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The Effects of Smoke-drying on the Nutritional Quality and Microbial Load of Apple Watersnail (*Lanistes libycus*) in Ikpoba River, Edo-State

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Authors' contributions

This work was carried out in collaboration between both authors. Author KO designed the study and wrote the draft manuscript. Author GWE performed the laboratory experiments and statistical analysis under the supervision of author KO. Both authors read and approved the final manuscript.

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ABSTRACT

The study was aimed at determining the effects of smoke-drying on the nutritional quality and microbial load of Apple Watersnail (*Lanistes libycus*) from Ikpoba River, Edo State. Thirty two (32) *Lanistis libycus* specimens were bought from a fish seller and taken to Faculty of Agriculture Laboratory where the microbial loads of the Apple Watersnail were analysed using Potato Dextrose Agar and Nutrient Agar for fungi and bacteria, respectively. A total of 25 isolates were obtained and identified as bacteria and fungi. The number of bacteria counts (9.3 X 10^3 cfu/gm) and fungi counts (18.0 X 10^3 cfu/gm) in sample A (fresh *Lanistes libycus*) was higher than that of sample B (smoke-dried Apple Watersnail) which were (5.0 X 10^3 cfu/gm) and (5.0 X 10^3 cfu/gm) for bacteria and fungi, respectively. The highest bacteria counts occurred in Sample C (44.3 X 10^3 cfu/gm) Apple Watersnail after two months of storage, at ambient temperature and the highest fungi counts occurred in Sample C Apple Watersnail after two months of storage (42.7 X 10^3 cfu/gm). After smoke drying, the sample was high in percentage crude protein and ash contents which suggested that smoke drying enhanced the nutritional value of the sample. The study also revealed that the Apple Watersnail from Ikpoba River was highly contaminated with microorganisms but when processed and stored at 25-29°C, the micro-organism reduced and prolonged shelf-life.

Keywords: Lanistis lybicus; bacteria load; fungi load; proximate composition.

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1. INTRODUCTION

Shellfish is a fisheries and culinary term for exoskeleton–bearing aquatic invertebrate used as food. They comprise crustaceans and molluscs and include various families, orders, genera and species of aquatic animals and a few land representatives [1].

Snails are the largest group of Molluscs constituting the largest animal group after arthropods. They have soft bodies and lack skeleton. They are also classified under the class Gastropoda because they appear to walk on their belly [2] and [1].

Fish and shellfish are one of the most important sources of animal protein and have been widely accepted as a good protein source and other elements for the maintenance of healthy body as they have great demand in national and international markets [3]. Apart from serving as a source of food, other economic importance of shellfish (*Lanistes libycus)* includes foreign exchange, income and provision of employment [4].

Shellfish are extremely perishable commodity and quality loss can occur very rapidly after catch [5], and [6]. The spoilage of any food product is attributed to microbial growth due to improper handling, inadequate processing and frozen storage. The sources of pathogenic bacteria may be from natural and unhygienic handling. Unsafe water used in processing seafood products pose a global public health threat, placing person at risk for a host of diarrhoea and other diseases [7]. The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication [8].

The evaluation of the general quality of shellfish and shellfish products is based on organoleptic, chemical and microbiological tests. The appearance, odour, colour and texture of shellfish are fundamental to shellfish quality. An estimate of freshness can be obtained by defining criteria related to changes in the sensory attributes that can be measured or quantified by sensory or instrumental methods [9].

Preservation and processing are means of prolonging the shelf life of fish products in acceptable quality through changes in texture, taste and appearance without adversely affecting the chemical nature of the products. Preservation itself does not help in the prevention but reduces spoilage to a large extent [10]. Different processing methods, including boiling and roasting, influence the proximate, mineral and toxicant composition of foods (11]. Processing methods apply the principle of temperature elevation and these include drying either through solar energy (sun drying) smoking, freeze-drying, canning, salting and boiling [12]. Smoking of fish is a major way of preserving fish in Nigeria. The smoking of fish has the objective of preservation basically due to dehydration and high temperature of smoking (50 -180°C) [13].

