EVALUATION OF INSECT INFESTATION AND BACTERIAL LOAD IN SMOKE- DRIED *Clarias gariepinus* IN WASE, PLATEAU - NIGERIA

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Abstract
Evaluation of insect infestation and bacterial load in smoked-dried Clarias gariepinus was carried out to determine insect pest and bacterial contaminants in Wase, Bashar and Kampani Zurak markets which constituted a dietary intake of inhabitants of Wase, Plateau State, Nigeria. Ninety smoked-dried Clarias gariepinus (1500g) were bought from the markets. Dermestes maculatus had 1666 (96%), Necrobia rufipes, 59 (3%) and Ahasverus advena: 9 (1%). Insect pest populations of their developmental stages show that larvae had the highest: 1584 (91%) with pupae least, 9 (1%). Bashar had highest 263 (15%) insect: Kampani zurak: 1098 (63%) and lowest in Wase: 82 (91.1%). There was significance difference (P < 0.05) between insect populations in smoked-dried C. gariepinus in the markets. Bacterial load in fish samples was determined using pour plate method and characterization of various isolates were based on biochemical tests. Mean bacteria count in smoked- dried C. gariepinus revealed that Wase had the highest: 5.01x10^{6}cfu/g while Bashar was lowest: 1.41x10^{7}cfu/g. Bacteria species isolated revealed that Staphylococcus spp had the highest 48(58%) while Bacillus species was lowest: 4(5%). Retailed smoked, dried Clarias gariepinus were highly infected with the insect Dermestes maculatus and Staphylococcus spp. Clarias gariepinus obtained from fishers should be properly preserved.
Key words: *Dermestes maculatus*, infestation, bacteria, smoke Clarias, Wase

Introduction

Inland fresh water provides mankind with major sources of edible fish and other aquatic foods (Tait, 1981).Fish is one of the most important animal proteins in terms of food available in the tropics, poverty alleviation, employment, income generation and foreign exchange earnings (FDF, 2005). Developing countries capture 80% of the world harvest and a large proportion of these catches are consumed locally (FAO, 1985). In many Asian countries, over 50% of the animal protein intake comes from fish, while in Africa the proportion is only 17.5% (Willman *et al*., 1998).

Nigerians are large fish consumers; the demand estimated was 1.4 million metric tons/year (FAO, 1985). This means that any shortfall in fish availability will affect the animal protein intake of the people in the country (Eyo, 2000).Eyo and Awoyemi (1998) reported that insects infest dried fish during storage by feeding extensively on the flesh. The authors found out that insect activities may cause up to 50% loss in weight of dried fish during storage. Insect larvae *Dermestes maculatus* and *Necrobia rufipes* are known to attack stored dried fish. Odeyemi *et al.*,(2000) ascertained that microbial and insect pest infestation in the tropics is put at between 20% and 50%.

Bacterial infections of fish and fish products may enhance its spoilage (Osuji, 1976).This can affect the health of consumers, especially when contaminated smoked-dried fish is ingested it may cause abdominal pain, acute gastroenteritis, bloody mucous, diarrhea, nausea and vomiting (Daramola *et al.*, 2007;Akinjogunla *et al.*, 2009). Smoked fish are contaminated with bacteria among other organisms associated with man, such as Enterobacteriaceae and Staphylococcus which grow well at 30 – 37^{o}C (Adam and Moss, 1999).

According to Prescott *et al*, (1999) bacterial contamination in food often results in food spoilage as well as life – threatening health hazards like poisoning. Eyo (2000) suggested that effort made towards increased fish production and conservation should be matched with efficient post harvest fish handling, preservation and processing to prevent spoilage and subsequent losses of fish. To reduce post harvest loss of fish there is need to evaluate the level of infestation by insect and microorganism of smoked-dried fish sold in the markets.

The main goal of the reseach is to provide base line information on the most common types and levels of insect population and bacteria load in ready to -eat- smoked-dried *C. Gariepinus* in Wase markets, similar reseach should be carried out in different markets in the Local Goverment Areas of the state, that will eventuallly culminate into establishing monitoring reference point.

Materials and Methods

Collection of Fish Samples

Ninety ready to - eat- smoke-dried adult *Clarias gariepinus* (15000 g) were bought from fish retailers on market day, 30 (5000g) each with

three replicate from three randomly selected markets in Wase Local Government Area of Plateau State: Bahar, Kamfani Zurak and Wase, as treatments A, B and C. The fish samples were packed in polyethylene bags and taken to Animal Production laboratory, Abubakar Tafawa Balewa University, Bauchi. The thirty (30) smoked-dried samples and their replicates were each kept in a labeled opened card board box (0.38m x 0.50m) screened with wire mesh. The levels of development of insect pests in the samples were observed with magnifying glass.

