Proximate compositions and microbial diversity of smoked-dried edible frogs in ado-ekiti and ikare-akoko, south western nigeria.

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Abstract

Microbial safety of food cannot be disregarded during its preparation and preservation. Therefore the proximate and mineral compositions cum microbial diversity of smoked-dried edible frogs in Ado-Ekiti and Ikare-Akoko, South-Western, Nigeria that were preserved differently for 2 weeks were investigated. Data were analysed using descriptive statistics and ANOVA at α0.05. The highest moisture, fibre and proteins values of 7.08 ± 0.02%; 0.27 ± 0.06% and 60.90 ± 0.16% were obtained from IKF samples and the least values of 6.13 ± 0.00%; 0.00 ± 0.00% and 52.53 ± 0.00% were from ADU, IKU and IKN frog samples respectively. However, the crude ash and crude lipid followed an opposite trends, with the highest values of 15.10 ± 0.00% and 13.11 ± 0.01% from IKU and ADF samples, while the least values of 10.37 ± 0.01% and 7.35 ± 0.00% were from ADF and IKU frog samples respectively. The frog from ADU had the highest Na ion values of 35.30 ± 0.17 mg and the lowest value of 17.53 ± 0.06 from IKN samples, while the magnesium ion highest values of 12.19 ± 0.01 mg was from IKF and the least value of 7.14 ± 0.01 mg was from ADU samples. There were significant differences (P>0.05) in the proximate and mineral ions in the frogs from the markets. The average bacterial count ranged from 3.90 × 106 to 5.80 × 106 cfu/g and the average fungi count ranged from 3.20 × 104 to 5.15 × 104 cfu/g. The presence of microorganisms: Bacillus cereus, *Listeria monocytogenes*, Sporobolomyces roseus and *Fusarium solani* in the dried frog samples is of public health concerns and proper attention is needed for quality control and adequate preservation before sales for consumption.

Keywords: Nutritive value, Preservation, Rana esculenta, Microbial diversity, Food safety.

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Introduction

World demand for food is increasingly higher, especially for those which provide high nutritional value such as proteins. Meat is important to human beings and could be obtained from various sources. Also, it is a very good source of nutrients and vitamins to the body. Animal protein in developing countries has over the years been in short supply, due to inadequate production, high cost of conventional sources of animal protein such as poultry, beef, mutton and pork and some health problems associated with red meat. Current research trends is now focused on other alternatives especially, from other animals such as snail, frog, etc [1] which would help to take care of these health challenges and at the same time be cheaper and safer for consumption [2,3].

Anura species are eaten in many parts of the world, the meat from frogs; popularly called frog meat has become an alternative source of animal protein for the ever increasing Nigerian population. In Nigeria, and many other African countries, frogs are heavily hunted and traded majorly for their nutritional and medicinal benefits [4]. Their meat is becoming popular as a source of protein in many countries including Nigeria. The meat serves as food as well as a source of income or foreign exchange [5]. Frog legs are rich in protein, omega-3 fatty acids, vitamin A and potassium. They are often said to taste like chicken because of their mild flavor, with a texture most similar to chicken wings. The taste and texture of frog meat is approximately between chicken and fish. Oliveira et al. [6,7] carried out a survey which indicated that frog meat is a highly digestible food, which justifies its use in special diets. Oduntan et al. [8] proposed that the consumption of edible frog (Rana esculenta) to substitute bush meat is feasible since it is reliable source of animal proteins and other vital nutrients for human in great abundance.

Several studies have shown that frog meat had good nutritional composition and it is used as protein source in the diet of many consumers [9]. Frogs meat provide between 5% and 45% of daily mineral requirements of a human body, from the consumption of one hundred grams of meat [10]. Generally, food products from aquaculture are known to consist of different nutrients and chemical compositions such as moisture, fibre, carbohydrate, ash, fat, protein, vitamins and mineral elements. Thus, the percentage of these chemicals and nutrients composition in a food product, to a large extent, determines the acceptability or fitness for consumption Burubai [11].

However, the factors influencing the microbial safety of food cannot be disregarded during its preparation [12]. It is very important to ensure that the product's nutritional integrity and necessary hygienic conditions are not compromised, so as to avoid Food Borne Diseases (FBDs), especially from most

of these street foods that have been established as modes of transmission of food borne infections that can be very severe and life threatening [13].

Furthermore, prevention of aquatic products from spoilage may be achieved by different processing and preservation techniques such as chilling, canning, salting, and drying and smoking [14]. These amphibians are obtained in different forms such as fresh, sun dried, smoked and smoked dried. However, there is need to create awareness in Nigeria about safety and nutritional status of *R. esculenta*, so as to serve as an economical source of animal protein, since it is cheap, widespread, abundant and readily available in most eco-zones of Nigeria [15].

