BACTERIOLOGICAL ASSSESSEMENT OF SMOKED TILAPIA FISH (*Tilapia zilli*) FISH USING AN IMPROVED TRADITIONAL SMOKING KILN

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ABSTRACT

This experiment titled the Bacteriological assessment of Tilapia fish using an improved traditional smoking kiln compared the bacterial load between traditional smoking kiln and improved traditional smoking kiln. The data was subjected to ANOVA of variance, Duncan multiple test was used to separate the means using the SPPS *values obtained for the traditional smoking kiln ranges from* $1.53^{C} \times 10^{-6} \pm 0.1$, $9.75^{a} \times 10^{-6} \pm 0.38$, $10.22^{a} \times 10^{-6} \pm 0.57$ *and* $12.17^{a} \times 10^{-6} \pm 0.03$ *while that of improved traditional smoking kiln ranges from* $1.53^{C} \times 10^{-6} \pm 0.1$, $9.75^{a} \times 10^{-6} \pm 0.38$, *the result obtained were significantly different P>0.05,the bacterial isolated had the following frequency which are Staphylococcus aureus 30%, Sporosarcina species 18%, Bacillus substilis 34% and Clostridium botilium 18% respectively. The study concluded that bacterial are heavily implicated in the traditional smoking kiln(TSK) when compared to improved traditional smoking kiln (ITSK). The study recommends that other preservative measures should be undertaken such as addition of brine, proper handling processes to minimize the incidence of harmful bacterial load.*

Key words: Improved Traditional Smoking Kiln (ITSK), Bacteria, Temperature, *Tilapia zilli*

INTRODUCTION

Fish is a key ingredient on the global menu, a vital factor in the global environment and an important basis for livelihood worldwide (Bene and Heck, 2005). Fisheries make an important contribution to the animal protein supplies of many communities in both industrialized and developing world (Adewolu and Adeoti, 2010). Fish is also widely acceptable because of its high palatability, low cholesterol and tender flesh (Eyo, 2000). Fish however is an extremely perishable commodity which begins to deteriorate as soon as it is caught and eventually dies. It spoils very quickly because of intrinsic and extrinsic factors (Oyero, 2006) . According to (Joules 2011), freshly caught fish spoils easily and thus the reason for preservation and that there are four most popular methods of preserving fish namely: Freezing, Canning, Smoking and drying. However, smoking method is most preferred in preserving the fish when flavor and aroma is desired. According to (Joules 2011), freshly caught fish spoils easily and thus the reason for preservation and that there are four most popular methods of preserving fish namely: Freezing, Canning, Smoking and drying. However, Joules (2011) believes that smoking method is most preferred in preserving the fish when flavor and aroma is desired. Fish processing and handling using the traditional smoking kiln gives room for high bacteria activities, which can result to incidence of fish spoilage and food poisoning. Hence there is the need to develop improved smoking kiln that can reduce bacteria load which is responsible for fish spoilage as well as food poisoning. (Oyero, 2006).

MATERIALS AND METHODS

Improved traditional smoking kilns, 1 traditional smoking kiln (control), 100kg Fire wood, 32 pieces of tilapia purchased from Gwagwalada market, Thermometer.(calibrated from 0^{0}-350^{0}C).The following pre – smoking activities were carried out, the fish on arrival to the farm were had the scales removed, eviscerated and thoroughly washed with 0.5% salt solution to reduce the bacteria load

Description of both the Improved Traditional Smoking Kiln (Altona) and the traditional kiln used

The improved smoking kiln "Altona" is designed and fashioned after the traditional drum kiln with slight modification to conserve fuel and improve the quality of smoked fish.

The kiln is fabricated from a 44-gallon drum (fig 1), the height of which is 0.92m. The drum is cut vertically to create door of 0.67m x 0.48m. The improved kiln is equipped with steel rods drilled into the inner compartment and wire mesh placed on them to form three fish trays 0.18m apart.

At the bottom of the drum is located a stokehole which is 0.33m x 0.17m. A perforated metal sheet is incorporated at 0.1m above the stokehole. This act as a damper to prevent direct contact of fire with the fish loaded on the bottom trays to avoid them from being charred.