Insects Pest Evaluation

Week zero (0) of the experiment samples of smoked-dried *Clarias gariepinus* followed by the first, second and third weeks were obtained from the three markets. The samples were observed for presence of insect pest for up to 4weeks durations of the experimental periods. The insect and their stages of development (adult, larvae and pupae) were identified according to (FAO, 2010).

Bacterial Count and Characterization of Isolates

Portions of the fish flesh with replicates samples were taken from each of the samples for four (4) weeks of the experimental periods from the different markets. The samples were ground in a sterile porcelain mortar and pestle into homogenous powder. One gram of each sample was measured with electronic digital (EHA 251) meter; the sample was aseptically introduced inside a universal bottle containing 9ml of sterile nutrient broth. Ten (10) fold serial dilutions were adapted, homogenates 0.1, 0.2 and 0.3solutions were pipette by means of a sterile automated pipette, and each dilution was introduced into a prepared standard nutrients agar plate by means of a wire-loop and then incubated at 37ºC for 24 hours. The colonies formed were counted with counter as described by Monica, (1984). A colony was selected at random and was cultured in petri dish at 34ºC for 24 hours to obtain pure isolates.

All isolates were observed for their pigmentation and further, sub-cultured to obtain a pure culture and gram-staining was carried out for 24 hours. Identification of the isolates was carried out based on the methods described by Moshood and Tengkuhaziyamin (2012) and Cheesebrough (2002). Biochemical analysis: Catalase, Coagulase, Citrate utilization and Urease tests were conducted to characterize the biological characteristics of the culture samples (Monica, 1984).

Statistical analysis

Simple percentages, frequencies of occurrence and means were used to analyze the data collected. Mini tab soft was used for the analysis of variance (ANOVA) for the insect pest populations in the markets. Least significance difference was used to compare different sample treatments means, following the method described by Ogbeibu (2005).

Results

Insects Infestation in smoked *Clarias gariepinus*

Figure 1 shows the processed fish infested by insects based on the predominant population: *Dermestes maculates* had the highest: 1666 (96%) insect population in the smoked-dried *C. gariepinus* and lowest: 9 (1%) in *Ahasverus advena*. Assessment of insects according to their stages of development (Table 1**)** indicated that the smoked fish showed that larvae had the highest population: 1584 (91%), followed by adult: 141 (8%) and least pupae: 9 (1%). Population of insects observed on the processed fish in the markets (Figure 2) showed that Bashar had the highest 1098(63%) population of insect followed by Kampani Zurak 373(22%) and lowest in Wase 263(15%) markets.

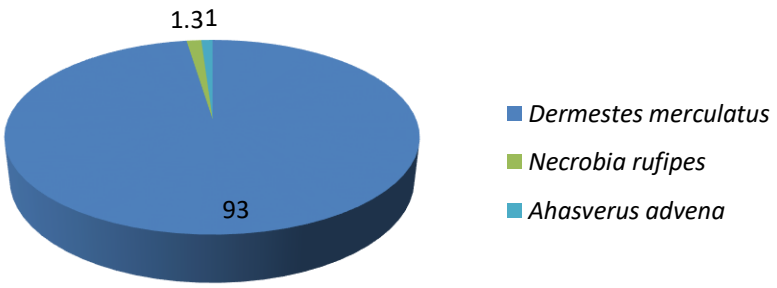

Fig1: Percentage insect population in smoked-dried *Clarias gariepinus*

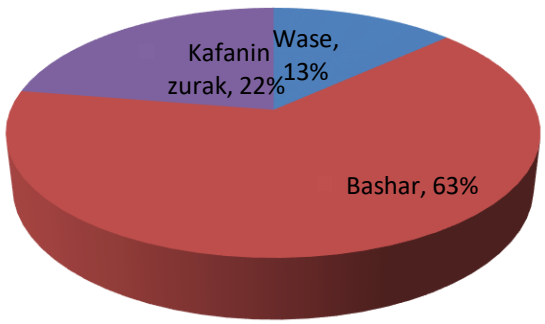

Fig 2: Percentage Population of Insect in the Markets

Microbial Analysis

Five species of bacteria were isolated from the smoked-dried *Clarias gariepinus*; *Kleseilla* spp, *Staphylococcus* spp, *Escheriachia coli*, *Bacillus* spp and *Proteaus* spp. *Staphylococcus spp* had the highest 58% bacteria isolated in the market, followed by *Klebseilla spp* 15%, *Proteus spp* 12% and *Bacillus* specie the least: 5% (Figure 3).

