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SHORT COMMUNICATION

EVALUATION OF THE LEVELS OF TOTAL VOLATILE BASES AND TRIMETHYLEAMINE FORMED IN FISH STORED AT LOW TEMPERATURE

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ABSTRACT. The levels of total volatile bases (TVB) and trimethylamine (TMA) formed in three species of saline water fish stored at - 4°C were investigated as indices of spoilage. The data showed that the concentration of TVB (mg/100g sample) in *Tilapia spp.* ranged from 19.40 – 61.00*; Mugil cephalus* 10.30 – 41.10 and *Carassius auratus* 12.50 – 66.7 during the maximum storage period of 20 days, while TMA levels (mg/100g sample) over the same storage period and conditions ranged from < 0.001 – 7.12 for *Tilapia spp.*, < 0.001 – 6.45 for *Mugil Cephalus* and < 0.001 – 7.28 for *Carassious auratus*. The data showed that the concentration of TVB and TMA increased with increasing storage time. These data may be used in formulating appropriate food safety limits for consumption of refrigerated fresh fish products in Nigeria.

KEY WORDS: Total volatile bases, Trimethyleamine, Food storage

INTRODUCTION

Total volatile bases (TVB) and trimethylamine (TMA) are a group of biogenic amines formed in non-fermented food products during storage [1]. Biogenic amines are toxic compounds found in fermented and non-fermented foods. [2, 3]. 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 [4]. Spoilage of fish is principally due to the activities of enzymes produced by *Pseudomonas patheromonas* strains [5]. 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 [6, 7]. Sores of *E. Clostoidium botulinum* will survive freezing and storage and may grow and produce toxin when temperature are 3.3°C and above. Spoilage bacteria critically utilize low molecular weight compounds such as nucleotides and amino acids present in fish muscle [8]. It is the breakdown of these molecules that are responsible for off-odours and other spoilage effects [9]. Studies by Miller *et al*. [10] using gas-liquid chromatography have identified some of the principal volatile compounds in spoiling fish as methylmercaptan, dimethylsulfide, hydrogen sulfide, trimethylamine, ethylacetate, ammonia and histamine, which are known as volatile bases. TMA formation is of great interest since it has long been used as a measure of the degree of fish spoilage [11]. TMA is produced by the reduction of

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trimethylamine oxide, which is present in appreciable quantities in most marine fish but not in other animals [12]. It is now clear that *Alteromonaces and A. putrefaciens* in particular are mainly responsible for this conversion, which enables these essentially aerobic bacteria to satisfy their oxygen requirements, and continue to grow in the depleted oxygen conditions developing in the fish tissues. The microbial conversion of trimethylamine oxide in fish to TMA during spoilage is shown below:

$$2(CH_3)_3N^+O^- \xrightarrow{\text{aerobic bacteria}} 2(CH_3)_3N: + O_2$$

The determination of the total volatile bases is used to support organoleptic analysis and physical appearance as to whether a sample is unfit for consumption. The presence of bacteria and the toxins they produce pose the greatest risk for food poisoning and other food-borne diseases, which are on the increase. In Nigeria, increasing incidents of food poisoning has been reported in recent years [7]. Details of the reports revealed that most of the incidents occurred after the victims had eaten meals prepared with frozen fresh fish.

It is therefore the intent of this study to determine the levels of TVB and TMA formation for three species of marine fish (*Tilapia spp., Mugil cephalus*, and *Carassius auratus)* stored at low temperature (- 4^{o}C) predominantly used in Nigeria over a time period.

EXPERIMENTAL

Sample collection. Fish samples used for the study were obtained from the IWOFE axis of the New Calabar River of Rivers State, Nigeria. The fishing process was systematically carried out to ensure individual species were caught at the same time on board a fishing boat and are immediately placed in an ice chest after washing thoroughly with the sea water and were taken to the laboratory for deep refrigeration. The species of fish used for the study are tilapia (*Tilapia spp.),* mullet *(Mugil cephlus)* and Goldfish (*Carassius auratus)*. Ten fishes of each species of the same size range (8.0 – 10.0 cm) were selected.

Sample storage. The fish samples were immediately deep-frozen at -4 °C in a Proline Crystal deep freezer model 4A and stored up to a total of 20 days during which fish samples were removed from the freezer and analyzed successively at intervals of 5 days beginning from day zero (Day-0), Day-5, Day-10, Day-15 to Day-20. Day-0 represents the fresh fish analysis without refrigeration. -4 °C is the temperature used in Nigeria for fish and meat products storage awaiting sales. To maintain the temperature at -4°C, an electricity generator was used as a backup to support the irregular public power.