Processing (smoking) of shellfish (*Lanistes libycus)* is not frequently practiced as much as that of fin fishes in this part of the world. This study is therefore aimed at revealing the effects of smoke-drying on the nutritional quality and microbial load of *Lanistes libycus* in Edo State Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Sample Size

A total of thirty two (32) Apple Watersnail were collected from Ikpoba River with an average weight of 21.2 g. After collection the shell and the viscera were removed with a knife and the average weight was now 6.6 g. Then the Apple Watersnail were washed with clean water with alum to remove the slime as practiced by the local consumers. The Apple Watersnail samples were rinsed twice to remove any trace of alum.

2.2 Description of the Smoking Kiln

The Magbon-Alade-Kiln was used to smoke-dry the snails. The kiln was constructed with steel with different partitions which includes the smoking chamber, charcoal chamber and the chimney. The charcoal chamber supplied heat to the snail samples, the chimney remove excess smoke from the kiln while the smoking chamber had trays where the snails were placed during smoking.

2.3 The Smoke-drying Process

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 8 hours at a temperature of 60.8°C, a temperature which was

twice that of cold smoking (30°C) [14] and an average constant weight of 2.2 g was gotten after eight hours.

The samples were labeled as;

Sample A - Fresh Apple water snail (*Lanistes libycus*) = 52 g
Sample B - Smoke-dried Apple water snail (*Lanistes libycus*) = 53 g

2.4 Storage

After smoking, the snail sample B was allowed to cool. It was wrapped with brown cartoon paper with both end of the paper sealed to prevent air into the Apple Watersnail samples, the brown cartoon paper was used because of its capacity to absorb moisture from the atmosphere.

After two months of storage it was labeled as sample C with two percent increase in moisture content.

2.5 Isolation of Bacteria and Fungi

Nutrient Agar and PDA Agar were used for the isolation of Bacteria and Fungi respectively. Media were prepared according to Manufacturer's instructions. One gram (1 g) of each of the samples was weighed out and blended, mixed with 9 ml of sterile distilled water into a test tube. 1 ml of the aliquot was obtained from each of the samples and transferred into the test tube labelled 10-1 and mixed properly.1ml aliquot was then transferred serially from the tube (10^{-1}) to tubes labelled10^{-2} and 10^{-3}, in that order. This was done for each of the samples A (Fresh Apple water snail (*Lanistes libycus*) = 52 g), B (Smoke-dried Apple water snail (*Lanistes libycus*) = 53 g), and C (Smoke-dried Apple water snail (*Lanistes libycus* after 2 months of ambient storage) = 28 g). 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 inoculated plates were then incubated at room temperature (28±2°C) for 24 to 48 hours for the bacteria and 3-5 days for fungi.

2.6 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 [15] Manual for the Identification of Medical Bacteria.

2.7 Morphology and Cultural Characteristics of Isolates on Media

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.

2.8 Proximate Analysis

Proximate composition was determined according to the method of AOAC (1994). This includes determination of percentage Ash, Crude Protein, Moisture and Crude fat contents. The moisture content of the fresh frozen samples was determined using air oven using a known weight at 105°C for 3 hours until a constant weight was obtained. Ash content was determined by incineration of° fresh frozen samples from moisture determination in a muffle furnace at 600°C for 3 hours. Crude protein was estimated by multiplying the nitrogen content of the fresh frozen sample by 6.25. Nitrogen content was determined by Kjedah's method. The analysis was carried out by extracting 2.0 g of each sample in a soxhlet apparatus using petroleum ether at 60-80°C as the extractant.

2.9 Statistical Analysis

The statistical analysis was done at 5% probability level using Analysis of Variance Table (ANOVA). The means comparison was done using the least significant difference (LSD) at 5% probability level to compare the microbial load and proximate composition of all the snail samples.

