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Research Paper

Total volatile base nitrogen (TVBN) and trimethylamine (TMA) content of "Bunyi youri" as influenced by the addition of glucose and clove during storage

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Abstract. The levels of total volatile base nitrogen (TVBN) and trimethylamine (TMA) formed in a traditional fermented solar tent dried fish during storage at ambient temperature were investigated as indices of spoilage. The results showed that the concentration of TVBN (mg N/100 g) in Bunyi youri treated with various concentration of glucose and clove ranged from 23.54 ± 0.58 to 30.1 ± 0.90 mg N/100 g, while the value for the control samples ranged from 36.45 ± 0.78 to 53.70 ± 0.22 mg N/100 g during the maximum storage period of 32 weeks. The corresponding values of TMA for the treated samples ranged from 10.34 ± 0.58 to 20.32 ± 0.63 mg N/100 g and the corresponding value for the controls ranged from 21.10 ± 0.49 to 45.37 ± 2.34 mg N/100 g. The results showed that in both cases, the concentration of these biogenic materials increased with increasing storage time. Thus these data may be useful in formulating necessary food safety limits for consumption of fermented solar tent dried fish products in Nigeria.

Keywords: Total volatile bases, trimethylamine, "bunyi youri", fermented, glucose, clove.

INTRODUCTION

Fish is a great source of protein, vitamins, minerals and omega-3 fatty acids, a key nutrient for brain development (Spencer *et al.*, 1971; Jaclyn *et al.*, 2010). Fishery plays an important role in international trades and food sector. Fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 and with a total value of US$217.5 billion, of which about 128 million tonnes was utilized as food for people. With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period of 1961 to 2009, outpacing the increase of 1.7 percent per year in the world's population (FAO, 2012). Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits and action of autolysis enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002).

In Nigeria, fish supply is from four major sources *viz.,* artisanal fisheries, industrial trawlers, aquaculture and imported frozen fish. Production from aquaculture is increasing compared to artisanal sources and supplied between 5 and 22% of total domestic fish production between 2000 and 2007 (FDF, 2007). Most of the fish harvested in the tropics is used for direct consumption but a great deal is processed into fish meal for use in compounding feeds. A significant quantity is also lost through the absence of adequate technology to prevent post harvest losses in most tropical countries. Post-harvest losses in fish products are a foremost nuisance of the Nigerian fish industry particularly at the artisanal level. Post-harvest losses occur at various points from capture to marketing. The demand for fish in Nigeria is

projected at 1.18 million tonnes (12 kg per capita) and capacity for Nigerian fish resources is estimated at 1.83 million tonnes (Tobor, 1993). Fish supply from all sectors is approximately 500,000 tonnes per annum. According to Adesehinwa *et al.* (2005), although captured fisheries is in control of over 60% of total domestic production per annum, the enormity of losses in this sector has been estimated at 30 to 50% of total catches. Postharvest losses caused by spoilage amount to about 10 to 12 million tonnes per year and in addition, it is estimated that 20 million tonnes of fish in a year are discarded at sea which is another form of post-harvest losses (FAO, 2010). Eyo and Mdaihili (1997) estimated a loss of 80 million Naira worth of fish through poor handling, processing, preservation and storage.

When fish undergoes bacteriological putrefaction, the loss in quality is attended by a decline in marketable value. This may bring the product within the scope of low-income groups who could not afford better quality fish. The people involved in fish marketing (and perhaps the fishermen) may have suffered a loss of potential income by not selling at the best possible price, but someone else may have gained by having access to a still nutritious food. Even when fish has deteriorated so much that it cannot be sold at all and it is thrown away, there are regrettably often people who are so impoverished that they would be glad to take the least spoilt fish. This can be regarded as a loss in value for the fisherman or trader, but a social gain for very low-income groups (Kumolu-Johnson and Ndimele, 2011), and much of this spoilage could be reduced if such fish are traditionally processed to products that will have extended shelf life such as “Bunyi youri.” “Bunyi youri” is a fermented sun-dried fish product processed mainly from *Lates niloticus*, that is, Nile perch or from Clarias fish species but Nile perch is however commonly used than Clarias species. The fermented fish is traditionally subjected to sun drying to enhance storage ability. It is widely consumed by the “Kanuri” of North Eastern Nigeria as food and condiment in the flavouring of soups (Negbenebor *et al.*, 1995). This fish product is believed to have resulted from an attempt to preserve the *Lates niloticus* which is not very sweet when consumed in the fresh state but tastes better when dried. During the initial stages of traditional processing of “Bunyi youri”, odour and off-flavour are produced, which attract flies and could lead to the contamination of the product. It is possible that the final product may not be microbiologically and biochemically wholesome. Total volatile bases nitrogen (TVBN) and trimethylamine (TMA) are a group of biogenic amines formed in fermented and non-fermented food products during storage (Davidek and Davidek, 1995). Biogenic amines are toxic compounds found in fermented and non-fermented foods (Horsfall *et al.*, 1999; Horsfall *et al.*, 2004). In foods such as fish, meat and certain vegetables, they are formed as a result of undesirable microbial activities.

