655

Estimation of biochemical parameter

Determination of trimethylamine nitrogen (TMA-N)

TMA-N was determined by the AOAC method [9]. The technique consisted in extracting the TMA from 20–25 muscle samples with trichloroacetic acid (7.5%) in a ratio of 1:9 (10 g:90 mL). The TMA was extracted with toluene and this extract was reacted with picric acid, which interacted with the primary and secondary amines to produce coloured reaction products (yellow picrates) with maximum absorption at 410 nm. Results were expressed as mg TMA-N per 100 g muscle.

Statistical analysis:

The results were expressed in Mean ± standard error of mean. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

***Leiognathus splendens* (Pony fish)**

RESULTS

TMA-N values of the specimen: *Leiognathus splendens* obtained from local fish market are shown in the (**Table 1)**. The highest TMA-N value recorded in market sample is 21.18 mg/100g and the lowest value recorded is 1.77 mg/100g. The increase in the amount of TMA-N in the fresh fish was highly significant (p< 0.001) from 2.721 ± 0.194 to 4.244 ± 0.276 after 4 hours of refrigerated storage and to 8.584± 0.921 after 24 hours of storage in refrigeration (**Table 2**). Between fresh and 72 hours, the last hours of refrigerated storage, there was a significant increase (p< 0.001) in TMA-N levels from 2.721±0.194 to 17.75 ± 1.966 in the fish samples stored with gut.

In degutted storage of *Leiognathus splendens* there was a steady and sustained significant increase (P< 0.001) in TMA-N content (**Table 3**) at 4 hours (4.66 ± 0.376) of refrigerated storage, followed by a rapid significant increase (P< 0.001) in TMA-N from fresh to 24 hours of storage (6.584± 0.891) and fresh to 72 hours of storage(8.75 ± 0.966) .

The formation of Trimethylamine in the muscle of *Leiognathus splendens* increased at the end of storage in both the groups. The TMA-N levels between the fish stored with gut and the fish stored without gut at 4. 244 and 72 hours.

The amount of TMA-N produced in the samples stored with gut was non significant (P>0.001) when compared to the samples stored without gut at 4 hours of refrigerated storage **(Table 2).** Significant increase (P< 0.001) in TMA-N values was noted in the samples stored

With gut than that of samples stored without gut at 24 hours of storage **(Table 2).** Where as highly significant (P< 0.001) increase in TMA-N were found in the samples stored with gut to that of the samples stored without gut at 72 hours of storage in refrigeration **(Table 3).**

Table 1: TMA-N values in the specimen *Leiognathus splendens* collected from market

S.No	mg/TMAN/100gmuscle
1	19.64
2	21.18
3	13.48
4	15.34
5	8.97
6	3.30
7	3.55
8	1.77
9	3.61
10	20.12
11	18.48
12	8.66
13	7.82
14	3.30
15	4.58
16	5.25

Table 2: TMA-N levels in the mg/100g Muscle Specimen *Leiognathus splendens*(With gut)stored at refrigerator conditions for different durations.

S.No	Groups	With gut
1.	Fresh	2.721±0.194
2.	4hrs	4.244±0.276***
3.	24hrs	8.584±0.921***
4.	72hrs	17.75±1.966***

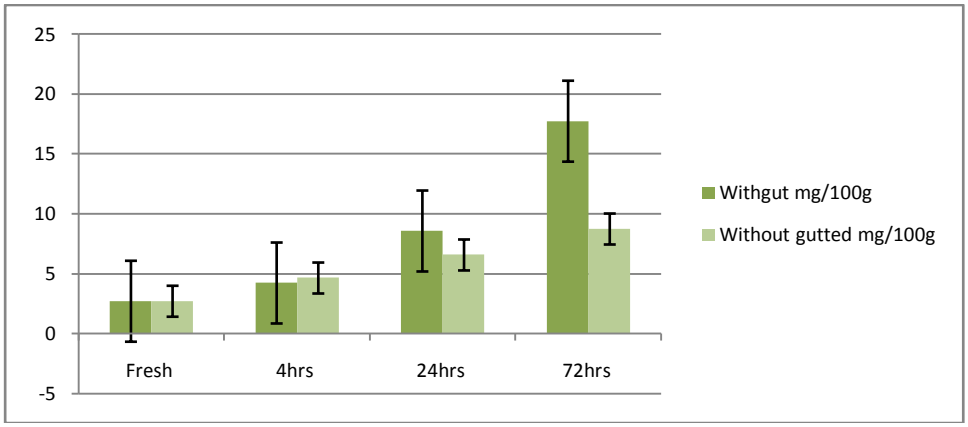
Data is expressed as Mean ±SEM of 12 individual observations. Statistical significance *** P<0.001

Table 3: TMA-N levels in the mg/100g Muscle Specimen *Leiognathus splendens* (Without gutted) stored at refrigerator conditions for different durations.

S.No	Groups	Degutted
1.	Fresh	2.721±0.194
2.	4hrs	4.66±0.376**
3.	24hrs	6.584±0.891***
4.	72hrs	8.75±0.966***

Data are expressed as Mean ±SEM of 12 individual observations. Statistical significance ** P<0.01, *** P<0.001

Fig 1: TMA-N levels in the mg/100g Muscle Specimen *Leiognathus splendens* (Withgut and without gutted) stored at refrigerator conditions for different durations.