The back top of the drum is drilled to provide a chimney where excess smoke and heat escape from. During smoking, the door of the kiln must be kept closed to conserve heat. On the other hand, the traditional smoking kiln was likewise constructed from a 44-gallon drum, with major contrasting features from that of the improved traditional kiln of it top completely removed, no door, no trays, no damper and no provision for a chimney. A Calibrated temperature gauge that ranges from 0-350^{0}Cand inserted into one of the holes of the chimney's at intervals of 2hours to determine at average temperature range from 60-90^{0}C, 90-120^{0}C, 120-150^{0}C for the 8 hours smoking duration respectively.

The smoking of the fish were carried out at four smoking treatments levels

Thirty-two (32) pieces of fresh Tilapia fish *(Tilapia zilli)* weighing about three kilograms were purcased from Gwagwalada market and transported in transparent polythene to the permanent site of the University where smoking activities were carried out.

LABORATORY ANALYSIS

Laboratory Analysis involved the following procedures as described by Oyeleke and mange (2008). Preparation of culture media (nutrient and mac conkey agar) was carried out by measuring 50.6g of sampled media into 250ml conical flasks and afterward diluted with 9ml of water. The solutions were then autoclave at a temperature 121^{0}C for 15minutes for the serial solution,each samples were soaked in sterile distilled water in a beaker to form the stock solution and the serial dilution were carried out by transferring 1ml from the stock to the first test tube containing 9ml of sterile distilled water, it was then mixed thoroughly which gives 10^{-2} then 1ml was transferred to the second test tubes given 10^{-3} ,it was then mixed thoroughly and 1ml was transferred to the third test tubes and repeated 1and 2 and the same was done up to 10 and from 10, 1ml was removed and discarded.

Plating was carried out using 0.04ml of 10^{-2} transferred onto an already prepared nutrient agar plate, with the use of an angular glass rod. It was spread all over the surface media and allowed to stayed for 20minutes.it was then labeled, turned to an inverted position. It was then incubated at 37^0 C for 4hours.Same procedure was done for mac conkey agar. The colonies were counted after 24hours of incubation by counting visible colonies that grew on the media and was recorded for each sample

Gram staining procedure was carried in this manner: Twenty four hours old bacterial culture was used to make a smear, air dried and heat fixed, it was then flooded with crystal violet for thirty seconds, after which the stain was washed off with Gram's iodine, the smear was covered with some quantities of the iodine for thirty seconds and this decolorized with 95% ethanol. The smear was counter stained with 1%safranin for one minute and washed with water, air dried and observed under oil immersion objective lens of the microscope.

STATISTICAL ANALYSIS

All statistical analysis were carried out using the completely randomized design (CRD), and were subjected to ANOVA of variance, Duncan multiple test was used to separate the means using the SPPS version 16 (2007)

RESULTS

The total bacterial count ranged from $0.00x10^{-6}$ to $12.17x10^{-6}$ (Total cfu/ml). The lower range came from one of the samples with longest temperature range. While the highest count came from the shortest

temperature range .which could mean the longer the exposure of the bacteria to heat the more they reduced in number.

The major types of bacteria found on the samples were include Staphylococcus aureus, Sporosarcina specie, Bacillus subtilis and Clostridium botulinum

DISCUSSION

From these results, it shows that the bacteria implicated in this study are thermophiles that strive best at elevated temperatures, as the temperature increases the number of bacteria reduces, these explain while traditional smoking kiln (TSK) had the highest value of 12.17^{a} $X10^{-6}\pm$ 0.33 for staphylococcus aureus and improved traditional smoking kiln(ITSK) has the lowest value of 0.00^{b} $X10^{-6}\pm0.00$ at a temperature of 120-150^{0}C for sporosarcina species, this implies that traditional smoking kiln has a high moisture content and in turns mean a high water activity when compared to improved traditional smoking kiln when it undergone heating.

