Assessment of Nutritional Qualities of Direct Contact Biogas Smoked Catfish (*ClariasGariepinus)*

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Abstract
Drying is a simple technology and it refers to reduction of moisture content of material to shelf stable levels. This study is aimed at studying the proximate, mineral, microbial and sensory quality of fish smoked with biogas. Live fresh Clarias gariepinus were sourced locally, killed, de-gutted, washed thoroughly with water and cut into pieces of 3 cm length each. Parts of the pieces were dried in the oven for comparison; other portion was smoked with biogas while the rest were used as control. Proximate, mineral, sensory and microbial analyses were carried out on the biogas-smoked and oven dried fish to compare the effect of heat source on the quality. Clarias gariepinus dried in the oven gave the highest value of protein (39.88), fat (22.47) and ash content (11.73) and was statistically different from that smoked with biogas and the fresh fish. However, proximate value of fish biogas-smoked (protein 37.83, fat 19.50, ash 8.69) conforms to minimum standard. The observation was similar for mineral content of the oven-dried and biogas-smoked (Na 118.76; K 20.56; Ca 54.63) pieces. The microbial loads of the biogas-smoked pieces (TCB 1.71 x 10^7, mould 1.25 x 10^7) were lower than the required minimum values. The sensory evaluation of the biogas-smoked pieces was satisfactory.

Keywords: *biogas; Clarias gariepinus; smoking; nutritive value; microbial load*

1.0 INTRODUCTION

One of the main products consumed in terms of animal protein is fish and it is largely consumed in Nigeria. It has an advantage over pork or beef because it is cheap and highly acceptable, with little or no religious bias (Eyo, 2001). Only about 50% of the demand for fish is currently being met by local supply. Fish is an important and the cheapest source of animal protein which accounts for about 37% of Nigeria's total protein intake. It accounts for 22% of the protein intake in Sub-Sahara Africa (Béné and Heck, 2005).

Fish is a highly perishable product and there is a need for immediate processing if unrefrigerated to prevent wastages. According to Orengoh and Kisumo (2007), 50% of total annual fish harvest goes to waste due to poor treatment, management and storage. In order to reduce the wastage and spoilage of fish during periods of oversupply, and to enhance long storage, it is necessary to adopt appropriate as well as affordable processing and preservation techniques for fish especially in the artisanal fishermen's environment.

Microbial spoilage of fish may be prevented by different methods such as drying, freezing, smoking, salting and use of modified atmospheric storage, (Fennema, 1982; Gupta and Gupta, 2006). Smoking is perhaps the simplest method among the several methods of long term preservation of fish as it does not require sophisticated equipment or highly skilled workers. One of the major ways of adding values to fish in the Tropics is by smoking and drying.

Food has been preserved by smoke-curing since before the dawn of recorded history. People in all cultures in the world have relied on the smoke curing of fish and meat products

for long term storage. Smoking apart reducing the moisture content of the product also impacts a desirable flavour, appearance and texture to the products (Olayemi *et al*., 2013). In an effort to develop an effective method of fish smoking, different models of improved ovens and kilns were developed in various parts of Africa (Davies *et al*., 2008)

Biogas typically refers to a gas produced by the anaerobic digestion or fermentation of organic matter including manure, sewage sludge, municipal solid waste, biodegradable waste, energy crops or any other biodegradable feedstock. Biogas is composed primarily of methane and carbon dioxide (Oyewole, 2010) .

The environmental advantages of using anaerobic digestion for dairy farm wastes include the reduction of odors, flies, and pathogens as well as decreasing greenhouse gas (GHG) and other undesirable air emissions (Oyewole, 2010).

In this work, biogas was used to smoke fish. The proximate, mineral, microbial and sensory quality of fish (*Clarias gariepinus*) smoked with biogas were determined.

2.0: MATERIALS AND METHOD

2.1 Materials

Five live fresh fishes of *Clarias gariepinus* specie were sourced locally in Akure, Ondo State, Nigeria. The fishes were killed, gutted, washed thoroughly with water, cut into pieces of 3 cm length each and placed on the wire gauze of the smoking kiln.

Smoking was done using blue-hot flame from bunsen burners placed in a smoking kiln powered by biogas. The fish chunks were turned at intervals and smoked to an average moisture content of 10.41 ± 0.02%. After smoking, the products were allowed to cool, packed in polythene bags to reduce infestation by microorganisms and kept in a freezer (Model Haier Thermocool) at 1 – 5 °C before laboratory analyses.

