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Research Article

The Effects of Bleeding and Gutting Procedures on the Microbial Load of Smoke-Dried *Clarias gariepinus* under Ambient Storage

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Abstract This study was carried out to determine the effects of bleeding and gutting procedures on the microbial load of smoke- dried *Clarias gariepinus*. Sixteen fish samples of *C. gariepinus* of average weight 0.9 kg were subjected to various bleeding and gutting treatments as follows; [A] Neither bled nor gutted, [B] bled but not gutted, [C] gutted but not bled and [D]bled and gutted. Microbial analyses were carried out on them. The mean total bacteria count of the fresh fish samples was highest in sample A which was neither bled nor gutted [28.0×10^3 cfu/g], while the lowest was observed in sample D which was bled and gutted [8.0×10^3 cfu/g]. The mean total fungi count was highest in sample A [9.3×10^3cfu/g] and the lowest was recorded in sample D [6.0×10^3cfu/g]. The mean total bacteria count of the smoke-dried samples was highest in sample B which was bled but not gutted [10.0×10^3 cfu/g] and lowest in Sample D [4.0×10^3cfu/g]. The mean total fungi count was highest in Sample A [8.3×10^3cfu/g], while the least was observed in Sample D [4.3×10^3 cfu/g]. The mean total bacteria count for the smoke-dried samples after two months of storage was highest in sample A [69.3×10^3cfu/g] and was lowest in Sample D [31.0×10^3cfu/g] Sample A [93.0×10^3cfu/g] had the highest mean fungi count while the least was seen in Sample C which was gutted but not bled [33.7×10^3 cfu/g]. Microbial count of the fresh fish samples was reduced after smoke-drying and increased during storage at ambient temperature. Gutting was more efficient in reducing microbial load of the samples than bleeding. This shows that gutted fish will have a longer shelf life than bled fish but both will last longer than fish species that are neither bled nor gutted when subjected to similar storage condition.

Keywords *Clarias gariepinus,* Bleeding, Gutting and Microbial Load

Introduction

The ever growing world population and the need to store and transport the food from one place to another where it is needed, have made food preservation become necessary in order to increase its shelf life and maintain its nutritional value, texture and flavor. Therefore, good food preservation techniques must prevent microbial spoilage of food without affecting its quality and nutritional qualities [1].

Fisheries make an important contribution to the animal protein supplies of many communities in both the industrialized and developing world [2]. Food security exists when all people at all times have both physical and economic access to the basic food they need, improving food securities requires making better use of fish produced by reducing post-harvest losses and increasing the percentage of fish used for direct consumption [3].

Fish is a highly perishable commodity that undergoes spoilage as soon as it is harvested. Once spoilage sets in, the odour/flavor, texture, colour and sometimes the chemical composition changes [4]. Fish provides between 30% and 80% of the total animal protein intake of the coastal people of West Africa. The amino composition of fish compares favourably well with egg, milk and meat and it contains high amount of unsaturated fatty acids, vitamins, proteins, minerals, and little or no saturated fat, and low carbohydrates [5].

A number of processing techniques are in operation in Nigeria. These include chilling, freezing, salting, canning, drying and smoking [6]. However, smoking is the most popular method of fish processing, Eyo, 2000 reported that smoking involves heat application to remove water and inhibit bacterial and enzymatic action on fish. Fish smoking is a traditional method of processing globally, it accounts for about 3% of the world's catch and also increases the shelf-life [4]. The flesh of smoked fish is delicate, succulent, and delicious and can be readily consumed without further processing [7]. Smoke dried fish is an important ingredient in the Nigerian traditional diet and is relished for its appetizing taste and flavour. The shelf-life of smoked fish product is usually extended primarily due to the reduced water activity [7]. Smoked seafood products vary widely in microbial stability, but this depends on the nature and degree of severity of smoking. Heavily salted, hard smoked products have water content that is too low to support microbial growth and present little or no public health hazards [8].

Spoilage of food products can be due to chemical, enzymatic or microbial activities. Chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year [9]. One-fourth of the world's food supply [10] and 30% of landed fish [11] are lost through microbial activity alone.

The degradation of fish is accelerated by microorganisms associated with aquatic environments as well as contaminants during post-harvest handling. When fish dies, microorganisms on the surface as well as gut and gills begin to utilize the fish protein and food nutrients resulting in loss of nutritional value. Microbial activities create undesirable changes like off-flavors, texture and appearance. Rate of bacterial spoilage is dependent on the initial microbial load, ambient temperature and handling practices [12].

Bleeding of fish is of high importance and should be a common practice in all fisheries. It removes the organic waste and helps to cool the fish's body [13]. Many researchers have found that the bleeding of fish leads to improvement in the quality of flesh in terms of appearance, odour and shelf life [13]. Bleeding is usually quicker and more effective when carried out at a relatively low temperature and when the fish are still alive [14]. It is good practice with some fish to bleed them prior to gutting. It is important to bleed and gut fish as soon as possible after harvest and slaughter. Some specialists believe that live fish should be bled, left in a rinse tank for a short period of time [about 20 minutes] and then quickly gutted. Others believe gutting and bleeding can be done together [13].

Materials and Methods

Collection of Samples

Sixteen [16] pieces of fresh *Clariasgariepinus* of the same size and weight (0.9kg) were carefully selected and purchased from the University of Benin Fisheries Department's experimental farm. All samples were collected in 15th March, 2016.