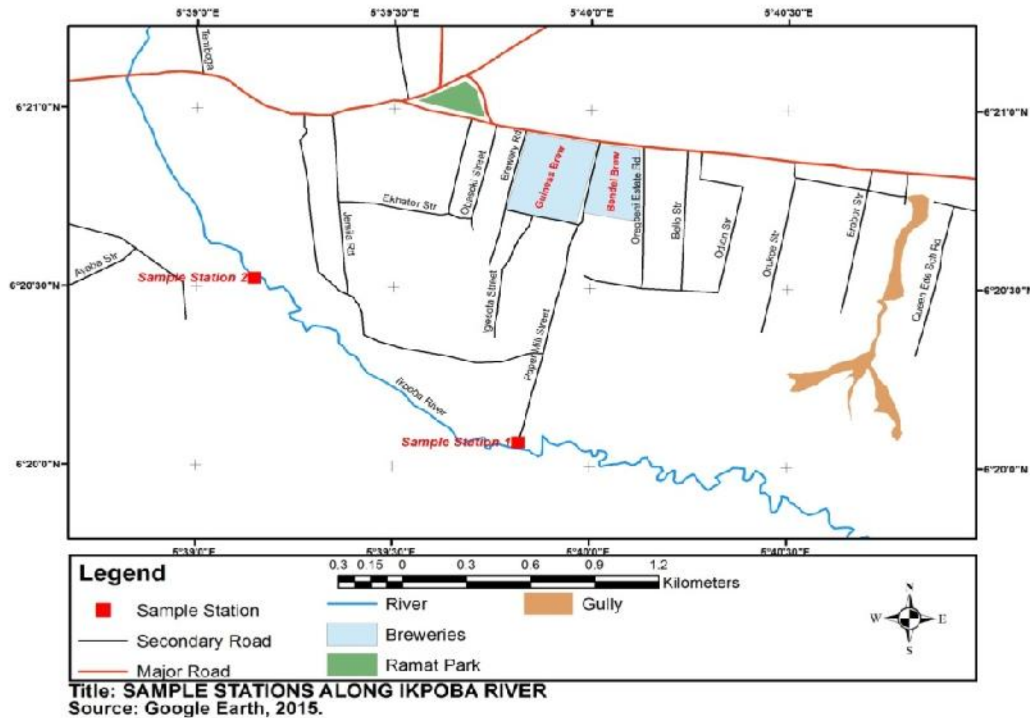

Figure 1. Map of Ikpoba River showing sampling stations

Figure 2. Apple water snail (*Lanistes libycus*) with shell

3. RESULTS

Table 1 shows the summary of weight changes during various smoking time and percentage moisture loss in the snail samples. The average weight loss for the smoke-dried Samples was 4.38 g. According to the table, the weight loss is the difference between the initial weight after Shucking (Shell + liquid wastes removed) and the final weight of each sample after drying while the percentage weight loss is the weight loss multiply by 100 and divided by initial weight after the viscera were removed and had an average weight of 6.6 g.

Tables 2 – 5 summarized the results obtained in the study of the comparative analysis of fresh and smoke-dried *Lanistes libycus* obtained from Ikpoba River. Tables 2 - 4 summarized the total heterotrophic microbial counts of all the samples which comprised of sample A - Fresh Apple water snail = 52 g, sample B - Smoke-dried Apple water snail = 25 g and sample C - Smoke-dried Apple water snail after 2 months of ambient storage) = 28 g.

Table 5 summarized the proximate results of Apple Watersnail obtained from Ikpoba River.

The bacteria counts (9.3 X 10^3) and fungi counts (18.0 X 10^3) in sample A was higher than that of sample B. The highest bacteria counts occurred in Sample C (44.3 X 10^3) and the lowest was in Sample B (5.0 X 10^3) (Table 2). The least fungi count was also obtained in Sample B (5.0 X 10^3) as seen in Table 3 and the highest fungi counts occurred in Sample C (42.7 X 10^3).

Proteus spp*, Mucus* spp*,* and *saccharomyces* spp. occurred frequently in all the snail samples. *Neurospora* spp and *Aspergillus niger* occurred

in sample A and B. while *Cladosporium* spp, and *Cryptomonas reoformis* occurred in sample A and C. The highest frequency of bacterial isolate were seen in Sample C [(6) 50%] while sample A and B had a fair share of the bacterial frequency isolates [(3) 25%]. For fungi, the highest fungi frequency was seen in Sample B [(10) 38.5%] and the lowest occurred in Sample C [(7) 26.9%] as shown in Table 4.

The percentage moisture content in Sample A (74.31%) was higher than that of Sample C (11.50%) which was also higher than that of sample B (8.50%). Sample A had the lowest value of percentage crude protein (11.42%) with sample B having the highest value (55.36%) and sample C (53.36%) just a little less than sample B. The percentage crude fat content in sample B (3.48%) was higher than that of sample C (3.34%) with sample A (0.08%) having the lowest value of percentage fat content. The percentage crude fiber for sample A, B and C were 0.04%, 0.07% and 0.06% respectively.