This agrees to the finding of Likhitrattanapaiboon *et al* (2009) who recorded the highest number in *staphylococcus aureus* inTabtim fish (hybrid tilapia Orechromis ssp). Furthermore, the traditional smoking kiln contains the highest number of bacteria when compared to the improved traditional smoking kiln because of their mode of construction, the improved traditional smoking has the following modification; a diffusion plate which controls and regulates the quantity and quality of smoke that dries the fish, whereas the traditional smoking kiln as no such, therefore direct flame and heat is been deposited on the fish sample, this agrees with the finding of Oyero(2006) and Eyo(2011) who reported that modification in smoking kiln brings about better sorption isotherm and lower moisture content.

The colony forming unit (CFU) is an indicator to threshold level of the acceptability of the safe level suitable of fish for human consumption , therefore the traditional smoking kiln has a higher forming colony unit (CFU) which ranges from $1.53^{c}\times 10^{-6}\pm0.1$, $9.75^{a}x\ 10^{-6}\pm0.38$, $10.2^{a}x\ 10^{-6}\pm0.57$ and 12.17^{a} x $10^{-6}\pm0.33$ meaning that the fish smoked using traditional smoking kiln are prone to food borne disease and food poisoning and it is in agreement with the finding of Ghasemi *et al* (2007) who stipulated that the total content of fish shall not contain a total mesophilic bacteria, feacal coliform and vibrio parahaemoliticus density of > 10^{6}-10^{7}, 4.0 x 10^{2} and 1.0x10^{2} colony forming unit (CFU)/g respectively.

The presence of *Clostridium botulinum* in the samples indicates a phenomenon called Botulism and by definition according to Koch (2000), Botulism is the name given to the disease which is triggered by the neurotoxin (a substance which damages nervous tissue) . In the case of a food borne botulism infection, symptoms occur between 12 and 36hours after intake, they include nausea, diarrhoea or constipation and also neurological symptoms. Depending on the amount of toxin taken in, there may be dryness of the mouth, optic dysfunction (double images, blurred vision, photophobia) dysphagia, acute ocular muscle paralysis and also rapidly progressing, symmetrical descending atonicparalysis. Though the patients could be fully conscious,

The presence of clostridium *Botulinum* in this study suggests that *Clostridium botulinum* could result into a case of food poisoning and food borne illness, especially the highest value recorded for traditional smoking kiln (TSK).

CONCLUSION

This experiment have shown the presence of four bacterial namely ; *staphylococcus aureus, sporosarcina spp, bacillu ssubtilis* and *clostridium botulinum* and that these four bacterial are heavily implicated in the traditional smoking kiln(TSK) when compared to improved traditional smoking kiln(ITSK)meaning that the traditional smoking kiln contains a lot of moisture content that makes these four bacteria's to strive best at these ranges of hours/time when compared to the improved traditional smoking kiln which shows a lower range level.

RECOMMENDATION

Based on the result obtained, the following recommendations are hereby advanced that:

Massive awareness should be undertaken to popularize the improved traditional smoking kiln at 8hours and above to minimize the incidence of thermophile bacteria's that can result to fish food poisoning and fish borne illness. In the light of the above mentioned point, improved traditional smoking kiln is comparatively cheaper and affordable than other improved smoking kiln like the kainji gas smoking kiln and more efficient than the traditional smoking kiln with a marginal increase in cost which a farmer can easily adopt as an appropriate technology rather than transferred technology.

Other preservative measures should be undertaken such as addition of brine, proper handling processes to minimize the incidence of *staphylococcus aureus* and *bacillus subtilus*, *sporosarcina spp* and *clostridium botulinum*. Finally, further research on this study should be undertaken beyond the four weeks (1month) that this experiment was carried in order to give an holistic result.

REFERENCE

Adewolu, M.A and A.J Adeoti (2011): Effects of mixed feeding schedules with varying crude protein levels on the growth and feed utilization of *Claris gariepinius.*

Bene, C and S. Heck (2005): Fish and Food Security in Africa NAGA, world fish quarterly bulletin vol.28 no3 December 2005.

Eyo, A.A (2000): Freshwater fish processing and preservation. In National workshop on post harvest food loss. Crop storage unit, Federal Department of Agricultural and Rural Development Kaduna Pp61-77.