2.2 Methods

2.2.1 Proximate determination of the smoked fish

The proximate compositions of the fish samples were determined according to the AOAC (2005) methods. The moisture content was determined by drying samples overnight at 105 °C for 3 hours. Crude protein content was determined using the Kjeldahl method. Fat content was determined by using soxhlet method and the fish samples were ashed at 500 °C to determine the ash content. Carbohydrate content was determined by calculating the difference. Each sample was analyzed in triplicate.

2.2.2 Mineral determination of the smoked fish

The mineral compositions of the fish samples were determined using the dry ash extraction method described by James (1995). An Atomic Absorption Spectrophotometer and Flame Photometer, following the manufacturer's specifications, were used. 2 g of the sample was weighed into small a porcelain crucible and ashed in the furnace at 650 °C for three hours. The ash was extracted by half filling the crucible with 2 ml HCl, boiled gently and the solution was transferred to a 50 ml beaker using Pasteur pipette. The precipitates were washed with distilled water, filtered into the filtrate and solution made up to 50 ml mark distilled water. Blanks were prepared using only distilled water. Potassium was determined using Flame Photometer (FP) (Model Jenway PFP 7) with standard solutions while Iron was determined by Atomic Absorption Spectrophotometer (AAS) (Bulk Scientific model 210/211 VGP) with standard solutions. This was used to analyze Mn, Na, Fe, Zn, Ca, Pb, Al and K content.

The standard solutions were prepared separately for each of the elements and values of each element were then determined using AAS or FP as necessary. The values measured were then plotted against the strengths of the standard solutions. The values of the various

digest were measured from the AAS or FP and the strength traced from the respective standard curve to give the corresponding values which would give the original values of the elements present in the digest (James, 1995; AOAC, 2005). Each sample was analysed in triplicate.

2.2.3 Microbial analysis of the smoked fish

Culture media:

Nutrient Agar (NA) was used for the culture of bacteria while Potato Dextrose Agar (PDA) was used for fungi. In preparing the media, 14 g of NA was dispersed in 1litre of sterile water and 39 g of PDA in 1litre of sterile water. Sterilization of the media was done with both media in a conical flask and sealed with foil paper to prevent contamination and then sterilized in the autoclave at 121 °C for 15 minutes after which it was allowed to cool. The media was shaken vigorously before use (Taylor *et al.,* 1998).

Isolation of microbial isolates:

The standard aerobic pour plate count technique was used which is based on the assumption that each viable cell will yield a colony forming unit per gram of sample (cfu/gm). The aerobic colony counts of the fish sample were done in accordance with Peter *et al.,* (1992) by the pour-plate method in the following procedure. Sterile knife was used to cut fish samples from the left side of the fish. 1.0 gm of the fish samples was weighed out using the top loading balance, blended and suspended in 10ml sterile water to make a stock suspension. 1.0ml of the stock was transferred by pippetting to a 9.0 ml diluent of sterile water. This process was repeated for four other test tubes to make 10^{-5} dilution. 10^{-5} was used as the dilution factor and 1.0 ml of the last test tube was discarded in order to achieve equal dilution.

Bacteria isolates were identified based on their cultural characteristics, Gram staining reaction and various identification tests. Isolates were identified according to Holt et al., (1994). The young cultures of the mould isolates were stained with lactophenol-blue and identified to the genus level by colony and cell morphology and biochemical tests according to Alexopoulos and Mims (1979).

For *E. Coli*; The amount of *E. coli* was determined using Eosin Methylene Blue (EMB) agar as culture medium. The medium contained fish that was incubated at 37 °C for 24 hr. Colonies with pinkish red growth having a metallic sheen or reflection confirms the presence of *E. coli.*

For Bacteria; Total count of bacteria was determined using nutrient agar (NA) by pour-plate technique. Aliquots of 1.0 ml of the 10^{-1}, 10^{-3}, and 10^{-5} dilutions of each of the fish stock dilutions of the samples were inoculated in sterilized petri dishes in duplicates and 0.2 ml of antifungal mixture was added to discourage fungi growth. Cooled molten nutrient agar was poured into inoculated plates and swirled gently to mix and solidify. Plates were incubated at room temperature for 24hours in inverted position to prevent condensation. The mean count of bacteria in colonies forming units per gram of sample was determined.

For Fungi; Aliquots of 1.0 ml of the 10^{-1}, 10^{-3} and 10-5 dilutions of the fish stock mixture were inoculated into potato dextrose agar (PDA) in duplicates amended with antibiotic mixture to discourage bacteria growth. Plates were inoculated at room temperature for 72 hours. Developing colonies were then counted.