Processing Procedures

Bleeding and gutting procedures

The fish was bled using the throat cut method, partially cutting the throat and allowing to bleed for ten minutes in clean water [13]. The fish was gutted by cutting the belly open from the anal opening and removal the gills in the head region.

Preparation of samples

All the samples collected were killed. Four each were neither bled nor gutted [A bled and not gutted [B], four were gutted but not bled [C] and bled and gutted [D]. All samples were washed thoroughly with clean water.

Smoking of the samples

The samples were placed for drying in the Magbon-alade-smoking kiln in the Fisheries Department, University of Benin. The smoke-drying process was carried out for 20 hrs at a temperature of 80 °C.

Storage

After smoking, the fish samples were allowed to cool. They were wrapped in polyethylene bags, sealed by tying the loose end in order to reduce microbial proliferation and stored at ambient temperature [27°- 32°] prior to analysis. The polyethylene bags were used because of its capacity to reduce absorbing of moisture from the environment.

Microbial Analysis (Methodology)

Isolation of bacteria and Fungi

The serial dilution method of Harrigan and McCance was aseptically carried out in tubes. Sterile test tubes were used for the ten-fold dilution. Test tubes labelled: 10^{-1},10^{-2},10^{-3}, were used for each of the samples and one gram [1.0 gm] of each of the samples was weighed out and blended, mixed with nine milliliters of sterile distilled water into a test tube. One millilitre [1.0 ml] of the aliquot was obtained from each of the samples and transferred into the test tube labelled 10^{-1} and mixed properly. One millilitre [1 ml] aliquot was then transferred serially from the tube [10^{-1}] to tubes labelled10^{-2}, 10^{-3}, in that order. This was done for each of the samples ABC and D and at the end of each serial dilution, the 1 ml left in the pipette tip was discarded. Aliquots from the appropriate tubes were then used to inoculate appropriate media for isolation and/or detection of target bacteria and fungi using the pour plate method. The pour plate technique was used for the isolation of bacteria during the study. The agar media used were prepared according to manufacturers' instruction. The plates were labelled appropriately, and with the aid of a 0.1 ml pipette aliquots of the appropriate dilutions were inoculated into the agar plates. The agar plates were then incubated at room temperature (28±2 ^{o}C) for 24 to 48 hrs for the bacteria and 3- 5 days for fungi.

Identification and Characterization of Isolates

The identification of bacterial isolates was based on their morphological, cultural and biochemical characteristics. Gram reaction, oxidase, catalase, sugar fermentation [glucose, maltose, sucrose, and mannose], indole, urease, citrate utilization, methyl red [MR] and Voges-Proskauer [VP] tests were carried out. The identification of the isolates was carried out using Cowan and Steel's [1974] Manual for the Identification of Medical Bacteria.

Morphology and Cultural Characteristics of Isolates on Media

Twenty four [24] to 48 hours agar cultures of each isolate were used in determining their cultural characteristics. The features examined in the colonies include; edge, shape, colour, opacity and surface appearance while 3 to 5 days cultures of fungi plates were used to study the culture, plate culture reversed and nature of growth.

Statistical Analysis

The statistical analysis was done at 5% probability level using SPSS Version 20. The means separation was done using the Duncan multiple range test [DMRT] at 5% probability level to compare the microbial load of all the fish samples.

Results

Tables 1-10 summarize the results obtained in the study of the effects of bleeding and gutting on the microbial load of smoked-dried *Clarias gariepinus.* Table 1 shows the weight loss of samples after subjecting them to various treatments. sample A weighed 0.9 kg, no weight was lost, sample B lost 0.07 kg [7.78 %] weighing 0.83 kg from the initial 0.9 kg after bleeding without gutting, sample C lost 0.05 kg [5.56 %] weighing 0.85 kg from the initial 0.90 kg after gutting without bleeding, while sample D lost 0.17 kg [18.89 %] weighing 0.73 kg from the initial 0.90 kg after bleeding gutting. According to Table 1, the weight loss is the difference between the initial before bleeding and gutting and the weight after bleeding and gutting while the % weight loss is the weight loss multiply by 100 and divided by initial weight.

Table 1: Weight loss in *Clariasgariepinus*before and after application of bleeding and gutting procedures

Samples	Treatments	Original weight [kg]	Weight after treatment [kg]	Weight loss	Weight loss [%]
A	Neither bled nor gutted	0.9	0.9	0	0
B	Bled but not gutted	0.9	0.83	0.07	7.78
C	Gutted but not bled	0.9	0.85	0.05	5.56
D	Bled and gutted	0.9	0.73	0.17	18.89

Results of the Initial Microbial load of Fresh Samples of *Clarias gariepinus*

The highest mean bacteria count in fresh *Clarias gariepinus* was observed in sample A which was neither bled nor gutted [28.0 $x10^3$cfu/g], nor gutted [28.0 $x10^3$cfu/g], it was not significantly different from sample B [P>0.05] but was significantly from sample C and D [P<0.05], while the lowest mean bacteria count was observed in sample D which was bled and gutted [8.0 $x10^3$cfu/g], it was from sample A and B [P<0.05] but was not significantly different from sample C [P>0.05] [Table 2]. It can be seen in Table 3 that the highest mean fungi count in fresh *Clarias gariepinus* was observed in sample A [$9.3x10^3$cfu/g], it was not significantly different from sample B, C and D while the lowest mean fungi count was observed in sample D [$6.0x10^3$cfu/g], it was not also significantly different from sample A, B and C [P>0.05]. *Proteus spp.*, *Micrococcus spp.*, and *Staphylococcus aureus* had the highest occurrence [11.1%], while *Aspergillus niger, Mucor mucido, Aspergillus clavate, Aspergillus flavus, Aspergillus nidulans, Penicilluim italicum and Sclerotium spp.* had the lowest occurrence [3.7%] in all the fish samples, the highest percentage frequency of bacteria isolates was observed in Sample A [14.8%], while the lowest was observed in both samples C and D [7.4%] as seen in Table 4. The highest fungi frequency was observed in both Sample A and C [22.2%], while the least was observed in both Sample B and D [7.4%].