Table 1. Average weight changes during various smoking time (smoke-drying process) and percentage moisture loss for *Lanistes libycus*

Treatment	Initial weight after gutting (g)	Weight after 1.6 hrs (g)	Weight after 3.2 hrs (g)	Weight after 4.8 hrs (g)	Weight after 6.4 hrs (g)	Weight after 8.0 hrs (g)	Weight loss (Initial weight- Final weight) (g)	% Weight loss
Salted and Smoke-dried *Lanistes libycus*	6.6	4.6	3.3	2.4	2.2	2.2	4.38	66

Table 2. Total estimated viable heterotrophic bacteria count in colony forming units per gram of fresh and smoke-dry *Lanistis libycus* (cfu/gm)

Samples	Dilution factors	Number of colonies/ plate	Average number of colonies per dilution $x \pm s_e$	Organism per gram of snail sample
A (Fresh Apple	10^3	8		$8_x 10^3 = 8.0_x 10^3$
watersnail	10^3	16	9.3 ± 0.8[a]	$16_x 10^3 = 1.6_x 10^4$
	10^3	4		$4_x 10^3 = 4.0_x 10^3$
B (Smoke-dry	10^3	05		$5_x 10^3 = 5.0_x 10^3$
Apple watersnail	10^3	04	5.0 ± 0.5[a]	$4_x 10^3 = 4.0_x 10^3$
	10^3	06		$6_x 10^3 = 6.0_x 10^3$
C (Smoke-dry	10^3	72		$72_x 10^3 = 7.2_x 10^4$
Apple watersnail	10^3	48	44.3 ± 1.8[a]	$48_x 10^3 = 4.8_x 10^4$
after 2 months)	10^3	13		$13_x 10^3 = 1.3_x 10^4$

Table 3. Total estimated viable heterotrophic fungi count in colony forming units per gram of fresh and somke-dried Apple Watersnail (cfu/gm)

Samples	Dilution factors	Number of colonies/plate	Average number of colonies/plate	Organism per gram of snail samples
A (Fresh Apple	10^3	26		$26_x 10^3 = 2.6_x 10^4$
watersnail)	10^3	25		$25_x 10^3 = 2.5_x 10^4$
	10^3	03	18.0 ± 1.2[a]	$3.0_x 10^3 = 3.0_x 10^3$
B (Smoke-dry	10^3	03		$3_x 10^3 = 3.0_x 10^3$
Apple watersnail)	10^3	04		$4_x 10^3 = 4.0_x 10^3$
	10^3	08	5.0 ± 0.5[a]	$8_x 10^3 = 8.0_x 10^3$
C (Smoke-dry	10^3	78		$78_x 10^3 = 7.8_x 10^4$
Apple watersnail	10^3	24		$24_x 10^3 = 2.4_x 10^4$
after 2 months)	10^3	26	42.7 ± 1.8[a]	$26_x 10^3 = 2.6_x 10^4$

Table 4. Frequency of occurrence of microbial isolates of Apple Watersnail samples obtained from Ikpoba River

Isolates	#	%	A(Fresh Apple watersnail *Lanistes libycus*)	B(Smoke-dry Apple watersnail *Lanistes libycus*)	C(Smoke-dry Apple watersnail *Lanistes libycus* after 2 months)
Proteus sp	3	8.3	✓	✓	✓
Micrococcus sp	2	5.6	✓	X	✓
Serratia sp	2	5.6	X	✓	✓
Staphylococcus aureus	2	5.6	X	✓	✓
Staphylococcus epidermis	1	2.8	X	X	✓
Escherichia coli	1	2.8	✓	X	X
Bacillus sp	1	2.8	X	X	✓
Bacteria frequency	12		3	3	6
Bacteria % frequency	33.5		25.0	25.0	50.0
Bacteria diversity	6		3	3	5
Bacteria % diversity	50.0		50.0	50.0	83.3
Mycosphacrella sp	1	2.8	X	✓	X
Penicillium oxalicum	1	2.8	X	✓	X
Mucor sp	3	8.3	✓	✓	✓
Trichoderma sp	1	2.8	X	X	✓
Aspergillus nidulans	1	2.8	X	✓	X
Cladosporium sp	2	5.6	✓	X	✓
Yeast sp	1	2.8	✓	X	X
Helminthosporium sp	1	2.8	X	✓	X
Cryptomonas reoformis	2	5.6	✓	X	✓
Aspergillus niger	2	5.6	✓	✓	X
Penicillium sp	1	2.8	X	✓	X
Aspergillus sp	1	2.8	X	✓	X
Geotrichum sp	1	2.8	X	X	✓
Rhizopus sp	1	2.8	X	X	✓
Neurospora sp	2	5.6	✓	✓	X
Mucus mucido	1	2.8	✓	X	X
Botrytis sp	1	2.8	✓	X	X
Saccharomyces sp	3	8.3	✓	✓	✓
Fungi frequency	26		9	10	7
Fungi % frequency	72.6		34.6	38.5	26.9
Fungi diversity	13		7	7	7
Fungi % diversity	50.0		53.8	53.8	53.8
Microbial frequency	38		12	13	13
Microbial % frequency	106.1		31.6	34.2	34.2
Microbial diversity	19		10	10	12
Microbial % diversity	50.0		52.6	52.6	63.2