This research was undertaken to determine the proximate composition of the smoked dried frog. Also to isolate and identify microbial contaminants associated with the smoked dried frog. Furthermore, to fill the gap between processing and post-harvest losses to post processing and post preservative losses in nutrient values and shelf life of smoke-dried edible frogs sold in Ikare-Akoko, Ondo State and Ado-Ekiti, Ekiti State, South western, Nigeria.

Materials and Methods

Experimental site

The research was carried out at the Faculty of Science Central Laboratory and Department of Microbiology Laboratory, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria from November, 2018 to January, 2019 for the various analyses.

Sample collection

A total of 20 samples each of edible dried smoked frogs were purchased from two markets: Ikare-Akoko market, Ondo State and Ado-Ekiti market, Ekiti State respectively.

Determination of shelf life of the frogs

Frog samples were separated into the different packing materials (the Aluminum foils and black polyethene nylon). The samples were left in the laboratory under ambient environmental conditions for two weeks, after which the samples were analysed for both the proximate, mineral and microbial analyses. The fresh samples were not preserved but analysed immediate.

Proximate and mineral analysis

The nutritive value analyses for moisture, ash, protein, fat and Nitrogen free extracts of the frog samples were determined according to Association of Official Analytical Chemists (AOAC, 2005) in triplicates. The mineral contents were determined using atomic absorption air-acetylene flame AAS 20 VARIAN. Sodium and Potassium ions were determined using Gallenkamp Flame analyzer, while Calcium, magnesium, iron, manganese, zinc and copper were determined using Buchs Model 205 Atomic Absorption Spectrophotometer. Phosphorus level was determined using the Phosphovanadommolybdate Colorimetric Technique on JENWAY 6100 Spectrophotometer (AOAC, 2005).

Microbial analysis

The samples were handled aseptically after being purchased and kept in separate sterile polythene bags and transported to the Department of Microbiology Laboratory for analyses.

Media preparation

Media used were prepared according to manufacturer's instruction and the amounts to be used were measured with metler balance (Mode FA 2104A weighing 0.0001 g-210 g). The measured agar powder was suspended in appropriate amount of distilled water with constants shaking to ensure complete dissolution. They were homogenized, corked with cotton wool, wrapped with aluminum foil and autoclaved at 121°C for 15 minutes and allowed to cool. About 20 ml of the media were poured into sterile Petri dish and allowed to gel. The media used were Nutrient agar, MacConkey Agar, Eosine Methylene Blue Agar and Sabouraud Dextrose Agar as adapted from Adewole et al. [16] and by Fawole and Oso [17].

Serial dilution

Serial dilutions of the sample were carried out by weighing 55 grams of each sample into a sterile conical flask containing 750 ml and 500 ml of sterilized peptone water. The conical flask was shaken properly and then 1 ml of the liquid from the mixture was aseptically dispensed with a pipette into a test tube (10^1) containing 9 ml of sterile peptone water and the same procedure continues up to the ninth test tube (10^9). From the third test tube (10^3), 1 ml of the dilution was dispensed with a pipette aseptically into a sterile Petri dish, same for the fifth (10^5) and seventh (10^7) test tube. Media that had been sterilized by autoclaving and allowed to cool was then poured aseptically into Petri dishes containing the dilutions. The plates were swirled gently on the work bench for even distribution of the inoculums and the plates were allowed to solidify, after which culture was followed as reported by Adewole et al. [16] and by Fawole and Oso [17].

Identification of bacterial isolates

Bacteria colonies, shape, colour, size, edge, elevation and surface texture were observed after 18-24 hours of incubation. Standard biochemical tests were conducted to identify bacterial isolates into species according to the description in Berge's Manual of Systematic Bacteriology [17,19].

Data analysis for mineral composition

The results obtained were subjected to statistical analysis using means and standard deviations. The results are expressed in triplicates. A one-way analysis of variance (ANOVA) at (α0.05) and Duncan's Multiple Range Test was employed to determine the significance of differences among the means using Statistical Analysis System by SAS [20].

Results

TThe percentage moisture contents of the frogs ranged from 6.13 ± 0. 00-7.08 ± 0.02%. The highest value was obtained from IKF samples and the least value was from ADU samples. There were significant differences (P>0.05) in the moisture contents within the treatments. The moisture content of the frog samples from ADN and IKF showed an increased values of -1.47% and -7.44% respectively, while there were decreases in values 2.88% and 8.65% respectively from the frog samples of IKN and ADF (Table 1).

The crude ash contents varied significantly (P>0.05) within the experiment, with the highest value of 15.10 ± 0.00% from IKU frog samples and the least value of 10.37 ± 0.01% was from ADF frog samples. The highest crude fibre value of 0.27 ± 0.06% was recorded in the frog sample IKF and the least value of 0.00 ± 0.00% was obtained from the frog samples IKU. There were no differences in the crude fibre of IKN and IKF frog samples. However, there were slight decrease in percentage differences of fibre content of the frog samples ADN and ADF respectively, which was 9.09% (Table 1).