Table 2: Total estimated viable heterotrophic bacteria counts of fresh *Clarias gariepinus* (cfu/g)

Samples	Dilution factor	Number of colonies per plate	Average number of colonies per dilution $x \pm s_e$	Organism per gram of sample = number of colonies× dilution factor
A	10^3	36		$36 \times 10^3 = 1.6 \times 10^4$
Neither bled	10^3	20	28.0 ± 4.6^a	$20 \times 10^3 = 2.4 \times 10^4$
nor gutted	10^3	28		$28 \times 10^3 = 2.8 \times 10^4$
B	10^3	16		$16 \times 10^3 = 3.6 \times 10^4$
Bled but not	10^3	24	21.3 ± 3.5^{ab}	$24 \times 10^3 = 2.0 \times 10^4$
gutted	10^3	28		$28 \times 10^3 = 2.8 \times 10^4$
C	10^3	16		$16 \times 10^3 = 1.6 \times 10^4$
Gutted but	10^3	8	10.7 ± 2.7^{bc}	$8 \times 10^3 = 8.0 \times 10^3$
not bled	10^3	8		$8 \times 10^3 = 8.0 \times 10^3$
D	10^3	12		$12 \times 10^3 = 1.2 \times 10^3$
Bled and	10^3	8	8.0 ± 2.3^c	$8 \times 10^3 = 8.0 \times 10^3$
gutted	10^3	4		$4 \times 10^3 = 4.0 \times 10^3$

*****Means with similar superscripts are not significantly different (P> 0.05).

Table 3: Total estimated viable heterotrophic fungi counts in fresh samples of *Clarias gariepinus* (cfu/g)

Samples	Dilution factors	Number of colonies per plate	Average number of colonies per dilution $x \pm s_e$	Organism per gram of sample = number of colonies× dilution factor
A	10^3	12		$12 \times 10^3 = 1.2 \times 10^4$
Neither bled nor	10^3	9	9.3 ± 1.5^a	$9 \times 10^3 = 9.0 \times 10^3$
gutted	10^3	7		$7 \times 10^3 = 7.0 \times 10^3$
B	10^3	14		$14 \times 10^3 = 1.4 \times 10^3$
Bled but not	10^3	7	9.0 ± 2.5^a	$7 \times 10^3 = 7.0 \times 10^3$
gutted	10^3	6		$6 \times 10^3 = 6.0 \times 10^3$
C	10^3	9		$9 \times 10^3 = 9.0 \times 10^3$
Gutted but not	10^3	6	6.0 ± 0.9^a	$6 \times 10^3 = 6.0 \times 10^3$
bled	10^3	3		$3 \times 10^3 = 3.0 \times 10^3$
D	10^3	7		$7 \times 10^3 = 7.0 \times 10^3$
Bled and gutted	10^3	9	8.7 ± 1.7^a	$9 \times 10^3 = 9.0 \times 10^3$
	10^3	10		$10 \times 10^3 = 1.0 \times 10^3$

*Means with similar superscripts are not significantly different (P> 0.05).

Table 4: Percentage frequency of occurrence and diversity of isolates obtained from fresh *Clarias gariepinus*

Isolates	#	%	A (Neither bled nor gutted)	B (Bled but not gutted)	C (Gutted but not bled)	D (Bled and gutted)
Proteus sp	3	11.1	1	1	1	0
Micrococcus sp	3	11.1	1	1	0	1
Staphylococcus aureus	3	11.1	1	1	1	0
Staphylococcus epidermidis	2	7.4	1	0	0	1
Bacteria frequency	11		4	3	2	2
Bacteria % frequency		40.7	14.8	11.1	7.4	7.4
Bacteria diversity	3		2	3	3	3
Bacteria % diversity		30	30	30	20	20
Aspergillu sniger	1	3.7	1	0	0	0
Cladosporium sp	2	7.4	1	0	1	0
Muco rmucido	1	3.7	0	0	1	0
Aspergillus fumigatus	1	3.7	1	0	0	0
Saccharomyces sp	2	7.4	0	1	0	1
Aspergillus flavus	1	3.7	0	0	1	0
Aspergillus nidularis	1	3.7	0	0	1	0
Penicillium italicum	1	3.7	0	0	1	0
Sclerotium sp	1	3.7	1	0	0	0
Botrytis sp	2	7.4	1	0	1	0
Mucorsp	3	11.1	1	1	0	1
Fungi frequency	16		6	2	6	2
Fungi %frequency		59.3	22.2	7.4	22.2	7.4
Fungi diversity	7		6	2	5	2
Fungi % diversity		70	60	20	50	20
Microbial frequency	27		10	5	8	4
Microbial % frequency			36.7	18.5	29.6	14.8
Microbial diversity			9	5	8	4
Microbial % diversity			90	50	80	40

1- Present; 0- Not present

Results of Samples of *Clarias gariepinus* immediately after Smoke-drying.