✓- Present X- Not present

4. DISCUSSION

[16], reported that bacteria are abundant in the environment in which fish live and it is therefore impossible to avoid them, being a component of their diet. In the course of this study, 14 fungi genera were isolated from the snail samples used. The isolates were identified as *Aspergillus, Penicillium, Mucor, Trichoderma, Cladosporium, yeast, Neurospora, Geotrichum, Rhizopus, Saccharomyces, Botrytis, Cryptomonas, Helminthosporium, Mycosphocrella.* A total of 6 bacteria genera were also identified from the snail samples which include; *Proteus,*

Table 5. Proximate results for *Lanistes libycus*

Samples	% Moisture content	% Dry matter	% Ash content	% Fat content	% Crude protein	%Nitrogn free extract (N.F.E)	% Crude fibre
A(Fresh *Lanistes libycus*)	74.31^a	25.69^a	4.36^c	0.08^c	11.42^c	9.79^b	0.04^a
B(Smoke-dried *Lanistes libycus*)	8.50^c	91.50^a	12.55^a	3.48^a	55.36^a	20.04^a	0.07^a
C(Smoke-dried *Lanistes libycus* after 2 months)	11.50^b	88.50^b	11.76^b	3.34^b	53.36^b	19.98^a	0.06^a

Note; Means with the same superscript on the same column are not statistically significant at (P>0.05) level of significance

Means with different superscript on the same column are statistically significant at (P>0.05) level of significance

Micrococcus, Bacillus, Staphylococcus, Serratia, and *Escherichia*. The microflora in the *Lanistes libycus* was similar to those identified by [17].

The mean counts of colonies per dilution of bacteria in Sample A (Fresh Apple Watersnail) (9.3×10^3) was higher than that of Sample B (Smoke-dried Apple Watersnail) (5.0×10^3) and the mean counts of colonies per dilution of fungi in Sample A (18.0×10^3) was also higher compared to sample B (5.0×10^3). The high level of bacteria and fungi counts in sample A may be due to the handling process during harvesting and transportation of the fresh Apple Watersnail samples. While the low bacteria and fungi mean counts of colonies per dilution of bacteria and fungi in sample B may be due to the smoke-drying process (smoke-drying to constant weight) to expel some moisture. This is in agreement with the findings of [18] who stressed that the microbial and chemical stability of fish and fish products during processing and storage is highly dependent on the water content of the product.

The mean counts of colonies per dilution of bacteria after 2 months in Sample C (44.3×10^3), was higher than Sample B (5.0×10^3). Also the mean counts of colonies per dilution of fungi in Sample C (44.7×10^3) was also higher than that of Sample B (5.0×10^3). This may be due to the long period of storage which led to increased moisture content in sample C for micro-organisms to proliferate. This was corroborated by [19] who said that smoked fish samples may have a relatively higher water activity level during storage which is a prerequisite for microbial growth. However, the total bacterial and fungi load counts recorded in sample A and C from this work exceeded the international commission on microbiological specification of 5×10^5cfu/g for food and food products [20]. Only sample B had a lower value of 5.0×10^3cfu as a result of the effectiveness of smoke-drying.

A total of 25 isolates were obtained and identified as bacteria and fungi, the bacteria isolates which includes *Proteus* spp, *Bacillus* spp, *Micrococcus* spp, *Staphylococcus aureus, S. epidermis, Echerichia coli,* and *Serratia* spp were isolated on nutrient agar. The fungi isolate includes *Aspergillus niger, Mucor* spp, *Neurospora* spp, *Penicillium* spp *Geotrichum* spp, *Rhizopus* spp, *Mucor mucedo, Saccharomyces* spp, *Aspergillus* spp, *Penicillium oxalicum, trichoderma* spp, *Aspergillus nidulans, Cladosporium* spp, *Yeast* spp, *Botrytis* spp, *Cryptomonas reoformis, Helminthosporium* spp and *Mycosphacrella* spp were isolated on potato dextrose agar (PDA) as seen in Table 4.

