Original research article

THE EFFECTS OF SMOKE-DRYING ON THE NUTRITIONAL QUALITY AND MICROBIAL LOAD OF APPLE WATERSNAIL (Lanistis libycus) IN IKPOBA RIVER, EDO-STATE.

ABSTRACT
The study was aimed at determining the effects of smoke-drying on the nutritional quality and microbial load of Apple Watersnail (Lanistis 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 Lanistis libycus 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) and fungi counts (18.0 X 10^3) in sample A (fresh Lanistes libycus) was higher than that of sample B (smoke-dried Lanistes libycus) which were (5.0 X 10^3) and (5.0 X 10^3) for bacteria and fungi, respectively. The highest bacteria counts occurred in Sample C (44.3 X 10^3) Lanistes libycus after two months of storage, at ambient temperature and the highest fungi counts occurred in Sample C Lanistes libycus after two months of storage (42.7 X 10^3). 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 Lanistas libicus 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: Lanistes libicus, Bacteria load, Fungi load, Proximate composition

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 (Ramzy, 2009). 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 (Barker, 2001 and Ramzy, 2009). 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 (Ranvichandran et al., 2012). Apart from serving as a source of food, other economic importance of shellfish (Lanistes libycus) includes foreign exchange, income and provision of employment (Ezeama, 2000). Shellfish are extremely perishable commodity and quality loss can occur very rapidly after catch (Khan and Khan, 2001 and Dewi et al., 2011). 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 (Hughes and Koplan, 2005). 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 (Edema et al., 2005). 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 sensor y or instrumental methods (Olafsdotter et al., 1997).

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 (Bagamboula et al., 2005). Different processing methods, including boiling and roasting, influence the proximate, mineral and toxicant composition of foods (Onyeike and Oguike, 2003). 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 (Tiamiyu et al., 2004). 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) (Abraham and Oramadike, 2011). 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.

MATERIALS AND METHODS
Collection of Samples and Sample Size
A total of thirty two (32) Lanistis libycus were collected from Ikpoba River with an average weight of 21.2g. After collection the shell and the viscera were removed with a knife and the average weight was now 6.6g. Then the Lanistes libycus were washed with clean water with a lum to remove the slime as practiced by the local consumers. The Lanistes libycus samples were rinsed twice to remove any trace of alum.

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.

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 60.80°C, a temperature which was twice that of cold smoking (30°C) (Sigurgisladotirr et al., 2000) and an average constant weight of 2.2g was gotten after eight hours.

The samples were labeled as;
Sample A - Fresh Apple water snail (Lanistes libycus) = 52g
Sample B - Smoke-dried Apple water snail (Lanistes libycus) = 53g

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 Lanistes libycus 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.

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 (1g) of each of the samples was weighed out and blended, mixed with 9ml of sterile distilled water into a test tube. 1ml 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 labelled 10^{-2} and 10^{-3}, in that order. This was done for each of the samples A (Fresh Apple water snail (Lanistes libycus) = 52g), B (Smoke-dried Apple water snail (Lanistes libycus) = 53g), and C (Smoke-dried Apple water snail (Lanistes libycus after 2 months of ambient storage) = 28g). 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.

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

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.

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 frozen sample by 6.25. Nitrogen content was determined by Kjedah’s method. The analysis was carried out by extracting 2.0g of each sample in a soxhlet apparatus using petroleum ether at 60-80°C as the extractant.

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.
Map of Ikpoba River showing sampling stations.

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

**RESULTS**

Table 1 below 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 Sample
s was 4.38g. 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 multiplied by 100 and divided by initial weight after the viscera were removed and had an average weight of 6.6g.

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. Table 2–4 summarized the total heterotrophic microbial counts of all the samples which comprised of sample A - Fresh Apple water snail (*Lanistes libycus*) = 52g, sample B - Smoke-dried Apple water snail (*Lanistes libycus*) = 25g and sample C - Smoke-dried Apple water snail (*Lanistes libycus* after 2 months of ambient storage) = 28g.