Table 5 shows that the highest mean bacteria count of *Clarias gariepinus* immediately after smoking was observed in sample B [10.0 $\times 10^3$], it was not significantly different from sample A [P<0.05] but was from sample C and D [P>0.05] while the lowest count was observed in sample D [4.0 x 10^3], it was from sample A and B [P<0.05] but was not significantly different from sample C [P>0.05]. It was also seen in Table 6 that the highest mean fungi count of smoked-dried *Clarias gariepinus* was observed in sample A [8.3 × 10^3], it was not significantly different from sample B, C and D [P>0.05], while the lowest mean fungi count was observed in sample D [4.3 $\times 10^3$], which was not significantly different from sample A, B and C [P>0.05]. Table 7 shows that *Proteus spp., Staphylococcus aureus, Escherichia coli and Botrytis spp.,* had the highest occurrence [10.0%] in all the samples while *Bacillus spp., Penicilliuim spp., Penicilluim spp., Aspergillus clavate,*

Rhizoctonia spp., Yeast orange and Yeast yellow had the least [1.6%]. Table 7 also indicates that Sample A, B, and D had the highest bacteria percentage frequency [6.5%], while Sample C had the least [4.9%].

Table 5: Total estimated viable heterotrophic bacteria counts of smoked-dried samples of *Clarias gariepinus* immediately after smoke-drying (cfu/g).

Samples	Dilution factors	Number of colonies per plate	Average number of colonies per dilution x ± s_e	Organism per gram of sample = number of colonies× dilution factor
A	10^3	13		$13 \times 10^3 = 1.3 \times 10^4$
Neither bled	10^3	6	9.0 ± 0.5^a	$6 \times 10^3 = 6.0 \times 10^3$
nor gutted	10^3	8		$8 \times 10^3 = 8.0 \times 10^3$
B	10^3	12		$12 \times 10 = 1.2 \times 10^4$
Bled but not	10^3	8	10.0 ± 0.5^a	$8 \times 10^3 = 8.0 \times 10^3$
gutted	10^3	10		$10 \times 10^3 = 1.0 \times 10^3$
C	10^3	7		$7 \times 10^3 = 7.0 \times 10^3$
Gutted but	10^3	4	5.3 ± 0.9^b	$4 \times 10^3 = 4.0 \times 10^3$
not bled	10^3	5		$5 \times 10^3 = 5.0 \times 10^3$
D	10^3	5		$5 \times 10^3 = 5.0 \times 10^3$
Bled and	10^3	4	4.0 ± 1.3^b	$4 \times 10^3 = 4.0 \times 10^3$
gutted	10^3	3		$3 \times 10^3 = 3.0 \times 10^3$

*****Means with similar superscripts are not significantly different (P> 0.05).

Table 6: Total estimated viable heterotrophic fungi counts in smoke-dried samples of *Clarias gariepinus* immediately after smoke-drying (cfu/g)

Samples	Dilution factor	Number of colonies per plate	Average number of colonies per dilution x ± s_e	Organism per gram of sample = number of colonies × dilution factor
A	10^3	21		$21 \times 10^3 = 2.1 \times 10^4$
Neitherbled nor	10^3	3	8.3 ± 0.5^a	$3 \times 10^3 = 3.0 \times 10^3$
gutted	10^3	1		$1 \times 10^3 = 1.0 \times 10^3$
B	10^3	4		$4 \times 10^3 = 4.0 \times 10^3$
Bled but not	10^3	15	7.3 ± 0.5^a	$15 \times 10^3 = 1.5 \times 10^4$
gutted	10^3	3		$3 \times 10^3 = 3.0 \times 10^3$
C	10^3	8		$8 \times 10^3 = 8.0 \times 10^3$
Gutted but not	10^3	3	4.7 ± 0.9^a	$3 \times 10^3 = 3.0 \times 10^3$
bled	10^3	3		$3 \times 10^3 = 3.0 \times 10^3$
D	10^3	4		$4 \times 10^3 = 4.0 \times 10^3$
Bled and gutted	10^3	3	4.3 ± 0.6^a	$3 \times 10^3 = 3.0 \times 10^3$
	10^3	6		$6 \times 10^3 = 6.0 \times 10^3$

*****Means with similar superscripts are not significantly different (P> 0.05).

Table 7: Percentage frequency of occurrence and diversity of microbial isolates obtained from smoke-dried fish samples immediately after smoke-drying

Isolates	#	%	A (Neither bled nor gutted)	B (Bled but not gutted)	C (Gutted but not bled)	D (Bled and gutted)
Staphylococcus epidermidis	2	3.2	1	1	0	0
Staphylococcus aureus	4	6.5	1	1	1	1
Proteus sp	4	6.5	1	1	1	1
Escherachia coli	4	6.5	1	1	1	1
Bacillus sp	1	1.6	0	0	0	1
Bacteria frequency	15		4	4	3	4