The most frequently isolated fungi from the entire snail samples were *Mucus* spp and *Saccharomyces* spp and both had a frequency of (3(8.3%) this was followed by *Aspergillus niger, Cryptomonas reoformis, Cladosporium* spp and *Neurospora* spp with a frequency of (2 (5.6%). Others, *Trichoderma* spp, *Rhizopus* spp, *Mucor mucedo, Geotrichum* spp, *Aspergillus* spp, *Aspergillus* spp, *Aspergillus nidulan*, *Botrytis* spp, *yeast* spp, *Helminthosporium* spp, *Penicillium oxalicium, Penicillium* spp, *and Mycosphacrella* spp. had (1(2.8%) each. For the bacteria *Bacillus* spp, *Staphylococcus epidermis*, *Echerichia coli* (1(2.8%), *Micrococcus* spp, *Serratia* spp, and *Staphylococcus aureus* (2 (2.8%) while *Proteus* spp 3(8.3%) was the most frequent bacteria isolated from this study. All these micro-organism except for sample B isolated from this study were not safe for human health if consumed unless they are properly cooked. The occurrence of

bacteria such as *Staphylococcus aureus, Proteus* spp*,* and fungi *Neurospora* spp, *Penicillium* spp and *Aspergillus niger* in the smoke-dried fish sample were in accordance with [21] when he stated that these organisms were the commonest micro-organisms associated with smoked fish and these micro-organism were also reported by [22] in smoked fish sold in Benin metropolis. [23] reported that the occurrence of *Proteus* species may be due to polluted water or run-off from contaminated water bodies to where these fishes are found. *Proteus* is an opportunistic etiological agent in the infection of the respiratory tract and wounds, burns, skin, eyes, ears, nose and throat, as well as gastroenteritis resulting from the consumption of contaminated snails and other fish products [23].

The occurrence of coliform bacteria like *Escherichia coli* in samples A, might be as a result of possible contamination of the snail habitat by Guinness brewery effluents and faecal contamination by residents in the area. This is in agreement with the report by [24] who reported that *Escherichia coli* was an indication of faecal contamination of seawater and this might have adverse effect on the health of the consumers. [25] reported that *E. coli* cause urinary tract infection, pneumonia, meningitis, diarrhoea and kidney damage due to the consumption of the contaminated seafood. The occurrence of *Bacillus* spp in sample treatment (C) can be said to be as a result of prevalence of their spore in the environment [26]. *Staphylococcus* spp which occur in all the treatments except sample (A) is as a result of contamination by snail handlers [26]. *S. aureus* causes chicken pox, Epiglottis, skin rashes and Toxic shock syndrome [27]. The occurrence of *Micrococcus* spp in fresh snail sample may be as a result of contamination during harvesting and temporary storage [28]. The occurrence of *Cladosporium, Mucor, Saccharomyce, Rhizopus, Aspergillus* spp *and penicillum* spp could be due to contamination during harvesting leading to air born transmission [29]. *Aspergillus* spp produces toxins known as aflatoxin which causes mycotoxicosis, liver cancer, cirrhosis and hepatitis [26]. *Penicillium* spp produces mycotoxins that are harmful to man and may result in renal damage/ necrosis of the kidney [30]. *Neurospora* spp produces spores that may cause asthma [30]. *Aspergillus niger*, *Rhizopus* spp and *penicillum* spp are contaminated from water, insect and contaminated hand, personal hygiene of the sellers [31].

Separation of the means using analysis of variance showed that there was no significant difference (P>0.05) in terms of microbial load in the snail samples as shown in Tables 2 and 3. This study revealed that Apple Watersnail obtained from Ikpoba River are highly contaminated with microorganism but when processed and stored properly (wrapped with brown cartoon paper), the micro-organism reduces drastically and prolong the shelf-life of the snail.

The proximate analysis results had shown that Apple Watersnail from Ikpopa River to be nutritionally rich. The fat, fiber and moisture contents are constituents in shellfish which provide an energy source to the consumers. Shellfishes have been reported to serve as a source of protein and mineral elements [3], which helps in the repair of worn-out tissue and body building.