The mean bacteria counts of smoked-dried *Clarias gariepinus* were found to be higher with mean value 5.01 x 10^6cfu/g and lowest mean value: 1.19 x 10^8 cfu/g in the first week of sample collection. Mean bacteria count of the smoked fish in Bashar market had the highest mean counts 1.48x10^7cfu/g in the second week and lowest mean counts: 1.41x10^7 in thefirst week of sample collection. Furthermore, mean bacteria counts of smoked-dried *Clarias gariepinus* obtained in Kanfani Zurak market had the highest mean counts 2.77 x10^6 cfu/g in the second week and lowest:1.58 x10^8in the first week of sample collection (Table 2).

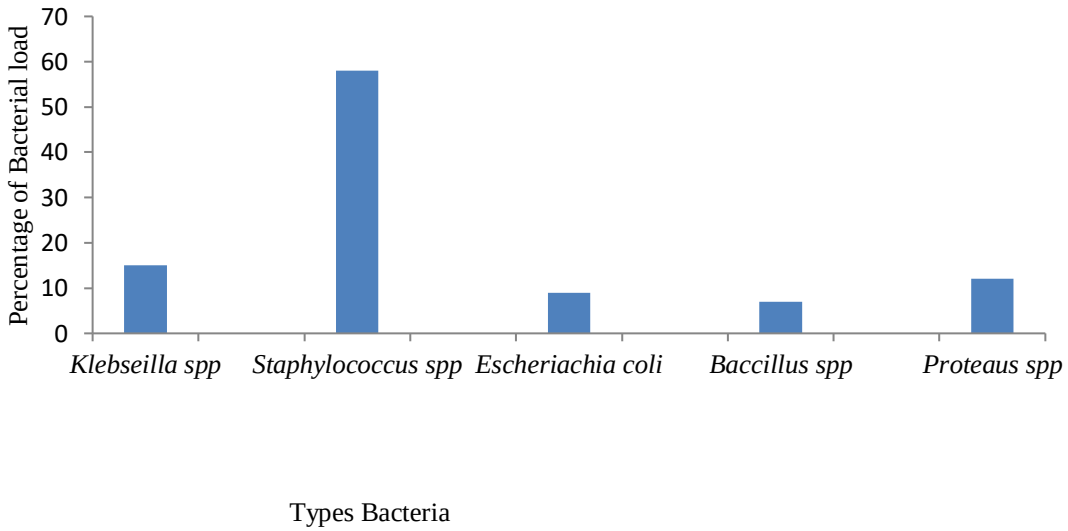

Fig 3: Bacteria Population in Smoked Dried *Clarias gariepinus* from Wase Markets

Table 1: Population of Insect Pests Based on the Stages of Development

Stageof development	Number of occurrence	Percentage of occurrence (%)
Adult	141	8
Larvae	1584	91
Pupae	9	1
Total	**1734**	**100**

Table 2: Mean Bacterial Count in the Smoke-dried *Clarias gariepinus* in Some Markets

Markets	Sample mean count value			
	Weeks			
	0	1	2	3
Wase	1.14×10^7	1.19×10^8	2.87×10^7	5.01×10^6
Bashar	1.39×10^7	1.41×10^7	1.48×10^7	1.41×10^7
Kanfani zurak	1.52×10^6	1.58×10^8	2.77×10^7	2.64×10^8

Table 3 One-Way Analysis of Variance

Analysis of Variance					
Source	DF	SS	MS	F	P
Factor	2	2334585	1167293	6.8E+04	0.000
Error	9	154	17		
Total	**11**	**2334739**			

Table 4: Comparison of Critical LSD (168.32) Value

Comparison	Difference	Significance
Kanfani zurak - Wase	1095.75 - 263.00 = 832.75*	H_O Rejected
Kanfani zurak – Bashar	1095.75 - 83.25 = 1012.5*	H_O Rejected
Bashar – Wase	263.00 - 83.25 = 179.75	H_O Accepted

Discussion

Insects Infestation in Processed *Clarias gariepinus*

Figure 1 shows that *Dermestes maculates* had the highest insect pest population encountered in the samples; this may be connected to already infest insect populations in the markets. Osuji (1976) and Eyo (2000) reported that *Dermestes maculatus* and *Necrobia rufipes* were the most common insect pests of dried fish. Ugwu *et al.*, (2005) observed that *D. maculatus* was responsible for the greatest loss in tissue weight in smoked *C. gariepinus*. According to the author the well-developed biting mouth parts of adult *D. maculates* was responsible for the loss of tissue weight of fish samples.