Bacteria % frequency	24.3		6.5	6.5	4.9	6.5
Bacteria diversity	4		3	3	3	4
Bacteria % diversity	23.5		17.6	17.6	17.6	23.5
Penicillium sp	1	1.6	1	0	0	0
Penicillium brown.	1	1.6	1	0	0	0
P.oxalicum	2	3.2	0	1	1	0
Mucor sp	3	4.8	1	1	0	1
Mucor mucido	3	4.8	1	1	1	0
Aspergillus niger	3	4.8	1	1	1	0
Aspergillus flavus	3	4.8	1	1	1	0
Aspergillus tamari	2	3.2	1	0	0	1
Aspergillus niduraris	2	3.2	0	0	1	1
Aspergillus fumigatus	1	1.6	0	0	0	1
Helminthesporium sp	2	3.2	0	1	1	0
Neurospora sp	2	3.2	0	1	1	0
Cladosporium sp	3	4.8	1	1	1	0
Trichoderma sp	3	4.8	1	1	0	1
Botrytis sp	4	6.5	1	1	1	1
Saccharomyces sp	3	4.8	0	1	1	1
Sclerotium sp	2	3.2	1	0	0	1
Rhizoctoria sp	1	1.6	1	0	0	0
Cryptomonas neoformis	2	3.2	0	1	1	0
Yeast	1	1.6	1	0	0	0
Fungi frequency	44		13	12	11	8
Fungi % frequency		75.3	22.4	20.8	17.6	14.4
Fungi diversity	13		9	11	9	7
Fungi% diversity		76.5	60.0	64.7	60.0	41.2
Microbial frequency	62		18	17	15	12
Microbial % frequency		99.6	28.9	27.3	24.1	19.3
Microbial diversity	17		12	15	13	15
Microbial % diversity		100.0	70.6	88.2	76.5	88.2

Results of Smoke-dried *Clarias gariepinus* after two Months of Storage

It can be seen in table 8 that the highest mean bacteria count was observed in sample A (69.3×10^3), it was not significantly different from samples B and C ($P>0.05$) but was significantly different from sample D ($P<0.05$) while while the least was observed in sample D (31.0×10^3), which was not significantly different from samples B and C ($P>0.05$) but was from sample A ($P<0.05$). The highest mean fungi count as shown in Table 9 was observed in sample A (93.0×10^3), it was significantly different from samples B, C and D ($P<0.05$), while sample C (33.7×10^3) had the lowest, and was not significantly different from sample D ($P>0.05$) but was from sample A and B ($P<0.05$). The highest occurrence (10.0%) was observed in *Proteus spp., Saccaromyces spp. and Penicillium italicum* and the lowest occurrence (2.5%) in *Serratia spp., Rhizopus spp., Sclerotium spp., Penicillium oxalicum, Cladosporium spp., Penicillium spp. and Trichoderma spp.* (2.5%). Table 8 also shows that the highest bacteria percentage frequency was observed in sample A (11.1%) and the lowest in sample D (5.6%). Sample B had the highest fungi frequency (19.5%), while the lowest was recorded in sample D (13.9%).

Table 8: Total estimated viable heterotrophic bacteria count (cfu/g) of *Clarias gariepinus* after 2 months

Samples	Dilution factor	Number of colonies per plate	Average number of colonies per dilution $x \pm s_e$	Organism per gram of sample = number of colonies × dilution factor
A	10^3	124		$124 \times 10^3 = 1.24 \times 10^5$
Neither bled	10^3	32	69.3 ± 27.9^a	$32 \times 10^3 = 3.2 \times 10^4$
nor gutted	10^3	52		$52 \times 10^3 = 5.2 \times 10^4$
B	10^3	80		$80 \times 10^3 = 8.0 \times 10^4$
Bled but not	10^3	52	54.7 ± 13.9^{ab}	$52 \times 10^3 = 5.2 \times 10^4$
gutted	10^3	32		$32 \times 10^3 = 3.2 \times 10^4$
C	10^3	68		$68 \times 10^3 = 6.8 \times 10^4$
Gutted but not	10^3	28	46.7 ± 11.6^{ab}	$28 \times 10^3 = 2.8 \times 10^4$
bled	10^3	44		$44 \times 10^3 = 4.4 \times 10^4$
D	10^3	60		$60 \times 10^3 = 6.0 \times 10^3$
Bled and	10^3	21	31.0 ± 1.7^b	$21 \times 10^3 = 2.1 \times 10^3$
gutted	10^3	12		$12 \times 10^3 = 1.2 \times 10^3$

*****Means with similar superscripts are not significantly different (P> 0.05).

Table 9: Total estimated viable heterotrophic fungi counts (cfu/g) in smoke-dried *Clarias gariepinus*

Samples	Dilution factor	Number of colonies per plate	Average number of colonies per dilution x $\pm s_e$	Organism per gram of sample = number of colonies × dilution factor
A	10^3	120		$120 \times 10^3 = 1.2 \times 10^5$
Neither bled	10^3	89	93.0 ± 14.6^a	$89 \times 10^3 = 8.9 \times 10^4$
nor gutted	10^3	70		$70 \times 10^3 = 7.0 \times 10^4$
B	10^3	86		$86 \times 10^3 = 8.6 \times 10^4$
Bled but not	10^3	72	63.3 ± 16.2^b	$72 \times 10^3 = 7.2 \times 10^4$
gutted	10^3	32		$32 \times 10^3 = 3.2 \times 10^4$
C	10^3	60		$60 \times 10^3 = 6.0 \times 10^4$
Gutted but	10^3	23	33.7 ± 9.4^c	$23 \times 10^3 = 2.3 \times 10^4$
not bled	10^3	18		$18 \times 10^3 = 1.8 \times 10^4$
D	10^3	63		$63 \times 10^3 = 6.3 \times 10^4$
Bled and	10^3	42	45.3 ± 13.3^c	$42 \times 10^3 = 4.2 \times 10^4$
gutted	10^3	31		$31 \times 10^3 = 3.1 \times 10^4$

*****Means with similar superscripts are not significantly different (P> 0.05).

Table 10: Percentage frequency of occurrence and diversity of isolates obtained from smoke-dried fish samples after 2 months.