The proximate results as shown in Table 5 agreed with other analysis carried out by earlier researchers such as [32], [33] and [34]. Separation of the means using analysis of variance showed that there was significant difference (P<0.05) in percentage moisture content. Further separation using duncan's multiple range test showed that there was significant difference (P<0.05) in sample A, B and C in percentage moisture content.

The high level of percentage moisture content observed in sample A (fresh Apple Watersnail) (74.31%) agreed with that reported by [35] who found out that fresh snail samples had high moisture content. The high moisture content observed in raw snail meat is also comparable to raw beef and other raw meat products [36]. Sample B had moisture content of 8.50%. The decrease in moisture content of sample B was as a result of smoke-drying which also led to increased fat and crude protein content [37]. The moisture content of the smoke-dried Apple Watersnail [(sample B) 8.50%] was similar to the recommended safe moisture content of smoke-dried snail (6 to 8%). Sample C had moisture content of 11.50%. The increase in percentage moisture content may be due to absorption of moisture during storage.

Separation of the means using analysis of variance showed that there was significant difference (P<0.05) in percentage crude protein content. Further separation using duncan's multiple range test also showed that there was

significant difference (P<0.05) in sample A, B and C in percentage crude protein content. The protein content of Sample B was higher than that of Sample A and this was in accordance with [38] who reported that increase in protein may be due to the dehydration of water molecules present between the proteins, thereby causing aggregation of protein and thus resulting in increased percentage protein content of the smoke-dried snail. Protein content increased as a result of the reduced moisture content in the snail sample [39] as seen in Table 5. Sample C had a crude protein of 53.36%. The decrease in the protein content in sample C during storage may be due to an increase in percentage moisture content.

Separation of the means using analysis of variance showed that there was significant difference (P<0.05) in percentage fat content. Further separation using duncan's multiple range test also showed that there was significant difference (P<0.05) in sample A, B and C in percentage fat content.

Sample A had a fat content of 0.08% the low percentage fat content in sample A compare to sample B (3.48%) and C (3.34%) may be due to the high moisture content of sample A [37]. The percentage fat content of sample B (3.48) was within the range previously detected for snail (3.18 to 4.25%) by [17]. The percentage fat content of sample B (3.48%) which was found to be significantly higher than sample A (0.08%). This was supported by [40] that stated that the fat content of smoked fish is significantly higher than that of fresh fish of the same species.

Separation of the means using analysis of variance showed that there was significant difference (P<0.05) in percentage ash content. Further separation using duncan's multiple range test also showed that there was significant difference (P<0.05) in sample A, B and C in percentage ash content. The percentage ash content of sample A (4.36%) was low compared to the percentage ash content of the smoke-dried sample B (12.55). This confirmed the findings of [41]. The increased in ash content of smoked snail samples may be due to loss of humidity [42]. The range for the ash percentage content for sample B (12.55%) and sample C (11.76%) gave an indication that the snail samples may be good sources of minerals such as calcium, potassium, zinc, iron and magnesium [43].

For percentage nitrogen free extract, separation of the means using analysis of variance showed that there was significant difference (P<0.05) in percentage nitrogen free extract content. But further separation using duncan's multiple range test showed that there was no significant difference (P>0.05) between sample B and C in percentage nitrogen free extract content. While for percentage crude fiber, separation of the means using analysis of variance showed that there was no significant difference (P>0.05) in percentage crude fiber content.

5. CONCLUSION AND RECOMMENDATIONS

Micro-organism occurs everywhere in nature. Fungi and bacteria including the non-pathogenic and pathogenic forms are usually present in many snail species and this ultimately results to one of the limiting factors in snail production.

Smoke-drying is a common method of preserving fish in Nigeria and its product are highly appreciated by consumers. The study revealed that there was a drastic reduction in the microbial load from an unacceptable to an acceptable limit. The proximate composition was also enhanced by virtue of smoke-drying.

It is therefore recommended that the microbial load can be reduced by practicing the following:

- ❖ Precautions should be taken to prevent water contamination during harvesting as well as post-harvest handling of snail.
- ❖ Smoke-drying practices should be adopted as a means to reducing microbial load.
- ❖ The sanitary conditions under which snails are reared should be improved by following standards or good practices: such as use of good quality water, use of feed with low microbial load etc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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