The crude lipid contents had the highest value of 13.11 ± 0.01% from ADF frog samples and the least value of 7.35 ± 0.00% from IKU frog samples. The crude lipid varied significantly (P>0.05) within the experiments. All the fat contents showed positive trends in percentage difference at the end of the preservation period with the highest value of -68.16% from IKN frog samples and the lowest value of -14.29% from ADN frog samples respectively. The crude protein contents varied significantly (P>0.05) within the frog samples analysed. The highest protein content of 62.52 ± 0.00% was from ADU frog samples and the least 52.53 ± 0.00% from the IKN frog samples.

The carbohydrate values ranged from 5.81 ± 0.01%-14.45 ± 0.17%, the highest value was from IKN frog samples and the least was from ADU frog samples. There were significant differences (P.0.05) among the treatments. However, all the Carbohydrate contents showed an increase in values after the preservation periods except IKF frog samples. The percentage difference ranged from -142.86%-12.21% (Table 1).

The percentage Sodium (Na+) ions of dried edible frog varied significantly (P>0.05) within the samples analyzed. The ADU frog samples had highest percentage of 35.30 ± 0.06% and least value of 17.53 ± 0.06% was from IKN frog sample. There was reduction in the Na ion contents except for the frog IKF which had an increased value. The highest percentage difference of 37.48% for Na ion was from ADF frog sample, while, the lowest percentage difference of 1.42% was from ADN frog samples, but the frog IKF had -14.68 percentage difference (Table 2). The Calcium (Ca^{2+}) ion ranged from 48.63% ± 0.06% to 71.33% ± 0.15%. The highest value was from ADU frog samples and the least was from ADF frog samples respectively.

The ADF frog samples had the highest Potassium (K^{+}) component of 74.97 ± 0.41%, while the lowest value of 56.77 ± 0.06% was recorded in ADU frog samples. There was no significant difference (P<0.05) in K^{+} contents of all the frogs from the treatments except ADU frog samples that was significantly (P>0.05) from the other treatments. The highest percentage difference of -32.06% was from ADF and least -11.93% was from IKN frog samples (Table 2). The Magnesium (Mg^{2+}) ion contents of IKF frog samples were the highest value of 12.19 ± 0.01% and the least value of 7.14 ± 0.01% was from ADU samples. The Mg ions showed significant differences (P>0.05) among the different groups (Table 3). There were observed slight increases in the elemental Mg ions from the frog samples after storage. The highest percentage difference of -52.10% was in the ADF frog samples and the lowest percentage difference of -5.60% was in the ADN frog samples. However, the frog samples from IKF had higher percentage difference than IKN frog samples respectively (Table 2).

The Iron (Fe^{3+}) ranged from 0.05 ± 0.01% from IKU frog samples to 1.35 ± 0.01% recorded from IKN frog samples respectively. The counts (4.15 × 106; 9.5 × 106), wh ions were significantly different (P>0.05) within the treatments. There were increases in the Fe contents of the preserved frogs except for ADF samples that showed a decrease in Fe contents. IKN frog sample had the highest percentage difference of -170% for Fe and the least value of 33.33% was observed in ADF frog samples. The Manganese (Mn) ions contents ranged from 0.40

Table 1. *Proximate compositions of smoke-dried edible frogs from Ado-Ekiti and Ikare-Akoko markets.*

Parameters (%)	ADU	ADF	AND	IKU	IKF	IKN
Moisture	6.13 ± 0.00^{b}	5.60 ± 0.02^{a} (8.65%)	6.22 ± 0.00^{b} (-1.47%)	6.59 ± 0.36	7.08 ± 0.02^{e} (-7.44%)	6.40 ± 0.17^{c} (2.88%)
Ash	14.98 ± 0.00^{e}	10.37 ± 0.01^{a} (30.77%)	13.69 ± 0.02^{c} (8.61%)	15.10 ± 0.00^{f}	12.49 ± 0.02^{b} (17.28%)	14.16 ± 0.00^{d} (6.23%)
Fibre	0.11 ± 0.01^{b}	0.10 ± 0.00^{b} (9.09%)	0.10 ± 0.00^{b} (9.09%)	0.00 ± 0.00^{a}	0.27 ± 0.06^{c} ND	0.10 ± 0.00^{b} ND
Fats	10.45 ± 0.01^{c}	13.11 ± 0.01^{f} (-25.45%)	11.96 ± 0.01^{d} (-14.45%)	7.35 ± 0.00^{a}	8.40 ± 0.01^{b} (-14.29%)	12.36 ± 0.01^{e} (-68.16%)
Protein	62.52 ± 0.00^{f}	56.71 ± 0.01^{c} (9.29%)	53.85 ± 0.01^{b} (13.87%)	58.73 ± 0.22^{d}	60.90 ± 0.16^{e} (-3.69%)	52.53 ± 0.00^{a} (10.56%)
Carbohydrate	5.81 ± 0.01^{a}	14.11 ± 0.03^{d} (-142.86%)	14.19 ± 0.02^{d} (-144.23%)	12.37 ± 0.02^{c}	10.86 ± 0.18^{b} (12.21%)	14.45 ± 0.17^{d} (-16.81%)

Note: Values with different superscripts in each row are significantly different (P<0.05)
Values in parenthesis are percentage difference in nutrient values after storage.