Judging from the stages of development of insect pests, table 1 revealed that larvae (91%) may be the most potential causes of tissues degradation in smoke-dried *C. gariepinus.* The high percentage of larvae in the ready to -eat-smoked-dried *C. gariepinus* may not be unconnected to higher moisture content of the product that provide suitable medium for the growth of insect pests. There was significance difference ($P < 0.05$) between insect populations in smoked-dried *C. gariepinus* in the markets. Ugwu *et al.*, (2005) observed that tissue degradation is responsible for the loss in weight of smoked *C. gariepinus* infested with adult *D. maculatus.* Mohammed *et al.*, (2017) observed that the high sustainability and deterioration of smoked fish products put storage losses to be higher. The same authors also reported that smoked *C. gariepinus* had the highest mean progeny and percentage weight losses (43.3% and 13.8%), followed by *Oreochromis niloticus* (40.5% and 10.30%) and *Synodontis nigrita* (33.0% and 6.10%) respectively.

Based on insect pests population in the market (Figure 2) Bashar had the highest percentage of insect pests, this may be as a result of high abundant of insects infestation in the market. The length of development period of *D. maculates* ranged from 50 – 57 days after infestation, if there is favourable condition of temperature and humidity (Mohammed *et al*., 2017). Table 3 indicated that Kanfani Zurak market is the main cause of significance difference in the population of insect pests in Wase local Government Areas.

Microbial Analysis

Staphylococcus spp had the highest population of bacteria in the markets (Figure 3). It is likely for a processed fish species to be contaminated with bacteria, especially when the fish processing procedures were not carried out under hygienic condition. WHO (2007) reported that the quality of smoked fish is essentially linked to processing and post processing procedures. According to the author, packaging techniques such as the use of old news prints, cement papers and polyethylene bags are all sources of contamination of fish.

The highest bacteria load in smoked-dried *Clarias gariepinus* obtained in Wase market: 5.01×10^6cfu/g (Table 2) was above the values obtained by Akinjogunla *et al*.,(2009) in fresh Bonga fish species and smoked skins: 4.3×10^5 cfu/g and 3.1×10^5cfu/g respectively. According to Akingogunla *et al.*, (2009) spoilage and pathogenic bacteria commonly found on preserved fish species may be due to contamination by faecal and environmental pollutants that occur during processing, storage and transportation of fish products to the market.

The mean bacterial count in the smoke-dried *Clarias gariepinus* in the study areas does not compared favorably with acceptable limits of 1.0×10^6cfu/g recommended by Ghana Standards Board (GSB) (Nyarko *et al.*, 2011). The mean bacteria count recorded in this study (6.2×10^4 to 3.3×10^5, 7.2×10^4 to 4.1×10^7cfu/g) were found to be lower than the values 7.2×10^4 to 4.1×10^7cfu/g reported by (Nyarko *et al.*, 2011). Within three market centers: located within Tema metropolis, Ghana. ICMS (1986) recommeded maximum value of bacteria count for good qualiity fish products to be 5×10^5 .

Furthermore, mean bacteria count of Smoked-dried *Clarias gariepinus* obtained in Kanfani zurak market was highest: 2.77×10^6 cfu/g in the second week and lowest: 1.58×10^8 in the first week of sample collection (Table 2). The mean weekly bacteria count obtained in the smoked-dried *C. gariepinus* in this research were not within the acceptable limits of GSB (Nyarko *et al.*, 2011). Moshood *et al.*, (2012) reported that *Staphylococcus* spp had the highest 47% frequency in smoked-dried fish sold in Bauchi metropolis.

Conclusion

Larvae and Adult insect pests were the potential fish destroyer organism of the smoked-dried *Clarias gariepinus* sold in Wase markets. *D. maculates* and *Staphylococcus spp* were the commonest insects and bacteria in smoke-dried *C. gariepinus* sold in the markets. Owing to high population of insect pests and bacterial load in the samples, it is recommended that freshly caught *C. Gariepinus* should be properly processed, preserved and handled under hygienic condition.

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