Although clove and glucose have been used separately or in combination with other spices to enhance the quality of meat and fish products, there is not much evidence in the literature on the combined effects of clove and glucose on TVBN and TMA of fermented fish products. The use of a combination of clove and glucose could therefore have a beneficial effect on TVBN and TMA of solar tent dried “Bunyi youri” and this needs to be investigated.

MATERIALS AND METHODS

Materials

Fresh fish samples of Nile perch (*Lates niloticus)* species were obtained from River Benue fresh fish market in Yola, Adamawa State, Nigeria. Samples were collected using a clean plastic container (80 × 20 × 45 cm) with tight lid. The container was packed with crushed ice to minimize deterioration of the fish during transportation to the laboratory in Maiduguri.

The fresh fish samples were cut open longitudinally from one side through the ventral surface. The fish was gutted and thoroughly washed with potable water. Five treatments were used in each experimental processing. For each of the five treatments, three replicates were used. The prepared fish was divided into five different groups and four groups were treated separately with 2.0% glucose (Glu) plus 0.1, 0.3, 0.5 and 0.6% of clove (Clo) respectively by dipping the fish samples into the glucose-clove solution for 20 min and the fifth group which served as the laboratory control was treated by dipping in only distilled water. A 2.0% glucose solution was utilized for the fermentation because in a similar study, Adams *et al.* (1987) observed that this concentration gave the optimum fermentation and fast reduction in pH during the fermentation of glucose-fish-cassava mixture. The prepared samples were allowed to ferment for 24 h at mean ambient temperature of 38.5 ± 1.7°C and at the mean relative humidity of 25.9 ± 2.1% as described by Adams *et al.* (1987). The fermented fish were dried in the solar tent drier at the mean temperature of 65.0 ± 5.2°C and at mean relative humidity of 21.9 ± 0.35% for two to three days until the samples were crisp dried. The dried samples were packed in plastic containers until required for further analysis.

Methods

Standard methods recommended by Food and Agriculture Organization of the United Nations (FAO, 1986) were used for the determination of TVBN and TMA.

Determination of TVBN

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 ×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 Sodium hydroxide (NaOH) was added. This was steam distilled into 15 ml of standard 0.01 M hydrochloric acid (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 TVBN (in mg N/100 g sample) was computed as follows:

$$\text{TVBN (mg N/100 g sample)} = \frac{(M)(VB - VS)(14)(300 + W)}{5} \quad (1)$$

Where VB = ml NaOH used for blank titration, W = water content of sample in g/100 g, M = molarity of NaOH standard solution, and VS = 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 to 3000 × 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 of Perchloric Acid ($HClO_4$) (20%), 10 ml anhydrous toluene and 4 ml of Potassium Carbonate (K_2CO_3) solution (10%) was added. The content of the test tube was well shaken with 0.1 g anhydrous Sodium Sulphate (Na_2SO_4) to dry the toluene. 5 ml picric acid working solution (0.02%) was added. This was properly mixed and transferred to a spectro-photometer cell, and the absorbance recorded at 410 nm against the blank. The levels of TMA (mg N/100g sample) were computed as follows:

$$\text{TMA} = \frac{A/A1\ (Vx)\ (Vt)\ (300)}{Vs} \quad (2)$$

Where A = absorbance of sample, A1 = absorbance of standard nearest to absorbance of sample,
Vx = mg TMA standard solution, Vt = volume (ml) of solution used, and Vs = volume (ml) of aliquot of sample used.