The microbial colonies were counted using colony counting machine. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation

was done for 1 ml of original sample. Plating was done in triplicate for each dilution and an average count was taken to obtain the total count.

2.2.4 Sensory properties

The organoleptic assessment of the fish samples was carried out using twenty semi-trained panelists. A 9-point hedonic scale ranging from 1(Dislike extremely) to 9 (Like extremely) was used (Olayemi *et al.*, 2011). The descriptive 9-point hedonic scale was used for the sensory attributes (taste, texture, aroma, colour and general acceptability) of the samples at 0.05 level of significant.

2.2.5 Statistical analysis

All measurements were carried out in triplicates and subjected to tests. All microbial counts were converted into base 10 logarithms of colony forming units per gram of sliced catfish samples (log 10 cfu/g). Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software of SAS Institute. Differences among the mean values of the various treatments were determined by the Least Significant Difference (LSD) test, and the significance was defined at $p < 0.05$. The differences which are equal to or more than the identified LSD values are considered statistically significant (Puwastien *et al.*, 1999; Olayemi *et al.*, 2011).

3.0: RESULTS AND DISCUSSION OF RESULTS

3.1 Effect of Drying Methods on the Proximate Composition of Clarias gariepinus Smoked with Biogas

The samples of *Clarias gariepinus* smoked using oven and biogas as energy sources varied in proximate composition. Statistical Analysis (ANOVA using SPSS) carried out on the proximate composition are presented in Table 1. Proximate analysis was also carried out on the fresh fish to ascertain the variation which occurred due to smoking.

Clarias gariepinus dried in the oven gave the highest protein, fat and ash content values of 39.88, 22.47 and 11.73%, respectively. The biogas-smoked fish proximate composition for protein, fat and ash content were 37.82, 19.50 and 8.69%, respectively while that of fresh fish showing the least protein, fat and ash content were 20.66, 4.54 and 7.09%, respectively. This implies that drying of *Clarias gariepinus* increases the protein and fat content. Also, the high value of protein, fat and ash content obtained from the oven could be attributed to the high drying temperature and enclosed system of drying. Higher temperature had often been associated with high values of ash content (Olayemi *et al.*, 2011). However, the carbohydrate content showed a deviation from the above trend. The fish smoked with biogas had the highest carbohydrate content of 23.52% while the oven drying gave a value of 16.65% with the fresh fish giving the least value of 11.28%.

Fish protein is of high quality and contains sufficient amounts of all the essential amino acids required by the body for growth, maintenance of lean muscle tissue and active metabolism (Talabi, 1995). In this study, there is significant increase in the protein level in oven dried and smoked catfish, when compared with the fresh catfish. This suggests that protein was not lost during drying and smoking as well. This finding is in agreement with the observations of Akinwumi (2014) and Puwastien *et al*. (1999). Similarly, Fapohunda and Ogunkoya (2006) reported that smoke drying methods increased the protein, ash and fat contents of *C. gariepinus*.

3.2 Effect of Drying Methods on the Mineral Content of Clarias Gariepinus Smoked with Biogas

The mineral analysis of *Clarias gariepinus* not only showed the presence of these compounds but also revealed variation in the concentration of such minerals as Sodium (Na), Potassium (K), calcium (Ca), Iron (Fe), Manganese (Mn), and Zinc (Zn) based on the energy source. The results obtained from the mineral analysis are presented in Table 2. The results shows the highest values of Sodium (Na), Potassium (K), and Calcium (Ca) of 188.00, 226.29 and 120.58 respectively for *Clarias gariepinus* dried in the oven; 118.76, 204.56 and 54.63, respectively for the fish smoked with biogas; and 116.26, 187.88 and 20.86, respectively for the fresh fish. Hence for Na, K and Ca, the oven dried fish gave the highest values while the fresh fish gave the least.

However, the order is different in the remaining minerals, that is, Iron (Fe), Manganese (Mn), and Zinc (Zn). In these minerals, Fe, Mn and Zn were highest with values of 12.35, 2.46 and 16.02, respectively for fresh fish; 5.28, 1.87 and 10.53, respectively for the oven dried fish; and 4.23, 1.27 and 7.48 respective for the fish smoked with biogas. Hence, the fresh fish gave the highest values of the latter minerals while the fish smoked with biogas gave the least values. Nevertheless, the values obtained were significantly different ($P < 0.05$).

