Microbial, Proximate and Heavy Metals Composition of Smoked Frog (*Rana esculenta*) From Selected Markets in Ilorin, Nigeria

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Abstract: The high cost of dietary protein from conventional sources such as cattle, fish and poultry has necessitated the demand for smoked frog as an alternative sources of protein by the common man. Microbial quality, proximate and heavy metal compositions of smoked frog samples from Ilorin markets (Ganmo, Idiape, Oja-Oba, Pata and Mandate) in Nigeria were studied using standard laboratory methods. The bacteria isolates were *Staphylococcus aureus, Escherichia coli* and S*almonella typhi*. The fungi isolates were *Fusarium oxysporum*, Aspergillus *flavus*, *Rhizopus* sp, *Aspergillus niger, Penicillium* sp and *Saccharomyces cerevisiae.* The total heterotrophic count of the samples ranged from $1.1\pm0.152x10^4$ cfu/g – $4.8\pm0.057x10^4$ cfu/g. The coliforms levels were generally high, ranging from $1.0\pm0.321x10^3$ cfu/g - $7.0\pm0.577x10^3$ cfu/g in Idiape [E] and Pata [B] respectively. The *Salmonella* counts ranged from $1.0\pm0.577x10^2$ cfu/g - $4.0\pm0.500x10^2$ cfu/g in Idiape [E] and Pata [B] respectively. The total *Staphylococci* count ranged from $2.0\pm0.259x10^2$cfu/g - 4.0±0.500x102 cfu/g in Mandete [D] and Ganmo [C] respectively. The fungi count ranged from $1.0\pm0.152x10^3$ cfu/g - $8.0\pm1.040x10^3$ cfu/g. The total *Escherichia coli* ranged from 2.0±0.500 - $6.0\pm0.288x10^2$cfu/g. The total microbial counts obtained from this work were found to be lesser than the specified limits [$1x10^5$ cfu/g] for bacteria but higher for fungi and for coliforms [$1x10^2$ cfu/g]. The crude protein content of the samples ranged from 8.2±0.378% -14.5±0.264% in Idiape [E] and Gamon [C] respectively. The crude fat, ash, carbohydrate and moisture content ranged from 50.50±0.520%-62.15±0.057%, 11.1±0.202% - 15.2±0.152%, 7.4±0.152% - 13.60±0.404% and 8.1±0.264% - 15.3±0.152%, respectively. The concentrations of Zn, Fe and Cu in the samples were 0.17±0.035mg/l - 0.22±0.115mg/L, 2.1±0.453mg/L - 2.3±0.230mg/L, 0.01±0.005mg/L - 0.03±0.002mg/L respectively while Pb and Cd were not detected. These heavy metals concentrations were found to be within the standard limits prescribed by WHO/SON. Smoked frog from Ilorin markets was found to be nutritionally rich but harbor pathogenic microorganism which can pose serious health hazard to consumers. Hence, they are not totally safe for human consumption.
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1. Introduction

Animal protein in developing countries has over the years been in shortage supply, and has been attributed to inadequate products and high cost of conventional sources of animal protein - poultry, goat meat, beef, mutton and pork. This has led to the sourcing of alternatives to provide a solution to the problem of protein deficiency in this regard, frogs are consumed in some part of the world as source of protein. These amphibians are obtained in different forms such as fresh, sun dried, smoked, and smoked-dried; a process in which the frog is treated by combined smoking and drying steps to such an extent that refrigeration can be avoided. The legs of frogs are considered by many to be a delicacy and have been collected on a local scale as an essential source of animal protein (Mohneke *et al*., 2009).