Statistical analysis

Analytical data were processed using analysis of variance as described by Ihekoronye and Ngoddy (1985) and the difference in means were separated using Duncan's multiple range test (DMRT) as described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The results of the effects of glucose and clove on the concentration of TVBN and TMA of "Bunyi youri" are presented in Tables 1 and 2, respectively. The effect of glucose and clove on the TVBN of "Bunyi youri" is presented in Table 1. The TVBN increased for the controls and the treated samples during storage. The result indicated significant differences ($P \leq 0.05$) between the treated samples and the controls. Pearson (1976) recommended that the limit of acceptability of TVBN for fish is 20 to 30 mg N/100 g while Kirk and Sawyer (1991) suggested a value of 30 to 40 mg N/100 g as the upper limit. Also the limit of acceptability of fish was reported to be 30 mg N/100 g by Connell (1995).

Reilly *et al.* (1985) stated that TVBN are not reliable as indices of quality. Boee *et al.* (1982) working on the storage of shrimp observed that TVBN increased evenly. Beyond the recommended levels above, white fish and prawns are regarded as unacceptable; however, results from this study showed that "Bunyi youri" treated with glucose and clove still had their final TVBN within the acceptable limits, since they all had values less than 30 mg N/100 g. Normally TVBN increased during storage at ambient temperature (Estrada *et al*., 1985). These authors stated that the TVBN of whiting (*Sillago maculates*) showed slow increase during the first half of storage, and levels increased more rapidly during the later stage at ambient temperature.

In this study the highest value of TVBN (53.70 ± 0.22 mg N/100 g) was in the commercial control followed by the laboratory control (36.45 ± 0.78 mg N/100 g) at week 32 respectively. The values for the treated samples were between 30.1 ± 0.90 and 23.54 ± 0.58 mg N/100 g, with sample treated with 2%Glu + 0.5%Clo showing the least value of 23.54 ± 0.58 mg N/100 g. This gradual increase at the storage room temperature has been reported by Suchitra and Sarojnalini (2012) to be due to the elevation of temperature and subsequent microbiological and biochemical changes in the fish muscle. It also indicates the continuous production of volatile bases due to the breakdown of proteins by action of microbes (Babu *et al.*, 2005), this is believed to be responsible for the generation of typical flavour and aroma of the final product (Majumdar *et al.*, 2005) and also shows that

Table 1. Effect of glucose and clove on the TVBN (mg N/100 g) of "Bunyi youri" during storage.

Treatment	Storage time (weeks)				
	0	8	16	24	32
Control A	50.75 ± 1.28^{a}	53.78 ± 0.50^{a}	55.16 ± 1.33^{a}	61.63 ± 1.00^{a}	53.70 ± 0.22^{a}
Control B	31.36 ± 1.02^{b}	32.67 ± 0.59^{b}	35.03 ± 1.08^{b}	38.59 ± 0.42^{b}	36.45 ± 0.78^{b}
2%Glu + 0.1% Clo	21.66 ± 1.14^{c}	21.89 ± 0.91^{d}	23.77 ± 1.63^{c}	28.63 ± 0.48^{c}	30.10 ± 0.90^{c}
2%Glu + 0.3% Clo	20.64 ±0.43^{cd}	21.07 ± 0.47^{d}	21.39 ±1.51^{d}	25.47 ± 0.23^{d}	24.47 ± 0.21^{d}
2%Glu + 0.5% Clo	19.60 ± 0.25^{d}	19.86 ± 0.72^{e}	20.08 ± 0.24^{d}	22.87 ± 0.38^{e}	23.54 ± 0.58^{e}
2%Glu + 0.6% Clo	22.37 ± 1.18^{c}	22.91 ± 0.14^{c}	24.00 ± 0.80^{c}	27.79 ± 0.74^{c}	29.82 ± 0.35^{c}

Values are mean ± standard deviation of triplicate determinations. Means followed by the same letter within the same column are not significantly different at P ≥0.05. Control A = Commercial "Bunyi youri" TVBN = Total volatile base nitrogen. Control B = Laboratory prepared "Bunyi youri".

Table 2. Effect of Glucose and Clove on the TMA (mg N/100 g) of "Bunyi youri" during storage.