This is in agreement with Effiong and Fakunle (2012) who observed low Iron contents in the three tropical smoked freshwater studied. However, Eyo (2001) reported low Iron contents but high Potassium contents in frozen fish. Freshwater fish meat is a particularly valuable source of Calcium and Phosphorus as well as Iron, Copper and Selenium (FAO, 2014). Onyia *et al*. (2010) reported similar findings and observed that the dominance of mineral elements in a fish also depends on the water body where the fish lives. In general FAO (2014) reported that the vitamin content of white fish muscle is similar to that of lean meat and, with the exception of vitamin C, can usually make a significant contribution to the total vitamin intake of man and domestic animals. This vitamin content of fish is not markedly affected by smoking or sun drying, provided storage is not very prolonged.

The trend of results obtained in this study is similar to those obtained by earlier researchers. Akinneye *et al*. (2007) reported the values of the major elements were obtained in the decreasing order K>Na>Mg>Ca in *Sardinella* (oven dried), *H. niloticus*, (oven dried), *H. niloticus* (smoke dried) and *Sardinella sp* (smoke dried). In smoke-dried samples, lower values of major elements were reported compared with other drying methods. Similarly, Adewumi, Ogunlade and Coker (2015) reported that the values of elements in oven and smoked-dried catfish samples were in the decreasing order P> K>Fe. Gopakumar (2000) reported the values of major elements of Indian fishes in the order P> K>Na>Mg>Ca. Their abundant presence may be due to the facts that fish body needs these macro elements in more amounts than the micro elements in the structure and function of the body. In fish, Calcium and Phosphorus together accounts for 60 – 70% of the minerals in the skeleton. Apart from being a constituent of the skeleton, Phosphorous has many roles in fish. It is present in Adosine polyphosphates, the key substances for energy release and also in phospholipids (Nair and Mathew, 2001).

Also, the highest values of the micro elements in hill-stream fishes reported by Abdul and Sarojnalini (2012) are in the decreasing order Fe>Co>Zn>Ni>Cu>Mn>Cr. In the results reported by Fafioye *et al*. (2008) of traditionally smoke-dried fresh water fishes, the order of magnitude of the three trace elements are Fe>Zn>Cu and the same order of magnitude was reported by Kinsella *et al*. (1977) in fillets of several species of fresh water fishes. Fawole *et al*. (2010) reported decreasing order of Zn>Fe>Ni>Cu>As in the studies of some fresh water species. The results of some fishes reported by Ghosh *et al*. (2004) and Nurulla *et al*. (2003) were in the decreasing order Fe>Zn>Mn>Co>Cu. Other researchers have similar observation

with regards to lack of agreement of different reports on the order of magnitudes of mineral contents of a given species of fishes (Akinneye *et al*., 2007). It may be right to say that mineral elemental contents of each species is function of the availability of these elements in their local environment, diet absorptive capability and as well as their preferential accumulation.

The variation recorded in the concentration of minerals in fish muscles examined could have been a result of the rate in which they are available in the water body. The functions of inorganic elements include the formation of skeleton structure, electron transfer, regulation of acid base equilibrium and osmo-regulation. Minerals are important components of hormones, enzymes and vitamins; they activate complex biochemical mechanisms, control, and regulate the uptake, storage and excretion of various inorganic elements allowing fish to live in a dynamic equilibrium with their aquatic medium (Underwood, 1971; Reinhold, 1975). The functions and values of these elements are many and varied and their deficiency causes diseases in the body. Iron has the longest and best history among all the micronutrients. It is key elements in the metabolism of almost all living organisms. Cobalt is known for its component of the vitamin B-complex while numerous aspects of cellular metabolism are Zinc dependent.

Table 1 Proximate analysis of *Clarias gariepinus* (%wet basis)

Drying method	Moisture	Ash	Fat	Protein	Fibre	CHO
Fresh fish	55.32±0.53^{c}	7.09±0.18^{a}	4.54±0.46^{a}	20.66±0.57^{a}	0.00±0.00	11.28±0.46^{a}
Oven dried	9.22±0.66^{a}	11.73±0.09^{c}	22.47±0.96^{c}	39.88±0.35^{c}	0.00±0.00	16.65±1.14^{b}
Biogas smoked	10.41±0.07^{b}	8.69±0.38^{b}	19.50±0.05^{b}	37.82±0.59^{b}	0.00±0.00	23.52±0.94^{c}

Table 2 Mineral analysis of *Clarias gariepinus*

Drying method	Na	K	Ca	Fe	Mn	Zn
Fresh fish	116.26±0.49^{a}	187.88±0.37^{a}	20.86±0.30^{a}	12.35±0.35^{c}	2.46±0.41^{c}	16.02±0.41^{c}
Oven dried	188.00±0.68^{c}	226.29±0.68^{c}	120.58±0.58^{c}	5.2787±0.16^{b}	1.87±0.09^{b}	10.53±0.09^{b}
Biogas smoked	118.76±0.34^{b}	204.56±6.27^{b}	54.63±0.44^{b}	4.2300±0.10^{a}	1.27±0.01^{a}	7.48±0.20^{a}