Table 2. *Mineral contents of smoke-dried edible frog from Ado Ekiti and Ikare-Akoko markets.*

Minerals (%)	ADU	ADF	ADN	IKU	IKF	IKN
Na	35.30 ± 0.17^{f}	22.07 ± 0.06^{b} (37.48%)	34.80 ± 0.17^{e} (1.42%)	27.93 ± 0.15^{c}	32.03 ± 0.21^{d} (-14.68%)	17.53 ± 0.06^{a} (37.24%)
Ca	71.33 ± 0.15^{f}	48.63 ± 0.06^{a} (31.82%)	65.43 ± 0.15^{e} (8.27%)	$64.77 \pm 0.25a^{b}$	52.57 ± 0.51^{c} (-4.78%)	60.17 ± 0.12^{d} (-19.93%)
K	56.77 ± 0.06^{a}	74.97 ± 0.41^{b} (-32.06%)	72.67 ± 0.25^{b} (-28.01%)	$64.77 \pm 0.25a^{b}$	72.97 ± 17.55^{b} (-12.66%)	72.50 ± 0.10^{b} (-11.93%)
Mg	7.14 ± 0.01^{a}	10.86 ± 0.01^{e} (-52.10%)	7.54 ± 0.01^{b} (-5.60%)	8.65 ± 0.01^{c}	12.19 ± 0.01^{f} (-40.12%)	10.22 ± 0.00^{d} (-18.15%)
Fe	1.11 ± 0.03^{c}	1.21 ± 0.01^{d} (-9.01%)	1.21 ± 0.01^{d} (-9.01%)	0.50 ± 0.01^{a}	1.09 ± 0.02^{c} (-118.0%)	1.35 ± 0.01^{e} (-170.0)
Mn	0.58 ± 0.00^{c}	0.40 ± 0.01^{a} (31.03%)	0.64 ± 0.00^{d} (-10.34%)	1.20 ± 0.00^{f}	0.57 ± 0.00^{b} (52.50%)	0.91 ± 0.00^{e} (24.17%)
Zn	0.61 ± 0.00^{c}	$0.8\ 0 \pm 0.00^{e}$ (-31.15%)	0.38 ± 0.00^{a} (37.70%)	0.49 ± 0.00	0.71 ± 0.00^{d} (-44.90%)	1.13 ± 0.00^{d} (-130.61)
P	44.62 ± 0.00^{d}	30.87 ± 0.06^{a} (30.82%)	55.54 ± 1.14^{e} (-24.47%)	38.20 ± 0.00^{c}	44.70 ± 0.01^{d} (-17.02%)	36.19 ± 0.03^{b} (5.26%)

Note: Values with different superscripts in each row are significantly different (P<0.05)
Figures in parenthesis are percentage difference in nutrient values after storage.

± 0.01% in the ADF frog samples -1.20 ± 0.00% from IKU frog samples. There were significant differences (P>0.05) in the Mn contents of frog samples (Table 2). However, the Mn ions had a decreased value in all the preserved frogs except ADN samples that showed an increase percentage of -10.34%. The highest percentage difference of 52.50% was present in IKF frog samples and the lowest percentage difference of 10.34% was from ADN frog samples (Table 2).

The Zinc (Zn) contents showed that ADF frog samples had the highest value of 0.80 ± 0.00% and the least value of 0.38 ± 0.00% was gotten from ADN frog samples respectively. The elemental Zn varied significantly (P>0.05 within the frogs sampled. There were increases in the Zn ions in all the preserved frogs except ADN frog samples. The highest percentage difference of -130.61% was recorded from IKN frog samples and the least percentage difference of 22.79% was observed in ADF frog samples respectively. The Phosphorus (P) ion from ADN frog samples was the highest with 55.54 ± 1.14% and the least 30.87 ± 0.06% was present in ADF frog samples. The elemental P ions were significantly different (P>0.05) among the samples (Table 2).

The bacterial counts for both Ado-Ekiti (FAE) frog and Ikare-Akoko (FIA) frog samples showed the highest bacteria counts (4.15×10^6; 9.5×10^6), while the lowest bacteria counts (3.7×10^6; 2.1×10^6) respectively. The mean bacteria counts ranged from 3.9×10^6-5.8×10^6. The fungal counts revealed the highest counts of 7.7×10^4 and 3.4×10^6, while the lowest fungal counts of 2.4×10^6 and 3.0×10^6 respectively for FAE and FIA frog samples. The mean fungal counts ranged from 3.2×10^4-5.0×10^4 (Table 3).