Isolates	#	%	A (Neither bled nor gutted)	B (Bled but not gutted)	C (Gutted but not bled)	D (Bled and gutted)
Micrococcus sp	3	8.3	1	1	1	0
Proteus sp	4	11.1	1	1	1	1
Serratia sp	1	2.8	0	1	0	0
Staphylococcus sp	2	5.6	1	0	0	1
Staphylococcus aureus	2	5.6	1	0	1	0
Bacteria frequency	12		4	3	3	2
Bacteria % frequency	33.3		11.1	8.3	8.3	5.6
Bacteria diversity	4		3	3	3	2

Bacteria % diversity	28.6		21.5	21.5	21.5	14.5
Rhizopus sp	1	2.8	1	0	0	0
Sclerotium sp	1	2.8	1	0	0	0
Aspergillus flavus	2	5.6	0	0	1	1
Aspergillus niger	2	5.6	1	0	1	0
Mucor sp	3	8.3	1	1	1	0
Saccharomyces sp	4	11.1	1	1	1	1
Penicillium oxalicum	1	2.8	0	0	0	1
Penicillium italicum	4	11.1	1	1	1	1
Cladosporium sp	1	2.8	1	0	0	0
Cryptomonas neoformis	2	5.6	0	1	0	1
Penicillium sp	1	2.8	0	1	0	0
Geotrichum sp	2	5.6	0	1	0	1
Trichoderma sp	1	2.8	0	0	1	0
Fungi frequency	24		7	6	6	5
Fungi % frequency		66.7	19.5	16.7	16.7	13.9
Fungi diversity	10		7	5	4	5
Fungi % diversity		71.4	50.0	35.7	28.6	35.7
Microbial frequency	36		11	9	9	7
Microbial % frequency		100.0	30.6	25	25	19.4
Microbial diversity	14		10	12	12	9
Microbial % diversity		100.0	71.4	85.7	85.7	64.3

KEYS, 1-Present; 0-Absent

Discussion

Table 1 shows that there was a significant weight loss in samples of fresh *Clarias gariepinus* (B, C and D) which were bled but not gutted, gutted but not bled and bled and gutted respectively. Blood contains 92% water (www.Unaab.edu.ng), therefore its removal from fish reduces moisture content which in turn reduces microbial activity in fish. Abbas *et al.*, (2007) states that lowering water activity (a_w) can minimize microbial activities and improve preservation of fish [15]. Bacteria are abundant in the diet and environment of fish and it is therefore impossible to avoid them [16]. in this study, six bacteria genera were isolated from *Clarias gariepinus*. The isolates were identified as *Proteus, Micrococcus, Bacillus, Staphylococcus, Serratia and Escherichia.* A total of fourteen fungi genera were also identified from the fish species which includes: *Aspergillus, Penicillium, Mucor, Trichoderma, Clasdosporium, Yeast, Neurospora, Geotrichum, Rhizopus, Saccaromyces, Cryptomonas, Scletorium, Rhizoctorria and Helminthesporium.* The total bacteria and fungi mean count recorded in all the Fish species (Fresh, Smoke-dried and Smoke-dried after 2 months) subjected to different treatment was observed to have exceeded the recommended count set by international commission on microbiological specification of 5×10^5 cfu/g for food and food products [17] despite the effect of smoke-drying. This could result from cumulative contamination from unhygienic handling practice before, during and after smoke-drying which permit the proliferation of the microbes, hence causing deterioration of the products. Processing of meat products using dirty equipment, polluted water, improper and direct handling with the hands increases microbial load in fish and other meat products [18-22].

The mean count of colonies per dilution of bacteria of the fresh samples was highest in Sample A which was neither bled nor gutted (28.0×10^3), it was not different from sample B but was significantly different from

samples C and D, and was least in the bled and gutted (sample D) (8×10^3), which was significantly different from samples A and B but not significantly different from sample C. The mean count of the colonies per dilution of fungi was highest in Sample A (9.3×10^3) and was not significantly different from samples B, C and D and least in Sample D which was bled and gutted (6.0×10^3), it was not significantly different from samples A, B and C. The high bacteria and fungi count in sample A may be as a result of the presence of visceral and blood in the fish and the low bacteria and fungi count in both samples C and D may be as a result of the absence of visceral and little or no blood in the fish samples. The bacteria entering along with the diet of fish during ingestion may adapt themselves in the gastro intestinal tract and form a symbiotic association within the digestive tract of fish in which large numbers of microbes are present [23-26] which is much higher than in the surrounding water indicating that the digestive tracts of fish provide favorable ecological niches for these organisms [27-28].

The mean count of the colonies per dilution of bacteria in the smoke-dried fish samples was highest in sample B (10.0×10^3) which was not significantly different from sample A but was significantly different from samples C and D and least in sample D (4.0×10^3), which was significantly different from sample A and B but not from sample C. The mean count of the colonies per dilution of fungi was highest in Sample A (8.3×10^3) which was not significantly different from samples B, C and D and seen to be lowest in sample D (4.3×10^3), and was also not significantly different from samples A, B and C. It was observed that the mean count of the colonies per dilution of the smoke-dried samples were reduced after smoke-drying.