The nutritional status of many people in developing countries such as Nigeria is characterized by low calorie and protein intake (Apantaku *et al*., 1998). Many of the rich, especially in urban areas of Nigeria also consider edible frog as a delicacy (Abulude, 2007) and therefore prefer it to conventional meat types. Edible frogs, therefore serves as an important source of animal protein in both rural and urban households throughout Africa. The population of many wild animal species in Africa is on the decrease while it is very difficult to come across many in the wild. However, edible frogs has a comparative advantage of abundance; as it is in great abundance and widely distributed in most West African countries especially in the swampy, rainforest, and savannah eco-zones. Abundance of frogs could be traced to the number of eggs it lays and hatched at once (Maliehe, 1993).

Frogs are amphibians in the order Anura (meaning “tail-less”, from Greek an- without; oura, tail), formerly referred to as Salientia (Latin salere (salio), “to jump”). As a result of increasing anthropogenic activities, heavy metals pollution of soil, water, and atmosphere represents a growing environmental problem affecting food quality and

human health especially cadmium and lead, as non-essential elements. Once these metals get absorbed by humans, they will accumulate inside the body throughout the life time (Manju, 2015).

The Aim of this study was to evaluate the microbial quality, proximate and heavy metals composition of smoked edible frogs.

2. Materials and Methods

Study Area

The study was carried out at Ilorin, Kwara State. Ilorin is densely populated with different types of market namely: Ganmo market, Idiape market, Mandate market, Ojaoba market, Pata market.

Sample Collection

Smoked-edible frogs samples were purchased at 5 different markets (Ganmo, Idiape, Oja-Oba, Pata and Mandate) located within Ilorin metropolis. Ten samples were collected in each market and the total samples were 50. All samples were collected in sterile polythene bags under aseptic condition tied carefully and taken to the laboratory for microbial analysis.

Microbial Analysis

The samples were homogenized using sterilized blender (Maxwell) and 10g was taken for microbiological analysis. Standard pour plates were prepared from 10-fold dilutions into nutrient agar medium for total heterotrophic bacteria counts, MacConkey agar for total Coliform counts, Salmonella/Shigella agar for total Salmonella/Shigella counts, Mannitol salt agar for Staphylococcal counts and Potato dextrose agar containing 0.1% streptomycin (for fungi isolation). The plates were prepared in duplicates and incubated under aerobic condition at 37°C for 24 hours, with the exception of Potato dextrose agar plates which was incubated at 25ºC for 3-5 day. The number of colonies in each plate were counted using the Quebec colony counter (Reichert, USA) and expressed as colony forming unit per gram of sample homogenate (cfu/g) (Clarence *et al*., 2009).

Fungal isolates were identified based on their morphological and cultural characteristics as recommended by Sampson *et al*. (1984).

Proximate and mineral analysis

Proximate composition was determined according to the method of AOAC (1998). This includes determination of Ash content, crude protein, carbohydrate, moisture content and crude fiber.

Metal concentration analysis

The edible portions of the meat from the samples were removed; homogenized and about 2.5 g was taken for analysis. Ten milliliters of nitric acid - perchloric acid (10:4) mixture was added to the sample, covered and left overnight at room temperature. The samples were digested, allowed to cool to room temperature, filtered (glass wool) and made up to 50mL. The filtered samples were analyzed in triplicate, using Buck 2000 Atomic Absorption Spectrophotometer (AAS) as per standard conditions. The blanks and calibration standard solution were also analyzed in the same way as the samples. All the chemical analysis was carried out in triplicate.

Statistical analysis

The results were expressed as mean ± SD of five samples from each group and the data were evaluated by one-way ANOVA. p values < 0.05 were considered statistically significant.

3. Results

The microbial load in the smoked frog obtained from different markets in Ilorin metropolis are shown in Table 1. The total heterotrophic count varied from from $1.1x10^4$ - $4.8x10^4$ cfu/g for the smoked frog in which the highest growth was recorded from Ganmo market (C). Coliform density ranged from $1.0x10^2$-$7.0x10^3$ cfu/g in which the highest was recorded from Ipata market (B). The *Salmonella/Shigella* counts varied from 1.0 -4.0 x 10^2 cfu/g in which the highest count was recorded from Pata market (B). The total *Staphylococci* count ranged from 2.0 – 4.0 $x10^2$ cfu/g in which Ganmo market (C) had the highest. The total *Escherichia coli* ranged from $2.0±0.500x10^2$-$6.0±0.288x10^2$cfu/g. The total fungi count ranges from 1.0 x 10^3 – 8.0 x 10^3cfu/g in which Pata Market (B) had the highest.