Table 3. *Bacteria and Fungi count (cfu/spu)/(cfu/ml) of frogs from Ado-Ekiti and Ikare markets.*

Bacteria counts			Fungi counts	
S/N	FAE	FIA	FAE	FIA
1	3.7×10^6	9.5×10^6	7.7×10^4	3.0×10^4
2	4.2×10^6	2.1×10^6	2.4×10^4	3.4×10^4
Total	7.8×10^6	1.6×10^7	1.1×10^5	6.4×10^4
Mean	3.9×10^6	5.8×10^6	5.0×10^4	3.2×10^4

Eight bacteria strains were isolated from both Ado-Ekiti (FAE) and Ikaria-Akoko (FIA) frog samples. Five bacteria isolates were gotten from FAE which includes *Bacillus cereus* and *Listeria monocytogenes* that had 28.60% respectively as the highest while *Bacillus subtitles*, *Listeria grayi* and *Kurthia gibsonia* had 14.20% respectively. Five bacteria isolates were gotten from FIA which includes *Bacillus cereus* that had 33.30% followed by *Bacillus subtitles*, *Bacillus badius*, *Staphylococcus aurous* and *Streptococcus galactic* co-jointed recorded 16.70% respectively.

Ten fungal strains were isolated from both Ado-Ekiti (FAE) and Ikare-Akoko (FIA) frog samples. The highest fungal frequency of occurrence was from FAE samples. Four fungal isolates were gotten from FAE which includes Sporobolomyces roseus and *Fusarium slain* that had 16.67% respectively and Rhodotorula minute, and Meniscus rubber had 33.33% respectively has the highest frequency of occurrence. However, seven fungal isolates were gotten from FIA which includes *Fusarium slain, Crysosporium xerophilum pitt, Cladopsorium cladosporiodes, Pichia membranifaciens, Alter aria insectaria, Aspergillums Niger* and *Byssochlamys fulva* in which all had equal frequency of occurrence (14.29%) (Table 4). The average fungal counts indicated higher values from FAE than FIA frog samples (Table 5, Figures 1 and 2).

Table 4. *Frequency of occurrence for bacteria isolated from Ado-Ekiti (FAE) and Ikare-Akoko (FIA) samples.*

S/N	Isolates	Frequency %	
		FAE	FIA
1	*Bacillus subtilize*	14.20	16.70
2	*Bacillus cereus*	28.60	33.30
3	*Listeria grayi*	14.20	-
4	*Listeria monocytogenes*	28.60	-
5	*Kurthia gibsonia*	14.20	-
6	*Bacillus badius*	-	16.70
7	*Staphylococcus aureus*	-	16.70
8	*Streptococcus galactic*	-	16.70
	TOTAL	100	100

Table 5. *Frequency of occurrence for bacteria isolated from Ado-Ekiti (FAE) and Ikare-Akoko (FIA) samples.*

S/N	Isolates	Frequency %	
		FAE	FIA
1	*Rhodotorula minute*	33.33	-
2	*Meniscus rubber*	33.33	-
3	*Sporobolomyces roseus*	16.67	-
4	*Fusarium solani*	16.67	14.29
5	*Crysosporium pit xerophilum*	-	14.29
6	*Cladosporium cladosporiodes*	-	14.29
7	*Pichia membranifaciens*	-	14.29
8	*Alternaria infectoria*	-	14.29
9	*Aspergillus niger*	-	14.29
10	*Byssochlamys fulva*	-	14.29
	TOTAL	100	100