Table 5 summarized the proximate results of *Lanistes libycus* obtained from Ikpoba River.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial weight after gutting (g)</th>
<th>Weight after 1.6 hrs (g)</th>
<th>Weight after 3.2hrs (g)</th>
<th>Weight after 4.8hrs (g)</th>
<th>Weight after 6.4hrs (g)</th>
<th>Weight after 8.0hrs (g)</th>
<th>Weight loss (Initial weight - Final weight) (g)</th>
<th>% Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salted and Smoke-dried <em>Lanistes libycus</em></td>
<td>4.6</td>
<td>3.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.2</td>
<td>4.38</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dilution factors</th>
<th>Number of colonies/ plate</th>
<th>Average number of colonies per dilution x ± se</th>
<th>Organism per gram of snail sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Fresh Apple watersnail <em>Lanistes libycus</em>)</td>
<td>$10^3$</td>
<td>8</td>
<td>$9.3 \pm 0.8^a$</td>
<td>$8 \times 10^3 = 8.0 \times 10^3$</td>
</tr>
<tr>
<td>B (Smoke-dry Apple watersnail <em>Lanistes libycus</em>)</td>
<td>$10^3$</td>
<td>16</td>
<td>$5.0 \pm 0.5^a$</td>
<td>$4 \times 10^3 = 4.0 \times 10^3$</td>
</tr>
<tr>
<td>C (Smoke-dry Apple watersnail <em>Lanistes libycus</em> after 2 months)</td>
<td>$10^3$</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Total estimated viable heterotrophic fungi count in colony forming units per gram of fresh and smoke-dried *Lanistes lybicus* (cfu/gm).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dilution factors</th>
<th>Number of colonies/ plate</th>
<th>Average number of colonies / plate</th>
<th>Organism per gram of snail samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Fresh Apple watersnail <em>Lanistes libycus</em>)</td>
<td>$10^3$</td>
<td>26</td>
<td>$25 \times 10^3 = 2.5 \times 10^4$</td>
<td></td>
</tr>
<tr>
<td>B (Smoke-dry Apple watersnail <em>Lanistes libycus</em>)</td>
<td>$10^3$</td>
<td>3</td>
<td>$8 \times 10^3 = 8.0 \times 10^3$</td>
<td></td>
</tr>
<tr>
<td>C (Smoke-dry Apple watersnail <em>Lanistes libycus</em> after 2 months)</td>
<td>$10^3$</td>
<td>24</td>
<td>$26 \times 10^3 = 2.6 \times 10^4$</td>
<td></td>
</tr>
</tbody>
</table>

Note:
Table 4: Frequency of occurrence of microbial isolates of *Lanistes libycus* samples obtained from Ikpoba River

<table>
<thead>
<tr>
<th>Isolates</th>
<th>#</th>
<th>%</th>
<th>A (Fresh Apple water snail <em>Lanistes libycus</em>)</th>
<th>B (Smoke-dry Apple watersnail <em>Lanistes libycus</em> after 2 months)</th>
<th>C (Smoke-dry Apple water snail <em>Lanistes libycus</em> after 2 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus sp</td>
<td>3</td>
<td>8.3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Micrococcus sp</td>
<td>2</td>
<td>5.6</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Serratia sp</td>
<td>2</td>
<td>5.6</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>5.6</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>2.8</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Mucor sp</td>
<td>3</td>
<td>8.3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Trichoderma sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Aspergillus nidulans</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Cladosporium sp</td>
<td>2</td>
<td>5.6</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Yeast sp</td>
<td>1</td>
<td>2.8</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Helminthosporium sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Cryptomonas reoformis</td>
<td>2</td>
<td>5.6</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Penicillium sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Aspergillus sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Geotrichum sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>1</td>
<td>2.8</td>
<td>X</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Neurospora sp</td>
<td>2</td>
<td>5.6</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>Mucor mucido</td>
<td>1</td>
<td>2.8</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Botrytis sp</td>
<td>1</td>
<td>2.8</td>
<td>✓</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saccharomyces sp</td>
<td>3</td>
<td>8.3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fungi frequency</td>
<td>26</td>
<td></td>
<td>9</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Fungi % frequency</td>
<td>72.6</td>
<td>34.6</td>
<td>38.5</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>Fungi diversity</td>
<td>13</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Fungi % diversity</td>
<td>50.0</td>
<td>53.8</td>
<td>53.8</td>
<td>53.8</td>
<td></td>
</tr>
</tbody>
</table>

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
Table 5: Proximate results for *Lanistes libycus*

<table>
<thead>
<tr>
<th>Samples</th>
<th>% Moisture Content</th>
<th>% Dry Matter</th>
<th>% Ash Content</th>
<th>% Fat Content</th>
<th>% Crude Protein</th>
<th>% Nitrogen Free Extract (N.F.E)</th>
<th>% Crude Fibr</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(Fresh <em>Lanistes libycus</em>)</td>
<td>74.31^a</td>
<td>25.69^a</td>
<td>4.36^c</td>
<td>0.08^c</td>
<td>11.42^c</td>
<td>9.79^b</td>
<td>0.04^a</td>
</tr>
<tr>
<td>B(Smoke-dried <em>Lanistes libycus</em>)</td>
<td>8.50^c</td>
<td>91.50^a</td>
<td>12.55^a</td>
<td>3.48^a</td>
<td>55.36^a</td>
<td>20.04^a</td>
<td>0.07^a</td>
</tr>
<tr>
<td>C(Smoke-dried <em>Lanistes libycus</em> after 2 months)</td>
<td>11.50^b</td>
<td>88.50^b</td>
<td>11.76^b</td>
<td>3.34^b</td>
<td>53.36^b</td>
<td>19.98^a</td>
<td>0.06^a</td>
</tr>
</tbody>
</table>

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.