A total of nine (9) microorganisms made up of three (3) bacterial species and six (6) fungal species were isolated from the samples. The bacteria were identified to be *Staphylococcus aureus, Escherichia coli* and S*almonella typhi*. The fungi isolates were *Fusarium oxysporum*, Aspergillus *flavus*, *Rhizopus* sp, *Aspergillus niger, Penicillium* sp and *Saccharomyces cerevisiae.* The occurrence of the microorganism isolated in the frog samples are as shown in Table 2. *Escherichia coli and Staphylococcus aureus* had the highest frequency of occurrence (100%) for bacteria while *Aspergillus niger and Penicillium* sp had the highest occurrence (80%) for the fungi.

The percentage (%) proximate composition of frog samples from various markets in Ilorin was shown in Table 3. Moisture content varies from 8.10 – 15.3. The crude protein ranged from 7.10 – 14.5% in which the highest was obtained from Ganmo (C). There is significant different across all the markets in Ilorin except Pata (B) and Oja Oba (A). Ash content ranged from 11.10- 15.20% while Carbohydrate content ranged from 4.05- 13.6%. There was no crude fiber content recorded in the smoked frogs' samples, the Crude fat in the smoked frogs' samples ranged from 50.50 – 62.15% in which the highest was recorded in Oja Oba (A), the crude ash ranged from

11.10 – 15.20%. The highest was recorded in Oja Oba (A) while the lowest was recorded in Gpanmo (C).

Table 4 shows the heavy metals detected in the samples [mg/L], there is no significance difference across all the samples at significant level of 0.05. The iron content of the smoked frog samples ranged from 2.10-2.30 mg/L, the highest was recorded in Mandate (D) and Oja Oba (A) market respectively. There was no cadmium and lead content in the smoked frog samples. The zinc content in the smoked frog samples ranged from 0.2-0.22 mg/L, the highest was recorded in Oja Oba (A), Copper was detected in all the samples which range from 0.01-0.03 [mg/l] the highest was recorded in Ganmo (C).

Table 1: Microbial Counts of Smoked Frog Samples from various Markets in Ilorin

Sample/Market	Total Viable Bacterial counts ($x10^4$ cfu/g)	Total *Escherichia coli* counts ($x10^2$ cfu/g)	Total *Staphylococcus* counts ($x10^2$cfu/g)	Total Coliform count ($x10^3$ cfu/g)	Total *Salmonella/Shigella* counts ($x10^2$ cfu/g)	Total Fungi counts ($x10^3$ cfu/g)
Oja Oba (A)	1.3 ± 0.10^{a}	5.0 ± 0.30^{b}	2.1 ± 0.00^{a}	1.4 ± 0.11^{ab}	3.0 ± 0.30^{bc}	3.0 ± 0.10^{ab}
Pata (B)	4.2 ± 0.30^{b}	2.0 ± 0.01^{a}	3.0 ± 0.50^{a}	7.0 ± 0.57^{d}	4.0 ± 0.50^{c}	7.0 ± 0.41^{c}
Ganmo (C)	4.8 ± 0.05^{c}	3.0 ± 0.02^{a}	4.0 ± 0.50^{a}	2.6 ± 0.11^{b}	2.0 ± 0.10^{ab}	4.0 ± 0.01^{b}
Mandate (D)	4.4 ± 0.41^{bc}	6.0 ± 0.21^{b}	2.0 ± 0.25^{a}	5.0 ± 0.20^{c}	3.0 ± 0.20^{bc}	1.0 ± 0.20^{a}
Idiape (E)	1.1 ± 0.15^{a}	2.0 ± 0.11^{a}	3.0 ± 0.10^{a}	1.0 ± 0.32^{a}	1.0 ± 0.57^{a}	8.0 ± 0.00^{c}