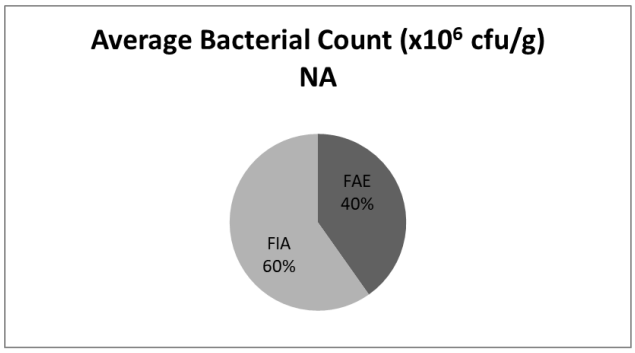

Figure 1. *Average bacterial counts for FAE and FIA frog samples.*

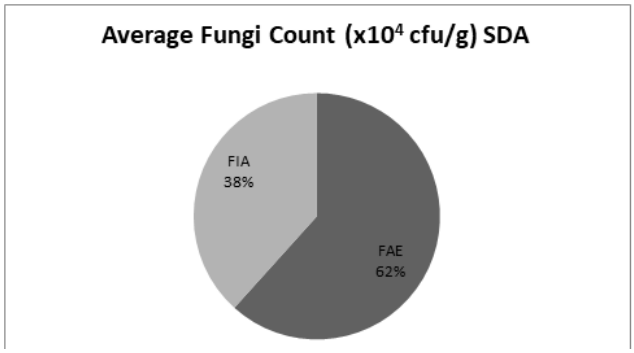

Figure 2. *Average fungi counts for FAE and FIA samples.*

Discussion

Aqua cultural products like fish and shellfishes are always subjected to heavy post-harvest lose, despite their importance in human nutrition and health Most of these products got perish before they get to the final consumers due to poor handling, preservation and processing practices adopted by the artisanal fishermen, fish farmers and fisheries entrepreneurs/vendors [21]. Fish and other aqua cultural products spoilage have been known to be influenced to a large extent by high ambient temperature, infrastructure for post-harvest processing and storage [22].

Proximate analysis refers to the determination of compounds contained in a mixture as distinguished from ultimate analysis, which is the determination of the elements contained in a compound by Wikipedia [7] or the determination of the major constituents of food and assessment of feed or animal products is within normal compositional parameters or somehow been adulterated It was observed that the unpreserved or freshly bought frogs obtained from Ado-Ekiti market (ADU) had the highest protein value which was significantly higher than the unpreserved/freshly bought ones from Ikaria market (IKU) and other preserved treatments, with the lowest protein contents from IKN frog samples. The nutritive value of finfish and other aquatic organisms have been the encouraging factors for investing in their fisheries and aquaculture [23].The protein and fat contents of the frogs in this study were higher than 19.46 ± 1.02% protein and 1.06 ± 0.15% fat for the same species [9] Also, higher than 6.95% protein and 2.09% lipid reported [8] for sun-dried *R. esculenta*. The differences observed may be due to inter species variation, nutrient composition of the environment, metabolic activities and the method of analysis used [23] The observed decreases in the protein contents of the frogs after the storage periods were similar to the reports of Onuoha et al. [22] that there were a slight decrease in the mean protein (63.33 ± 4.63%-59.10 ± 0.48%) of *C. gariepinus* preserved in plastic bucket after soaking in brine for 21 days. Also the observed increase in lipid is similar to reported increase in fat content (13.59 ± 0.09-17.20 ± 1.57%) of *F*[22].

Furthermore, the crude ash (43.23%) and moisture contents (9%) recorded by Oduntan et al. [8]. were found to be higher in *R. esculenta* meat than the moisture and ash contents in smoked dried edible frog in this study, however, the results were found to be lower than the findings of [3]. The low moisture content recorded indicated that the smoked frogs were sufficiently dried before selling and that could explain this difference. Furthermore, the slight increase in moisture of the preserved samples may be due to the absorption of moisture from the environment. Furthermore, the decrease in the moisture may be similar to the decreased moisture (11.80 ± 1.46%-8.18%) of C.gariepinus that was packaged in plastic buckets, after being soaked in brine for three hours and stored for 21 days [22].

Also the lower ash may be due to the protective advantages of the packaging media used in this study. If the ash contents were to be high, it showed that the frogs' samples were being exposed to dirt, during drying on the ground/displaying in the open market. This is similar to the observation of Torres et al. [24]. That reported that the ash content at the end of storage differs significantly to that of onset. This is also similar to the slight increase in the ash contents (10.62 ± 0.06%-15.03 ± 0.71) of *Clarias gariepinus* that was packaged in plastic buckets, after being soaked in brine for three hours and stored for 21 days [22].

Minerals are substances needed in the body for neural conduction, muscle contraction and relaxation plays an important role in the synthesis of amino acids and proteins and other specific biochemical roles in maintaining body functions [25]. Frogs obtained in Ado-Ekiti market had highest values for these mineral elements from unpreserved and preserved samples: calcium, sodium, potassium and phosphorous while other minerals Mg, Fe, Mn and Zn recorded the highest values for frog samples from Ikare market.

The mineral compositions of frogs in these studies were higher than those reported by Adeniyi, et al. [26] for Clarias which had

Ca (24.53 mg), Mg (29.61 mg), Fe (85.67 mg), Zn (38.24 mg) and tilapia with Ca (17.63 mg), Mg (41.44 mg), Fe (67.75 mg), and Zn (34.21 mg). Furthermore Oduntan et al. [8] obtained in mg per 100 g/DM frog meat: 1701% Mg, 982% K, 23371% Ca and 390% Fe. The micro-minerals such as calcium, potassium and magnesium obtained in the present study were higher than those of Rana esculenta reported by Özogul et al. [27] and by Cagiltay et al. [28] but lower to varied quantities of the mineral elements of 59.0 mg Fe; 429 mg Mg; and 210% Ca in different species of frog R. galamanensis Muhammad and Ajiboye [29]. The variations observed could be attributed to the geographical positions of the sampling sites [30].