After two months of storage, the highest mean count of colonies per dilution of bacteria was observed in sample A (69.3×10^3) which was not significantly different from samples B and C but was significantly different from sample D and the lowest in sample D (31.0×10^3) which was not significantly different from sample B and C but was, from sample A. The mean count of the colonies per dilution of fungi also increased in all samples, the highest value was observed in sample A (93.0×10^3) which was significantly different from samples B, C and D and the lowest value was observed in sample C (33.7×10^3) and was not significantly different from sample D but was, from samples A and B. The increase of microbial count in the samples was probably due to the high level of moisture content and proliferation of bacteria in the fish during storage. The occurrence of *Aspergillus, Rhizopus, and Penicillium*species indicates that contamination may be due to absorption of moisture during storage, the stored fish might have reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing and handling [29].

A total of 29 isolates were obtained and identified as bacteria and fungi in all samples of fresh, smoke-dried and Fish samples under ambient storage, the bacteria isolates which include *Proteus sp, Bacillus sp, Escherichia coli, Micrococcus sp, Staphylococcus aureus, Staphylococcus epidermidis* and *Serratia sp* were isolated on nutrient agar. The fungi isolate includes *Aspergillus niger, Mucor sp, Neurospora sp, Penicillium sp, Geotrichum sp, Rhizopus sp, Mucor mucedo, Saccaromyces sp, Aspergillus flavus, Penicillium oxalicum, Trichoderma sp, Aspergillus nidularis, Cladosporium sp, Yeast, Aspergillus tamari, Aspergillus fumigatus, Helminthosporium sp, Botrytis sp, Sclerotium sp, Rhizopus sp, Rhizoctorria sp, Cryptomonas neoformis,* they were isolated on potato dextrose agar (PDA). The bacteria isolates in fresh samples of *Clarias gariepinus* was highest in sample A (14.8%) and lowest in samples C and D (7.4%), the highest fungi isolates was observed in samples A and C (22.2%) and the lowest in samples B and D (7.4%). The bacteria isolates for smoke-dried sample was high in samples A, B and D (6.5%) and was low in sample C (4.9%), sample A had the highest fungi isolates (22.4%), and sample D had the lowest (14.4%). After two months of storage, sample A had the highest bacteria isolates (11.1%), while sample D had the lowest (5.6%). The highest fungi isolates was observed in sample B (16.7%), while the lowest was observed in sample D (13.9%).

The results show that all the gutted samples especially sample D (Bled and gutted) had low bacteria and fungi counts, this shows that gutting may have more effect on the microbial load of fish when compared to bleeding, this is in accordance with the findings of Olugbojo and Ayoola, (2015) who reported that bacteria occurs most in the gut and gills, therefore should be removed to reduce microbial load [30].

Conclusion and Recommendations

Fungi and bacteria including the non-pathogenic and pathogenic forms are usually present in fish species but the immune system of the fish fights against them. As soon as fish is caught and dies, the micro-organisms attack the fish and break down the fish into unwholesome and unacceptable forms. Mishandling, unhygienic smoking, poor processing and method of storage increases post-harvest losses and ultimately results to one the limiting factors in fish production.

It has been shown by the study that fresh and smoked-dried *C. gariepinus* is contaminated by various micro-organisms but these micro-organisms can be reduced by bleeding and gutting. Both bleeding and gutting had a positive effect in the reduction of the microbial load of fresh and smoke-dried fish throughout the study period. The study also revealed that gutting had more effect in microbial load reduction than bleeding.

Recommendations

- Bleeding and gutting practices should be carried out on fish prior to smoking to increase shelf life and reduce microbial load.
- Hygienic practices such as cleaning surfaces, equipment for fish processing prior to smoke-drying should be carried out.
- Hand gloves should be worn during preparation of fish for smoking and smoke-drying to avoid direct contact with the fish.

References

[1]. Ghaly, A.E. Dave, D. Budge, S., & Brooks, M.S. (2010). Department of process Engineering and Applied Science, Dalhousie University Halifax, Nova Scotia, Canada.

[2]. Adewolu, M.A., & Adeoti, A.J. (2010).Effect of mixed feeding schedules with varying dietary crude protein levels on the growth and feed utilization of *clarias gariepinus* (Burchell 1822) fingerlings. *Journal of fish Aquatic Science*. 5: 304-310.

[3]. FAO, (2010). Fisheries and aquaculture topics. Food security and fisheries topics facts sheets. Text by Peter Manning, FAO Fisheries and Aquaculture Department, Rome.

[4]. Gupta, S.K., & Gupta, P.C. (2006).General and Applied Ichthyology (Fish and Fisheries). S. Chand and Co. Ltd, Ram Nagar, New Dehli. 1045-1068.

[5]. Adenike O.M. (2014). The Effect of Different Processing Methods on the Nutritional Quality and Microbiological Status of Cat Fish (*Clarias lezera*). *Journal of Food Process Technology* 5(6): 333.

[6]. Komulo-Johnson, C.A., Aladetoun, N.F., & Ndimele, P.E. (2010). The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus* (BURCHELL 1822). *African Journal of Biotechnology* Vol. 9 (1), pp. 073-076.

[7]. Eyo, A.A., (2001).Fish processing Technology in the Tropics. University of Ilorin press. Pp. 112-129.

[8]. Idah, P.A., &Nwankwo, I. (2013). Effects of smoke-drying temperatures and time on physical and nutritional quality parameters of Tilapia (*Oreochromis niloticus*). *International Journal of fisheries and Aquaculture.* 5(3), pp. 29-34.