The values with the same alphabet are significantly the same at $p_{value} = 0.05$

Table 2: Occurrence of Microbial Contaminants in Smoked Frog Samples from various Markets in Ilorin

Isolates	Markets				
	Oja Oba (A)	Pata (B)	Ganmo (C)	Mandate (D)	Idiape (E)
Bacterial					
Staphylococcus aureus	+	+	+	+	+
Escherichia coli	+	+	+	+	+
Salmonella typhi.	+	+	_	_	+
Fungal					
Fusarium oxysporum,	+	+	_	_	+
Aspergillus *flavus*	_	_	+	_	+
Rhizopus sp	+	_	_	+	_
Aspergillus niger	+	+	_	+	+
Penicillium sp	+	+	+	+	_
Saccharomyces cerevisiae	+	+	_	_	+

Key: + = Present, - = Absent

Table 3: Proximate Composition (%) of Frog Samples from various Markets in Ilorin

Sample/Market	Moisture content	Crude Protein	Crude Fat	Ash Fiber	Available Carbohydrate
Oja Oba (A)	8.10 ± 0.12^{a}	8.2 ± 0.10^{b}	62.15 ± 0.10^{d}	15.20 ± 0.00^{c}	7.4 ± 0.10^{b}
Pata (B)	15.3 ± 0.10^{c}	7.1 ± 0.15^{a}	61.02 ± 0.15^{d}	13.05 ± 0.00^{b}	4.05 ± 0.00^{a}
Ganmo (C)	12.5 ± 0.11^{b}	14.5 ± 0.45^{e}	50.50 ± 0.55^{a}	11.10 ± 0.00^{a}	12.5 ± 0.25^{d}
Mandate (D)	9.2 ± 0.15^{a}	12.2 ± 0.55^{d}	54.05 ± 0.45^{b}	12.00 ± 0.10^{ab}	13.6 ± 0.12^{e}
Idiape (E)	12.5 ± 0.10^{b}	10.0 ± 0.11^{c}	56.20 ± 0.10^{c}	13.30 ± 0.00^{b}	9.2 ± 0.15^{c}

The values with the same alphabet are significantly the same at $p_{value} = 0.05$

Table 4: Heavy Metals Detection in Samples [mg/L]

Sample/Market	Copper	Zinc	Lead	Iron	Cadmium
Oja Oba (A)	0.01 ± 0.00^{a}	0.22 ± 0.15^{a}	0.00	2.30 ± 0.10^{a}	0.00
Pata (B)	0.01 ± 0.00^{a}	0.19 ± 0.00^{a}	0.00	2.10 ± 0.05^{a}	0.00
Ganmo (C)	0.03 ± 0.01^{a}	0.20 ± 0.00^{a}	0.00	2.30 ± 0.25^{a}	0.00
Mandate (D)	0.01 ± 0.00^{a}	0.17 ± 0.10^{a}	0.00	2.20 ± 0.00^{a}	0.00
Idiape (E)	0.02 ± 0.05^{a}	0.21 ± 0.00^{a}	0.00	2.10 ± 0.25^{a}	0.00

The values with the same alphabet are significantly the same at $p_{value} = 0.05$