Most aquatic products in Nigeria are usually processed to prevent economic losses. These products are highly susceptible to deteriorations immediately after harvesting. Smoking as a means of preservation is the most common and preferable method by which aquatic products are handled Falaye [31] the shelf life of smoked products is extremely at the mercy of the prevailing climatic conditions of that particular region. Cold smoked fish remained in good shape within a period of 1-3 days after smoking and 1-2 weeks in refrigerator and months in freezers [32]. The results showed progressive deteriorations of the protein contents of the frogs as the duration of the storage increases. This may be attributed to the fact that spoilage of fresh fish may be triggered by the actions of enzymes and bacteria that might be acquired exogenously and endogenously through handling processes from the source. This action is in line of thought of Salan et al. [33] that observed the deteriorations of fish by the actions of enzymes and bacterial can be slowed down when additives like salt/brine are added. In this work there was no addition of any additive to the preserved frogs or probably the quantity of any additive added from the source might be very low. This is in line with the report of Onuoha et al., that reported that 20% salt added to *Clarias gariepinus* before storage for 21 day slowed down the process of deteriorations.

The potential for the contamination of roadside foods like smoked dried-frogs with pathogenic micro-organisms have been well documented and several disease outbreaks have been traced to consumption of contaminated street foods by Sharma and Mazumdar [34].

Therefore, the presence of eight species of gram-positive bacteria and ten fungal strains that were isolated from FAE and FIA samples is inconformity with the various submission of different researchers that believe that frog can come in contact with microorganism such as bacteria and fungi which may be present in the soil, water body, in the arthropods that they eat by Rebollar et al. [35], and whenever they are stressed, leading to the build-up of pathogens and eventually causing infections. Furthermore, Microorganisms' are ubiquitous and our foods including smoked–dried frogs are not exempted. Food items could easily be contaminated with microorganisms in the environment, during handling and processing [33,36] and inadequate storage or preservation or serves as a medium for the growth of microorganisms after contamination [22]. This means these frogs must have been contaminated from the source, there have been several reports of unhygienic environmental conditions of the markets in Nigeria coupled with the fact that there are other factors that can affect the presence and growth of microorganisms on the frog meat such as lack of proper smoking on the side of the meat handlers or vendor and improper hygiene and handling processes adopted by the smoked frog meat sellers, which is in agreement with the findings of Adewole et al. [16,37]. Furthermore, [38] reported that bacterial organisms were the cause of contamination in a related study in suburb of Accra, Ghana and some of the organism was also present in this study. These authors concluded that unhygienic practices and poor handling by the sellers of the frog meat were the major cause of contamination. Therefore, contamination of the frog meats can be [16,22,39] from both intrinsic properties (i.e. physical properties of the frog meat and its extrinsic properties (i.ie environmental factors).

F which is a pathogenic micro-organism as detected in this study was also isolated by Sharma and Chattopadhyay [34] in the assessment of microbial load of raw meat samples sold in open markets of the city of Kolkata having a definite implication from the Public health point of view. The total bacterial count in this present study were higher than 2.1×10^6 to 9.5×10^6 cfu/g compared to the total bacterial count 1.3×10^5 to 7.9×10^7 cfu/g in the study of the microbial quality of pork and poultry meat with or without grill marinade according to Szosland-Fałtyn et al. [40]. The recommended microbiological limit for smoked-dried frog is 5×10^5 per gram for bacteria counts by ICMSF [41]. The average total bacteria counts on the frog samples of the two markets (FAE and FIA) showed that the frogs had above the acceptable limit for bacteria, making it unsafe for consumption. Contrarily, the reported bacterial load range in this study is however, described as tolerable [42] who stated that, in the standard microbial load specification in animal product, the total viable microbial counts of less than half a million is satisfactory, half a million to less than ten million and more is unsatisfactory. Furthermore, the values of microbial counts reported here were more than those observed [43]. In a similar study. Also there were slight differences in the microbial counts of the frogs from the two markets (Ado-Ekiti and Ikare-Akoko) respectively. The differences might be due to disparity in the processing methods (with or without the use of additives), sanitation of the processing area, handling as well as the personal hygiene of the sellers [16,22,40]. Although, smoking as a means of preservation increases the shelf life of the frog meat thereby reducing spoilage and help to inhibiting the activities of microorganism, however, when not properly carried out, microbial growth activities still continues, leading to the deterioration of the frog meat. Therefore, the processing line should be carefully monitored with the appropriate quality control system such as the principle of Good Manufacturing Practice (GMP), Total Quality Management (TQM) and Hazard Analysis and Critical Control Points (HACCP).