Ghasemi, M.S.A & Azadnia (2009): Bacteria counts in fresh south harvested fish while boarding in shiraz. *Research Journal of Biological Science*, 4(9). pp 982-984 ISSN: 1815-8846.

ICMSF (International Commission on Microbiology Specifications for foods (1998): Microorganism in Microbiology Ecology of food commodities. First Edition, Blackie Academic and professional, London pp 174.

Joules, G. (2011): Methods of preserving Meat and Fish. Bitting record, pp18-20.

Koch, R. (2007): Joint press release of Federal Institute of health protection of consumers and veterinary medicine.

Likhitrattanopaia B.M., Wiliaphan P. Lawhavint O. (2007) : Contamination of bacteria in chilled Fresh tabtim Fish(hybrid tiliapia *Oreochromis spp*) processing to assess the critical point of processing. *Kastsart Journal of Natural Science*, 41:pp110-113.

Oyero J.O (2006): Effect of different processing technique on the Nutritional quality of Nile Tilapia (*Oreochromis niloticus*) phd thesis pp20-25.

Table 1: Types of Bacteria

Smoking kiln	Smoking Time (Hrs)	Temp (0°C)	TOB	A	B	C	D
TSK	8hrs	-	$5.87^{a}x10^{-6}\pm0.37$	12.17^{a} $x10^{-6}\pm0.33$	9.75^{a} $x10^{-6}\pm0.38$	10.22^{a} $x10^{-6}\pm0.57$	10.22^{a} $x10^{-6}\pm$ 0.57
ITSK	8hrs	60-90	$4.77^{ab}x10^{-6}\pm0.37$	4.69^{b} $x10^{-6}\pm$ 1.56	2.09^{b} $x10^{-6}\pm1.04$	5.21^{ab} $x10^{-6}\pm2.08$	3.17^{b} $x10^{-6}\pm$ 2.03
ITSK	8hrs	90-120	$4.53^{b}x10^{-6}\pm0.47$	$3.13^{bc}x10^{-6}\pm$ 0.00	1.56^{b} $x10^{-6}\pm0.90$	5.21^{ab} $x10^{-6}\pm2.76$	3.65^{b} $x10^{-6}\pm$ 0.52
ITSK	8hrs	120-150	$1.53^{c}x10^{-6}\pm$ 0.1	2.09^{c} $x10^{-6}\pm$ 1.04	0.00^{b} $x10^{-6}\pm0.00$	2.08^{b} $x10^{-6}\pm0.52$	2.09^{b} $x10^{-6}\pm$ 1.16

Values in the same row and on the same column are significantly different $P \leq 0.05$

TSK: Traditional Smoking Kiln
ITSK: Improved Traditional Smoking Kiln
TOB: Total Number of Bacteria
A= *Staphylococcus aureus*
B= *Sporosarcina species*
C= *Clostridium substilius*

D= *Bacillus substilius*

Table 2 shows the colony forming units of the suspected bacteria as the highest value of 12.17^{a} $x10^{-6}\pm0.33$ for *Bacillus substillus* using TSK and least value of 0.00^{b} $x10^{-6}\pm0.00$ for *Sporosarcina* using ITSK
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Table 4: Comparison of the Bacteriological Status of the Smoked Tilapia Sample (Cfug-1) with the Recommendation Value (ICMSF 1998)

A	B	C	D	E	F	G	H	I
T1	8	8hrs	-	Skin	12	7	11	6
T2	8	8hrs	60-90	Skin	10	6	9	5
T3	8	8hrs	90-120	Skin	7	3	7	4
T4	8	8hrs	120-150	Skin	5	2	4	3

A –Treatment
B –No of samples
C –Hours/ Times
D –Temperature range
E –Sampling Areas
F –Sample exceeded recommendation value of total bacteria count.
G -Samples recommended value of *staphylococcus* count.
H- Samples recommend value of *sporosarcina* count.
I-Samples recommended value of *bacillus* count.

Table 4: Show the comparison of bacteria with a recommended standard by International commission on microbiological specifications for foods (ICMF 1998). Based on these tables these bacteria exceed the threshold limit of the acceptability of fresh fish.