4. Discussion

The high bacteria, coliform and fungi counts obtained may be due to poor hygienic standard of preparation and handling. All the organisms isolated from the smoked frog samples have health implications on man. The presence of *E. coli* in the samples is an indication of secondary contamination as *E. coli* are known to be associated with gastrointestinal tracts of warm-blooded animals and are known to be present in the environment as a natural flora (Prescott *et al*., 2005). This secondary contamination may be as a result of sewage contamination of river or ponds from where the frogs are harvested. *E. coli* is the causative agent of diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infection, septicemia, pneumonia and meningitis (Nestal *et al.,* 2001). The presence of *Salmonella* species one of the most important food-borne pathogens is an indication of sewage contamination and it is found to be associated with number of non-human hosts for example, reptiles (Winfield and Groisman 2003). It has been reported to survive and persist in the aquatic environment. Nestal *et al. (*2001) reported that *Shigella* sp. and *Salmonella* sp. are causative agents of illnesses such as shigellosis and salmonellosis in humans who are the only reservoir of these organisms. Evidence shows that smoked frog can be a reservoir of extra-intestinal infections caused by epidemic strains of *E. coli* causing uncomplicated urinary tract infections and other severe infections (Prescott *et al*., 2005). The presence of *Staphylococcus aureus* in smoked edible frogs is indicative of human contact such as constant touch by both the buyers and the sellers or indirectly through the addition of additives or utensils as reported by Madueke *et al.* (2014).

Fungi are common environmental contaminants due to their ability to produce spores; this could explain their presence in frog samples. Species of *Aspergillus, Penicillum* and *Fusarium* are known to produce deleterious mycotoxins under favorable conditions their presence in the smoked frog samples must therefore be treated with caution. *A. niger*, *A* and *flavus* have also been implicated in causing mycetoma in human (Cheesbrough, 2006). *Aspergillus flavus* is involved in allergic aspergillosis (pulmonary aspergillosis) and also produces aflatoxin that is highly carcinogenic (Prescott *et al*., 2005).

The total microbial counts obtained in this work were found to be lesser than the specified standard limits ($1x10^5$ cfu/g) for bacteria, while higher for fungi and for coliforms [$1x10^2$ cfu/g] by ICMSF (1982) and USFDA (1991). This high level of microbial loads could be as a result of human activities done in the rivers where the frogs are harvested. Lack of storage facilities could have heightened the chances of contamination. The frog samples were maintained throughout the day under room temperature, this encourages proliferation of contaminants.

There was no cadmium and lead in the smoked frog samples. Adults and growing children needs zinc in recommended daily allowance of 15 mg/day and 10 mg/day respectively. It is an essential element for both animals and humans. It has a protective effect against the toxication of both cadmium and lead (Calabrese *et al*. 1985). A deficiency of zinc is marked by retarded growth, loss of taste and hypogonadism, leading to decreased fertility. The maximum permissible limit by WHO is (4.0 mg/L). Copper is an essential part of several enzymes and necessary for the synthesis of haemoglobin but at high level it has been shown to cause stomach and intestinal distress, liver, kidney damage and anemia. The maximum permissible limit by WHO is 2.0 mg/L. Copper was detected in all the samples at a level below the permissible WHO standard. Iron is essential trace element, it play a major role in the metabolic processes that take place in human system and regulation of blood. Iron is a major component of the hemoglobin found in human blood. The level of iron detected in smoked frog samples was higher than maximum permissible limit by WHO (0.3mg/L).

The moisture content was found to be higher in the Pata samples which may be as a result of inadequate dryness of it compared to Oja Oba samples. The moisture content indicates that the smoked frog is sufficiently dried to minimize microbial growth. However microbial load may be due to moisture absorption from the environment which increases the growth of molds on the surface. The proximate composition of the smoked frog samples indicate that frog is a good diet containing protein, fat, ash, carbohydrate in varying percentages. Consumption of smoked frog can promote the defense mechanism for protection against invasion of human pathogens because smoked frog has antimicrobial peptide (Louise *et al.,* 2005). Ingesting smoked frog can reduce the risk of heart diseases and lower the risk of developing dementia, including Alzheimer's diseases. Breastfed babies of mothers who eat smoked frogs have better eyesight perhaps due to the omega-3-fatty acid transmitted in breast milk. Smoked frog oil may be useful in treating dyslipidemia in diabetes. Eating smoked frog during pregnancy may help to reduce the risk of delivery of a premature baby. Proximate composition is important in ensuring the requirements of food regulations and commercial specifications (Waterman, 2000). The smoked frogs, in addition to being source of protein, have appealing flavour and texture acquired on smoking.