The FAE and FIA samples cultured on MacConkey agar showed no growth. The result showed that FAE and FIA samples cultured on EMB has microbial growth and was also confirmed to be Gram positive. Generally, higher counts were obtained with nutrient agar. This is because Nutrient Agar is a general purpose agar which allows the growth of various physiological groups present.

In this present study, a total of 10 fungi strains were identified from both FAE and FIA samples of smoked-dried frog samples. These fungal isolates can be regarded as both field and storage

fungi. The field fungi isolated are *Fusarium oxysporum, Fusarium solani, Cladopsorium cladosporiodes* and *Crysosporium xerophilum* while the storage ones were *Aspergillums niger, Rhodotorula minute, Meniscus rubber, Sporobolomyces roseus, Pichia membranifaciens, Byssochlamys fulva* and *Alter aria insectaria* respectively. The environmental requirements for the growth of the field fungi are different from the storage ones. The field fungi are destroyed during processing for storage, while the populations of storage fungi increase [44]. The presence of these fungal isolates in the frog samples from the two areas were in agreement with the findings of Kana et al. [45] who isolated similar fungi species from food and poultry feed mixtures in Cameroon. These authors further stated that the fungi species that colonize the smoked products must have been present in the atmosphere in the form of spores during the processing or gained access during storage period as a result of inadequate storage facilities as well as in the market and also during transportation. Majority of these smoked dried frog samples are kept close to agricultural commodities, which are more susceptible to fungal contamination. They have been reported to be stored in poorly ventilated and generally dirt environment, where houseflies contaminate them with dirt from the surrounding environment. Smoked fish and other aquatic products are prone to microbial attack especially due to unhygienic methods during the smoke drying periods, which in turns encourages fungal attack [16,21].

Fusarium solani is one the most prominent fungi isolated from the market samples, is a field fungal with high occurrence in food and they produce microbial contamination in foods. *Fusarium salami* and *Aspergillums Niger* isolated in the present study were also detected, in the Mycoflora and mycotoxin contamination of smoke-dried frog (Aubria sp.) (Konko) sold in Ibadan. Furthermore, this is also similar to the observations of Kana et al. [45,46] Gautama et al. [47] Sekar et al. [48] that revealed that *Fusarium spp*. were screened from food and poultry feeds, dried meats, fruits and grains respectively.

The frog samples from Ikare-Akoko had the presence of *Aspergillums flavus* and A. alternaria Several *Aspergilus* species often contaminate food such *Aspergillums flavus*, *A. Niger,* and *A. versicolor* [49,50] due to their worldwide distribution and occurrence on a great variety of substrates, thus revealing them as the most common species of *Aspergillus* that are responsible for post-harvest decay. Suleiman et al. [51] pointed out that microbial contamination of Kejeik dried fish in Sudan is caused by *A. Niger* and other species of microbes. *A. Niger* can produce 0.01-2.960 µg/kg aflatoxin G1 in Tuticorin fish products [52].

Oladejo and Adebayo-Tayo [53] studied the moulds of Banda ("kundi"/"tinko") sold in Ibadan. The fungal isolates found in samples were *Aspergillus Niger, A. flavus, A. fumigatus, A. candidus and A. piperis* among which *A. Niger* had the highest frequency of occurrence. In this study, the total fungi count ranged from 2.4×10^4-7.9×10^4 cfu/g compared to the total fungi count 1.0×10^3-8.0×10^3 cfu/g in the study of Mycoflora and Mycotoxin contamination of Smoke-dried frog (*Aubria sp.*) (*Konko*) sold in Ibadan according to Oladejo and Adebayo-Tayo [3]. The level of fungal growth in the analysed frog samples exceeded the acceptable microbial counts (10^2/g for moulds), based on microbial recommendation of the by Food and Agriculture Organization FAO [54]. Smoked-dried frogs are prepared under unhygienic conditions and displayed openly to a high degree of contamination [55]. These street foods could be main vehicles for the transmission of severe food borne infections and fatal disease that could be life-threatening [56].

Conclusion

This study revealed that the smoked-dried edible frog has high protein content and contains acceptable levels of nutrients and minerals. Therefore, it can be used as a good source of crude protein as well as minerals. This study also revealed that the smoke-dried frogs were contaminated with micro-organisms such as bacteria and fungi that are pathogenic in nature, although in small amount, but prolonged consumption may lead to the occurrence of severe public health hazards. It also reveals that the populace in the studied areas would have been taking these products in partially unhealthy states; this is due to the fact that most of these consumers were illiterate and low income earners that consume the frog without further processing at the point of purchase. The two storage media did not affect the nutritive value exponentially, but better method of preservation with the use of additives for the smoked dried frog will reduce the presence and proliferation of these microorganisms or eliminate them. Furthermore, more health campaigns on the need to embrace improved personal hygiene, proper handling, processing and storage among the vendors and consumers and also, improve the hygienic condition of the frog meat to be sold in the area should be adopted.

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