Sample preparation. The fish samples were allowed to thaw by placing on a ceramic tile in the laboratory temperature of 29^{o}C, then equal weights of the fleshy muscles immediately below the head around the abdomen were carefully sliced off and blended using a food processor (Magimix Cuisine System 5000) to ensure that the sample is homogeneous.

Methods. Standard methods recommended by Food and Agriculture Organization of the United Nations [13] was used for the determination of TVB and TMA.

Determination of TVB. 100 g of flesh of fresh fish sample was weighed and blended with 300 mL of 5% tricholoroacetic acid. The blend was then centrifuged at 3000 x g for 1 h to obtain

clear extract. 5 mL of the extract was pipetted into the Markhan apparatus and 5 mL of 2 M NaOH added. This was steam distilled into 15 mL of standard 0.01 M HCl containing 0.1 mL rosolic indicator. After distillation, the excess acid was then titrated in the receiving flask using standard 0.01 M NaOH to a pale pink end point. A procedural blank was done using 5 mL trichloroacetic acid with no sample and titrated as before. The concentration of TVB (in mg/100 g sample) was computed as follows:

$$\text{TVB (mg/100 g sample)} = \frac{(M)(V_B - V_S)(14)(300 + W)}{5} \quad (1)$$

where V_B = mL NaOH used for blank titration, W = water content of sample in g/100 g, M = molarity of NaOH standard solution, and V_S = mL NaOH used for sample titration.

The water content (W) of the sample was obtained by drying an initial weight of fish sample at 77°C in an oven to constant weight. This temperature is used to dehydrate the material completely and to limit the vaporization of volatile materials.

Determination of TMA. 100 g of minced fresh fish sample were weighed into blender and 200 mL of 7.5% trichloroacetic acid solution added and blended. The homogenous solution was centrifuged at 2000 – 3000 x g until supernatant was clear and then decanted. 4 mL aliquot of supernatant was pipetted into a test tube. A blank and standards were prepared. For the blank, 4 mL distilled water was used and for the standard, 1.0, 2.0 and 3.0 mL of working standard solution (0.01 M TMA/mL) each, diluted to 4 mL with distilled water was used. To each tube (blank, standard, samples) was added 1 mL $HClO_4$ (20%), 10 mL anhydrous toluene and 4 mL K_2CO_3 solution (10%). The content of the test tube was well shaken with 0.1 g anhydrous Na_2SO_4 to dry the toluene. 5 mL picric acid working solution (0.02%) added. This was properly mixed and transferred to a spectrophotometric cell, and the absorbance recorded at 410 nm against the blank. The levels of TMA (mg/100g sample) were computed as below:

$$\text{TMA} = \frac{A/A_1(V_x)(V_t)(300)}{V_s} \quad (2)$$

where A = absorbance of sample, A_1 = absorbance of standard nearest to absorbance of sample, V_x = mg TMA standard solution, V_t = volume (mL) of solution used, and V_s = volume (mL) of aliquot of sample used.

RESULTS AND DISCUSSION

The concentrations (mg/100g) of the biogenic amines formed during storage at -4 °C for the three species of fish were 19.4 – 61.00 in *Tilapia spp*., 10.30 – 41.10 in *Mugil cephalus* and 12.50 – 66.70 in *Carassius auratus* for TVB and 0.0 – 7.12 in *Tilapia spp,* 0.0 – 6.45 in *Mugil cephalus* and 0.0 – 7.28 in *Carassius auratus* for TMA. Figure 1 show that the trend of TVB and TMA formation in the three fish species is time dependent and that formation of these biogenic toxic substances increased as storage period rises from zero to 20 days. An interesting feature of TVB is the amount present in the organisms even before storage. *Tilapia spp.* contain 19.40 mg/100g, *Mulgil cephalus* 10.3 mg/100g and *Carassius auratus* 12.5 mg/100g before storage. These levels increased significantly on storage. Malle *et al.* [11] had earlier reported that, the degree of fish spoilage may be measured as the extent of TVB and TAM formation before and during storage, and that 50% increase of initial level may produce deleterious effects on consumer. The result indicates that formation of TVB increased from 8.72 to 68.04% for

Tilapia spp., 19.72 to 70.23% for *Mulgil cephalus* and 19.86 to 81.28% for *Carassius auratus*, respectively. From the trend of TVB and TMA formation in the species, we can conclude that *Carassius auratus* with higher concentrations during storage would decay faster than *Tilapia spp*. *Mugil cephalus* having the lowest concentration might be stored longer at the same storage condition.