[9]. Baird-Parker, T.C. (2000). The Production of Microbiologically Safe and Stable Foods. In: The Microbiological Safety and Quality of Food, Lund, B.M. and T.C. Baird-Parker (Eds.). Aspen Publishers Inc., Gaithersburg, MD., USA. pp: 3-18.

[10]. Huisin't Veld, J.H.J., (1996). Microbial and biochemical spoilage of foods: An overview. *International Journal of Food Microbiology*. 33: 1-18.

[11]. Amos, B., (2007). Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement. United Nations University, Uganda. http://www.unuftp.is/static/fellows/document/amos06prf.pdf.

[12]. Begum, F., Saikia, M., &Boruah, R. (2014). Diversity of Bacterial Tissues in Fishes from two Traditional Fish Markets in Nagaon, Assam (India). *Journal of Biological and Chemical Research*31 (1) 495-505.

[13]. Tetteh E.N. (2010). Effect of Different Bleeding Conditions on the Colour tone of Fresh, Frozen and Salted Fillets of Atlantic Cod (*Gadus morhua).*

[14]. Smith, J.G.M., & Hardy, R. (2001). Handling and Processing of Saithe, Ministry of Technology, Torry Research Station, Torry Advisory Note No. 47 [http://www.fao.org/wairdocs/ tan/x5924e/x5924e00.htm].

[15]. Abbas, K.A., Saleh, A.M., Mohamed, A., & Lasekan, O. (2009). The relationship between water activity and fish spoilage during cold storage: A review. *Journal of Food, Agriculture Environment,*7: 86-90.

[16]. Strom, E., & Olafsen, J.A. (1990). The indigenous microflora of wild- captured juvenile cod in net-pen rearing. In: *Microbiology in poecilotherms (Lesel R, editor). Elsevier Science*, Amsterdam, Pp. 181-185.

[17]. ICMSF (International Commission on Microbial Specification for Foods) (2002). Micro-organism in food. Microbiological Testing in Food Safety Measurement. Kluwer Academic/Plenum, NY.

[18]. Adebolu, T.T., & Ifesan, B.O. (2001). Bacteriological quality of vegetables used in salads. *Nigeria Journal of Microbiology*. 15(1):81-85.

[19]. Dunn, R.A., Hall, W.N., Altamirano, J.V., Dietrich, S.E., Robinson-Dunn, B., & Johnson, D.R. (1995). Outbreak of *Shiegella flexneri* linked to salad prepared at a central commissary in Michigan. Public Health Rep. 110(5):580-586.

[20]. Okonko, I.O., Ogunjobi, A.A., Kolawale, O.O., Adejoye, O.D., & Ogunnusi, T.A. (2008). Comparative studies and microbial risk assessment of different water samples used for processing frozen seafoods in Ijora-olopa, Lagos State, Nigeria. *African Journal of Biotechnology*. 7(16):2902-2907.

[21]. Omemu, A.M., &Bankole, M.O. (2005). Ready-to-eat vegetable salad: effect of washing and storage temperature on the microbial quality and shel-life. In: (NSM). Book of Abstract of the 29th Annual Conference & General Meeting on microbes as agents of sustainable development, organized by Nigerian Society for Microbiology (NSM), Abeokuta from 6-10th November 2005, p. 28.

[22]. Anihouvi, D.G.H, Kayode, A.P.P., Anihouvi, V.B, Azokpota, P., Kotchoni, S.O., & Hounhovigan, D.J. (2013). Microbial contamination associated with the processing of *tchachanga*, a roasted meat product. *African Journal of Biotechnology*. 12 (18), 2449-2455.

[23]. Trust, T.J., Bull L.M., Currie, B.R., & Buckley, J.T. (1979). Obligate Anaerobic Bacteria in the Gastrointestinal microflora of the grass carp (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), and rainbow trout (*Salmo gairdneri*). *Journal of Fisheries Research Board of Canada,*36: 1174–1179.

[24]. Rimmer, D.W., & Wiebe, W.J. (1987). Fermentative microbial digestion in herbivorous fishes. *Journal of Fish Biology,* 31: 229–236.

[25]. Ringo, E., & Strom E. (1994). Microflora of Arctic charr, *Salvelinus alpinus*(L.). I. The gastrointestinal microflora of free-living fish and the effect of diet and salinity on intestinal microflora. *Aquaculture and Fisheries Management,*25: 623–629.

[26]. Clements, K.D., & Choat, J.H. **(**1995). Fermentation in tropical marine herbivorous fishes. *Physiology and Zoology,* 68(3):355–378.

[27]. Trust T. J., & Sparrow R.A.H. (1974).The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Canadian Journal of Microbiology,* 20: 1219–1228.

[28]. Sakata T (1990). Microflora in the digestive tract of fish and shell-fish. In*: Microbiology in poecilotherms [sic] (Lésel R, editor).* Elsevier Science, Amsterdam, Pp 171–176.

[29]. Ayolabi, C. I. & Fagade, O.E. (2010). Mycological evaluation of smoked fish from the retail outlets in Ago-Iwoye, Ogun State, Nigeria. *Journal of Life and Physical Science Acta SATECH,* 3 (2): 65-66.

[30]. Olugbojo, J.A., & Ayoola S.O. (2015). Comparative Studies of Bacteria Load in Fish Species of Commercial Importance at the Aquaculture Unit and Lagoon Front of the University of Lagos. *International Journal of Fisheries and Aquaculture*, 7 (4):37-46.