Conclusions

This study reveals that smoked edible frog, which man has substantially exploited for food in developing countries can serve as a competent source of animal protein and other vital nutrients in human diets. The result of heavy metals detected shows that copper, zinc and iron were within the acceptable limits recommended by WHO. However, it is recommended that a more close supervision of smoked edible frogs should be carried out by relevant authorities because of the presence of pathogenic organisms. There is also need to create awareness in developing countries about nutritional status of *Rana esculenta*, so as to serve as an economical source of animal protein, since it is widespread, abundant, cheap, and readily available in most developing countries.

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Reference

1. Abulude FO. Determination of the chemical composition of bush meats found in Nigeria *America Journal of Food Technology* 2007; 2: 153-160.
2. AOAC. Methods of analysis. 3rd ed., Association of Official Analytical Chemists (AOAC), Washinghton, DC, USA, 1998.
3. Apantaku SO, Apantaku FS, Oyesola OB. Rural poultry farmers' use of electronic mass media and level of productivity in Egbeda, Nigeria. Proceedings of the Silver Anniversary Conference of the NSAP *(Nigerian Society for Animal Production) and the Inaugural Conference of the WASAP (West African Society for Animal Production),* Abeokuta, Nigeria, 1998; 526-527.
4. Clarence SY, Nwinyi CO, Chinedu NS. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research* 2009; 3(6): 390-395.
5. Calabrese EJ, Canada AT, Sacco C. Trace elements and public health. Annual *Review of Public Health* 1985; 6: 131-46.
6. International Commission of Microbiological Specification for Food (ICMSF). Microorganisms in foods. Vol. 1: focus on West Africa. *Salamandra* 2009; 45:193– 203
7. Their significance and methods of Chadrick J. O'Learyenumeration. 2nd ed., F.S. Thatcher and D.S. Clark (eds.). University of Toronto Press, Toronto, ON, Canada, 1982; 19-30.
8. Louise A. R-S, Laura KR, Chadrick J. O'L, Laura EH, Douglas C. Antimicrobial Peptide Defenses in Amphibian Skin. *Integrative and Comparative Biology* 2005; Volume 45, Issue 1, 137–142, https://doi.org/10.1093/icb/45.1.137.
9. Douglas C. Woodhams Madueke SN, Awe S, Jonah AI Microbiological Analysis of Street Foods along Lokoja- Abuja Express Way, Lokoja. *American Journal of Research Communication* 2014; *2 (1) 196 -211.*
10. Maliehe TM. *Non Wood forest products in South Africa*. Paper prepared for the Regional Expert Consultation Non Wood forest products for English – Speaking African Countries, Arusha, Tanzania, and common wealth science council 1993; 52.
11. Manju M. Effects of Heavy Metals on Human Health *International Journal of Research* 2015; 1-7.
12. Mohneke M, Onadeko AB, Rodel MO. Exploitation of frogs a review with a.
13. Nester EW, Roberts CE, Nester MT. Microbiology: A human perspective. William C. Brown Publishers, Dubuque, IA, USA, 2005.
14. Prescott LM, Harley JP, Klein DA. Microbiology. 6th ed., McGraw-Hill Companies, Inc., New York, NY, USA, 2005.
15. Sampson RA, Hoekstra ES, VanOorschot CAN. Introduction to food-borne fungi. Centrealbureau Voor Schimmelcultures, Baarn, The Netherlands, 1984; 105-7.
16. United States Food and Drug Administration (USFDA). Sanitation of shellfish growing areas. Seafood safety. F.E. Ahmed (ed.). National Academic Press, Washington, DC, USA, 1991.
17. Waterman, JJ. Composition and quality of fish. *Torry Research Station* 2000; Edinburgh.
18. Winfield MD, Groisman EA. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* 69(7): 3,687-94

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