In preservation, fish is susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage. The results indicate that even at the low temperature storage some form of chemical conversion reactivity may be going on, even though bacterial actions are dormant and fish deterioration may be possible especially at longer period of storage.

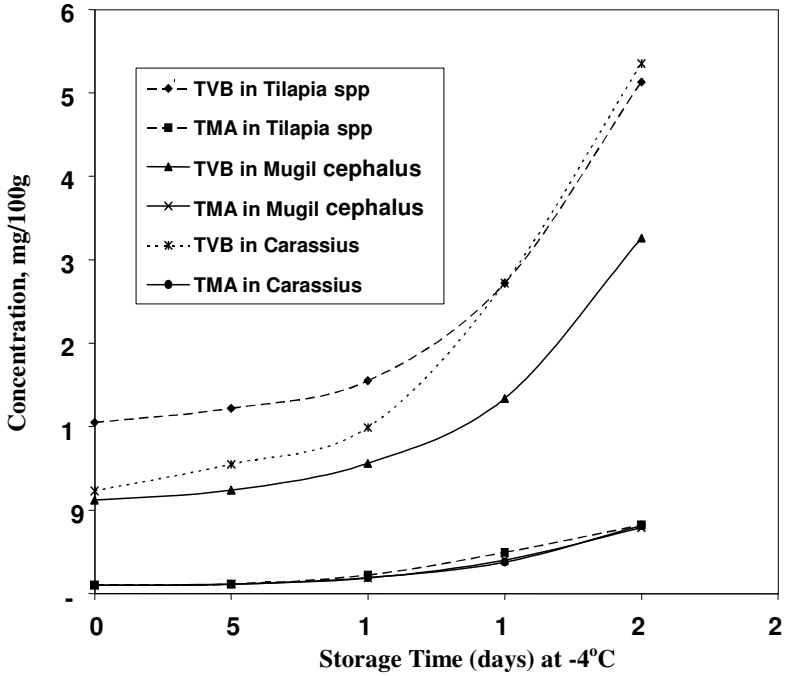

Figure 1. The effect of storage on the formation of TVB and TMA in the three species of fish at -4 °C.

The concentrations of TMA obtained within the study duration of 20 days for the three species of fish samples analyzed were low and may not be of health concern if such fish are consumed within the twenty days of storage. This was because these concentrations are higher than the safety limits suggested by AOAC [14] and corroborated by FAO [13], which set TMA values of 1 mg/100 g sample for fresh fish and 18 mg/100 g sample for spoiled fish. Values around 15 mg/100 g sample could be taken as indications of doubtful quality. Shimizu and Hibiki [15] had also suggested 100 mg/100 g sample as tentative limit for TVB as reported by FAO [12]. The maximum storage time in this study was 20 days at which the maximum concentrations of TVB and TMA were recorded.

In order to demonstrate the validity of the experimental data, a one-way ANOVA at $p < 0.05$ was conducted and the results summarized in Table 1 for TVB and TMA, respectively. The data showed no significant difference in the formation of TVB and TMA in the three species of fish studied at $p < 0.05$.

Table 1. One-way analysis of variance at $p < 0.05$ for TVB and TMA formation in the three species of fish.

Source of variation	Analyte	SS	df	MS	F	P-value	F-crit
Between groups	TVB	540.40	2	270.20	0.42	0.67	4.26
	TMA	0.86	2	0.43	0.05	0.95	3.88
Within groups	TVB	5764.61	9	640.51			
	TMA	104.75	12	8.73			
Total	TVB	6305.00	11				
	TMA	105.60	14				

In conclusion, formation of biogenic toxic chemical substances in stored fresh fish is dependent on storage time. In general, both TVB and TMA formation increased with increase in storage period. The extent of formation of these compounds with respect to time may be exploited as an alternative for minimizing the incidence of fresh fish food poisoning. TMA may be considered potential risk in setting food safety limits for consumption and importation of refrigerated fish products in Nigeria.

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