DISCUSSION

Fish contains Trimethylamine oxide (TMAO) as an osmolyte and its quantity depends on the species of the fish and the environment. Marine fish contains 1-100 mg TMAO in every 100g of muscular tissue whereas fresh water fish generally contains only 5-20 mg/ 100g [10]. After the death of the fish Trimethylamine oxide is degraded to Trimethylamine Nitrogen (TMA-N) by bacterial and enzymatic action. TMA-N is associated with the fishy odor of spoilage and is part of spoilage pattern of many fish. The small increase in TMA over the storage period in ice reflects the low level of Trimethylamine oxide (TMAO) in the flesh of fish.

TMA is not produced in significant amount at the early stage of chill storage of fish but it appears after 3 or 4 days after which the rate of production of TMA parallels the bacterial proliferation pattern [11]. Fresh fish has very low amounts of TMA, with values below 1.5 mg TMA/100g in fresh cod, but values increase during spoilage. The fish is considered stale when TMA is above 30mg/100g cod [12].

The level of TMA found in fresh fish rejected by sensory panels varies between species, but is around 10-15 mg TMA-N/100g in aerobically stored fish [13].

The formation of Trimethylamine nitrogen in the muscle of *Leiognathus splendens* was measured as a function of time under constant refrigeration temperature (-4^{o} C). The effect of degutting in the production of Trimethylamine nitrogen in fish was estimated by grouping the fish as with gut and degutted.

Fresh *Leiognathus splendens* showed 2.721 mg/ 100g of TMA-N/100g muscle. Survey of literature shows that there is no data available on the production of Trimethylamine nitrogen in *Leiognathus splendens.* This work is the attempt to measure the amount of TMA-N in *Leiognathus splendens* at fresh stage and after storage under refrigeration.

Similar studies for shelf-life assessment of other fish have been reported. Poli [14] reported a shelf-life for ungutted European sea bass (*D.labrax*) of 10days, while [15]. Reported a shelf-life for gutted and headed sea bass (*Lateolabrax japonicas*) of 19 days.

The average concentration of TMA-N in *Leiognathus splendens* at o hrs was 2.721 mg/100g. The contents of TMA-N continuously increased during refrigerated storage. After 72 hrs of storage, of TMA-N increased to 8-9 times its fresh value.

The pattern of TMA production in *Leiognathus splendens* stored in refrigeration at -4^{o}C with gut and after removing the gut at 4, 24 and 72 hours of storage was studied. The TMA-N values of the fish samples stored with gut were comparatively higher than those of the degutted fish.

In this study, the level of TMA in fish stored in refrigeration with gut and without gut was 4.244 ± 0.276 and 4.66 ± 0.376 mg/100g at 4 hrs of storage and increased to maximum levels of 17.75 ± 1.966 and 8.75 ± 0.966 at the end of 72 hrs of storage.

The production rate of TMA-N was slower in fish stored after removing the gut than in those stored without removing the gut. The natural bacterial load present in the fish gut had added to the fast degradation of TMAO to TMA- N in the fish stored without removing the gut.

TMA-N concentrations oscillated between 1.77mg/100g and 21.18mg/100g in market samples. The TMA-N values in the fresh lot were significantly different ($P<0.05$) from the market samples. Higher TMA-N values may be associated with the post handling conditions involving bacterial contamination during the

invasion of visceral fluid into the fish muscle enhanced by poor conditions of fish transportation and unhygienic market conditions and low quality ice.

ACKNOWLEDGMENT

Authors are thankful to Thiruvalluvar University, Serkadu, Vellore, Tamilnadu for providing laboratory facilities to carry out this study.

REFERENCES

1. Noel D, Rodrick GE, Otwell WS, Bacus J. Chicago. June 25-29.pp. 181.
2. Davidek, T.; Davidek, J. *Biogenic Amines*, in *Natural Toxic Compounds of Foods*, Davidek, J. (Ed.), CRC Press: Boca Roton; 1995; pp. 108-123.
3. Horsfall M, Jnr, Ayebaemi AI, Edmonds TE. *Bull. Chem. Soc. Ethiop.* 1999, 13: 167.
4. Horsfall M, Jnr, Ayebaemi AI, Edmonds TE. *Discov. Innov*. 2004, 16: 32.
5. Frazier WC, Westhoff DC. *Food Microbiology*, 3rd ed., McGraw-Hill: New Delhi; 1991; pp. 243-254.
6. Gillespie NC, Macrae JC. *J. Appl. Bacteriol.* **1995**, 39: 91.
7. Palumbo SA. *J. Food Protect.* 1997, 49: 1003.
8. Nnenna O. *Killer Fish in Nigeria Markets*, Guardian Newspaper of 10th May, Vol. 11, No. 422, 2000; p 10.
9. AOAC Offical method 971.14, 1995. Trimethylamine nitrogen in sea food AOAC official methods os analysis Arlington, VA, Chapter 35:7.
10. Stansby ME, Olcott HS. Composition of fish in Industrial fishery technology, Newyork, Rrinbold pub.b 1963.
11. Lindsay RC, Shahidi F, Botta JR. Great Britian, Chapman and hall: 1994, 75-85.
12. Bonnel AD. London; Chapman and hall, 1994, 74-75.
13. Dalgaarad P, Gram L, Huss HH. *Int J Food Microbial* 1993, 19:283-94.
14. Poli BM, Parisi G, Zampacavallo G, Mecatti M, Lupi, Gualtieri M. *Aquaculture*, 2001, 202:303-315.
15. Chhhang KLB, Chang J, Shiau CY, Pan BS. *Journal of agriculture and food chemistry*. 1998, 46: 682-686.