Treatment	Storage time (weeks)				
	0	8	16	24	32
Control A	23.10 ± 3.22^{a}	26.61 ± 1.00^{a}	39.37 ± 1.86^{a}	42.69 ± 2.77^{a}	45.37 ± 2.34^{a}
Control B	21.10 ± 0.49^{a}	22.65 ± 1.50^{b}	29.67 ± 0.64^{b}	36.99 ± 1.52^{b}	40.13 ± 0.45^{b}
2%Glu + 0.1% Clo	11.18 ± 1.00^{b}	11.31 ± 0.92^{cd}	15.27 ± 1.16^{c}	16.67 ± 1.45^{c}	20.30 ± 0.54^{c}
2%Glu + 0.3% Clo	10.34 ± 0.58^{b}	11.34 ± 0.36^{d}	12.95 ± 0.66^{d}	14.82 ± 1.42^{cd}	17.57 ± 1.20^{d}
2%Glu + 0.5% Clo	10.16 ± 0.14^{b}	10.32 ± 0.25^{d}	12.06 ± 0.42^{d}	13.24 ± 1.08^{d}	15.60 ± 0.91^{e}
2%Glu + 0.6% Clo	11.63 ± 0.91^{b}	12.24 ± 0.45^{c}	15.91 ± 0.51^{c}	17.05 ± 0.83^{c}	20.32 ± 0.63^{c}

Values are means ± standard deviations of triplicate determinations. Means followed by the same superscript within the same column are not significantly different at (P ≥ 0.05). Control A = Commercial" Bunyi youri" Control B = Laboratory prepared "Bunyi youri". TMA = Trimethylamine.

higher liberation of TVBN were correlated with bacterial activity (Vanderzant *et al.*, 1973). The effects of Trimethylamine (TMA) value on the processed "Bunyi youri" during storage are presented in Table 2. The TMA result indicated no significant difference (P ≥ 0.05) on week zero between the treated samples but showed significant difference (P≤ 0.05) between the treated samples and the two controls. Thereafter the TMA values increased steadily for all the treated samples and both controls. Samples treated with 2% Glu + 0.1% Clo and 2% Glu + 0.6% Clo showed no significant difference (P ≥ 0.05) in TMA values at the end of storage period. However, TMA values obtained (23.1 ± 3.22 and 45.37 ± 2.34 mg N/100g, 21.10 ± 0.49 and 40.13 ± 0.45 mg N/100 g for the control 1 and control 2 at week 0 and week 32 respectively as well as 10.34 ± 0.58 and 20.32 ± 0.63 mg N/100 g for the treated samples at week 0 and week 32 irrespective of treatments were in all cases lower than the TVBN results. This is in agreement with the report of Riquixo (1998). The product of TMA is dependent on the bacterial activity as well as from endogenous enzymes (Mohd'Yusuf *et al.*, 2010). The recommended level of TMA value for human consumption is 10 to 15 mg N/100 g (Connell, 1995). Huss (1995) attributed the sudden increase in TMA value in fish muscle to putrefaction by spoilage bacteria.

There is also wide variation in critical values suggested for individual species, like 5 to 7 mg N/100 g for haddock (Castell and Triggs, 1955) and Montogomery *et al.* (1970 stated the accepted limit of TMA as 12 to 15 mg N/100 g. In this study, all the samples irrespective of treatment showed the TMA values to be above the recommended value at week 32 (40.13 ± 0.45 and 45.37 ± 2.34 mg N/100 g) for the control samples and the treated samples had values that ranged between 15.60 ± 0.91 and 20.32 ± 0.63 mg N/100 g at the same week of storage. However sample treated with 2% Glu + 0.5% Clo showed lower level of TMA (10.1 6± 0.14, 10.32 ± 0.25, 12.06 ± 0.42 and 13.24 ± 1.08 mg N/100 g) at week 0 up to week 24, respectively. The implication of this is that all the treated samples met the recommended value of TMA only up to week 8 of storage period.

CONCLUSION

The formation of toxic chemical substances in stored "Bunyi youri" is seen to be dependent on the storage time and the processing treatment with glucose and clove. The TVBN and TMA formation increased gradually with increase in storage period and varied with the concentration of glucose and clove, and as observed by Horsfall *et al.* (2004), this extent of formation of these compounds with respect to time may be exploited as an

alternative for minimizing the incidence of fish food poisoning. Thus the TVBN and TMA may be considered potential risk in setting up food safety limits for consumption and international trades of fermented fish products in Nigeria.

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