**DISCUSSION**

Strom and Olafsen (1990), 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, Mycosphorella*. A total of 6 bacteria genera were also identified from the snail samples which include; *Proteus, Micrococcus, Bacillus, Staphylococcus, Serratia, and Escherichia*. The microflora in the *Lanistes libycus* was similar to those identified by Bukola et al., (2011).

The mean counts of colonies per dilution of bacteria in Sample A (Fresh *Lanistes libycus*) (9.3 x 10^3) was higher than that of Sample B (Smoke-dried *Lanistes libycus*) (5.0 x 10^3) and the mean counts of colonies per dilution of fungi in Sample A (18.0 x 10^3) was also higher compared to sample B (5.0 x 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 *Lanistes libycus* 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 Abbas et al., (2009) 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 x 10^3) was higher than Sample B (5.0 x 10^3). Also the mean counts of colonies per dilution of fungi in Sample C (44.7 x 10^3) was also higher than that of Sample B (5.0 x 10^3). This may be due to the long period of storage which led to increased moisture content in sample C for micro-organisms...
This was corroborated by Eyo (2001) 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 5x10^5 cfu/g for food and food products (ICMSF, 2002). Only sample B had a lower value of 0.0 x10^3 cfu 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 included Aspergillus niger, Mucor spp, Neurospora spp, Penicillium spp Geotrichum spp, Rhizopus spp, Mucor mucido, Saccharomyces spp, Aspergillus spp, Penicillium oxalicum, trichoderma ssp, Aspergillus nidulans, Cladosporium spp, Yeast spp, Botrytis spp, Cryptomonas reoformis, s, Helminthosporium spp and Mycosphacrella spp were isolated on potato dextrose agar (PD A) 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 mucido, Geotrichum spp, Aspergillus spp, Asp ergillus nidulans, Botrytis spp, yeast spp, Helminthosporium spp, Penicillium oxalidium, Penicillium spp, and Mucor 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 bacteri a 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 Martin (1994) when he stated that these organisms were the commonest micro-organisms associated with smoked fish and these micro-organism were also reported by Abolagba et al., (2009) in smoked fish sold in Benin metropolis. Chanal, (2000) 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 (Chanal, 2000).

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 reports by Eniola et al. (2007) and Okontal et al. (2008) 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. Chea (2015) reported that E.coli cause urinary tract infection, pneumonia, meningitis, diarrhea and kidney damage due to the consumption of the contaminated seafood. The occurrence of B acillus spp in sample treatment (C) can be said to be as a result of prevalence of their spore in the environment (Adebayo-Tayo et al., 2009). Staphylococcus spp which occur in all the treatments except sample (A) is as a result of contamination by snail handlers (Adebayo-Tayo et al., 2009). S. aureus causes chicken pox, Epiglottitis, skin rashes and Toxic shock syndrome (Milner, 2005). The occurrence of Micrococcus spp in fresh snail sample may be as a result of contamination during harvesting and temporary storage (Liu et al., 2010). The occurrence of Cladosporium, Mucor, Saccharomyce, Rhizopus, Aspergillus spp and penicillium spp could be due to contamination during harvesting leading to air born transmission (Nasser, 2002). Aspergillus spp produces toxins known as aflatoxin which causes mycotoxicosis, liver cancer, cir
hosis and hepatitis (Adebayo-Tayo et al., 2009). *Penicillium spp* produces mycotoxins that are harmful to man and may result in renal damage/ necrosis of the kidney (Adebayo-Tayo et al., 2012). *Neurospora spp* produces spores that may cause asthma (Adebayo-Tayo et al., 2012). *Aspergillus niger, Rhizopus spp* and *penicillium spp* are contaminated from water, insect and contaminated hand, personal hygiene of the sellers (Lass-flor, 2009).

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 Table 2 and 3. This study revealed that *Lanistis lybicus* 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 *Lynistis lybicus* from Ikpoba 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 (Ranvichandran et al., 2012), 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 Effiong and Mohammed (2008), Mumba and Jose (2005) and Abdullahi (2001). 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 *Lynistis lybicus*) (74.31%) agreed with that reported by Ajayi et al. (1978) 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 (Lawrie, 1991). 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 (Marias and Erasmus, 1997). The moisture content of the smoke-dried *Lynistis lybicus* [(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 Ninawe and Rathnakumar (2008) 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 (Ogbonnaya and Shuba, 2009) 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 compared to sample B (3.48%) and C (3.34%) may be due to the high moisture content of sample A (Marias and Erasmus, 1997). The percentage fat content of sample B (3.48) was within the range previously detected for snail (3.18 to 4.25%) by Bukola et al., 2011. 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 Abolagba et al., 2015 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 Ande et al., (2012). The increased in ash content of smoked snail samples may be due to loss of humidity (Salam, et al., 2006). 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 (Andrew, 2001).

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.

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.
- There should be an improvement of sanitation within Edo metropolis by provision of public toilet and enactment of effective policy for the collection and disposal of municipal solid waste as these would drastically reduce the pollution of rivers with human and domestic wastes.
- 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.

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