3.3 Organoleptic/Sensory Evaluation of *Clarias gariepinus* Smoked with Biogas

The sensory evaluation as shown in Table 3 was conducted on aroma, texture, colour, taste and general acceptability of the samples of the dried fishes. There was significant ($p<0.05$) difference in the sensory attributes of the samples assessed. This might be due to variations among individuals in responding to the same level of stimuli like colour, taste and sensitivity to chemical stimuli. The samples of fish dried with the oven were preferred to the samples that were dried with biogas in terms of colour, aroma and taste. The result of general acceptability, however, shows that the oven dried fish was most acceptable. The sensory

attributes as observed by the response of the taste panel showed that people preferred fish dried using oven than the ones smoked using biogas. However, there was no significant difference ($p<0.5$) in the data obtained from the people on the texture of fish smoked with either biogas or oven. This shows the fish were in good quality and standard conditions as expected in Nigerian smoked fish products.

Table 3 Organoleptic/ Sensory evaluation of biogas-smoked *Clarias gariepinus*

Drying method	**Colour**	**Texture**	**Aroma**	**Taste**	**Overall Accept.**
Oven dried	6.25±1.01	6.25±1.18	6.20±1.60	7.00±0.71	6.43±0.94
Biogas dried	5.05±1.32	6.00±0.85	5.70±0.59	5.10±1.05	5.46±0.74

3.4 Microbial assessment of Clarias gariepinus smoked with biogas

The data in Tables 4 showed that catfish smoked with biogas had the highest bacterial count of 1.71×10^7 cfu/ g while the oven dried catfish had a lower bacterial count of 0.347×10^7 cfu/g. Similarly, fungal (mould and yeast) count were also obtained as 1.24×10^7 cfu/ g and 0.173×10^7 cfu/ g for biogas-smoked and oven-dried catfish, respectively. These values fall wihin the maximum recommended value of bacteria count for good quality fish products which is 5×10^5 colony forming unit per gram (cfu/ g) or $< 10^6$ cfu/ g. Also, the value of yeast/ mould recorded after smoking was found to fall within the acceptable limit of acceptable number of colony forming unit of mould/yeast of smoked fish (ICMSF, 1986) and the (Microbiological Guideline for Ready–to–eat–Food, 2007).

The result also indicated that there was no contamination with enteric organisms during smoking as there was no coliform found after smoking. The absence of *E. coli* specie which is an indicative organism indicating contamination by microorganisms from enteric origin further confirms the effectiveness of the smoking system. According to Eyo (2001), microbial action plays a large part in the spoilage of fish and fish products. Fish smoking with biogas has been able to effectively reduce this main source of spoilage.

Table 4 Summary values for Microbial Analysis of biogas-smoked catfish

Microorganism	**Oven**	**Biogas**
Total count bacteria (TCB) (cfu/g)	3.47×10^6	1.71×10^7
E. coli (EMB) (cfu/g)	0×10^5	0×10^5
Yeast / Mould (PDA) (cfu/g)	1.73×10^6	1.25×10^7

The high count for the fish smoked with biogas could be attributed to improper pre/ post handling/ smoking procedures. This is in agreement with Schewan (1977) and Duke-Ndudim *et al.* (2014) who considered smoking process, a mild preservative treatment, which kills bacteria and prevents microbial proliferation due to combined effects of heating, drying, pH and antimicrobial smoke components. Hence, as a mild treatment, smoking does not achieve complete elimination of microbial load of a fresh fish which has been proved to be naturally high due to the high microbial load of their habitat (water) (Frazier and Westoff, 1995).

Another possible source of contamination is the exposure of the fish to the biogas via incomplete combustion or presence of raw gas in the surrounding environment where the smoking is done since the anaerobic process of biogas digestion involves bacteria. The presence and possibility of contamination of fish during smoking with biogas or other heat source has been reported by Duke-Ndudim *et al.* (2014).

It was observed that the catfish oven dried had far less microbial count compared to that of the biogas. This is possibly due to the enclosed system used for the drying which reduces exposure to contamination either from the surrounding or from the kiln. Also, no gas was present in the oven system.

4.0: CONCLUSION

Biogas is a safe source of energy and can be exploited for use in homes such as in cooking. Fish therefore could be smoked using biogas without any fear of acceptability by the consumers. However, great care should be taken in handling the fish during process in order to reduce contamination.

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