Journal of American Science

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Multidisciplinary Academic Journal Publisher

EFFECTS OF TWO FUEL SOURCES (RICE HUSK AND SAWDUST) ON THE PROXIMATE COMPOSITION AND MICROBIAL QUALITY OF *Heterobranchus Longifilis*

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Abstract: This study was designed to determine the effect of two fuel sources on the proximate composition and microbial quality of smoked *Heterobranchus longifilis.* The mean weight of 1.08kg fish was purchased from fishermen at fish landing site at Wadata in Makurdi, Benue State. They were washed, degutted, salted and divided into two and smoked using sawdust and Rice husk. The fish after drying to a constant weight was transported to the laboratory and stored for 8 weeks for storage experiment. Proximate composition of smoked fish was determined before and after storage, the result shows that the fish smoked with Rice husk has higher crude protein 62.25±0.49, Crude fibre 8.07±0.01 and ash 7.57±0.05 compare to those smoked with sawdust which had a Crude protein value of 60.94±0.34, Crude fibre 7.05±0.01 and Ash 7.44±0.06. Also, the result of the microbial analysis shows that fish smoked with Rice husk has high microbial load $9.51x10^{4}$**±**$0.01x10^{4}$cfu/g and $8.26x10^{6}$±$0.00x10^{6}$cfu/g before and after storage respectively as compare to sawdust $6.56x10^{4}$±**0.00** cfu/g and $5.37x10^{5}$±$0.01x10^{5}$cfu/g initial and final respectively. It is concluded that fish smoked with Rice husk had a higher nutritional profile which attracted more microbes under storage, implying that it will have a longer shelf life when compared to fish smoked with sawdust.

[George, U. Otoh, A. Abiaobo, N. Nwaneri, J. Tyovenda, DO**. Effects Of Two Fuel Sources (Rice Husk And Sawdust) On The Proximate Composition And Microbial Quality Of *Heterobranchus Longifilis*.** *J Am Sci* 2024;20(1):41-47]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 04. doi:10.7537/marsjas200124.04.

Keywords: Rice husk, Sawdust, Proximate Composition, Microbial Quality, *Heterobranchus longifilis*

11. Introduction

Fish is considered an excellent food of high-quality protein that can replace both red and white meat especially with the fast-growing aquaculture industry in recent years **(***Kari et al., 2020*). It contains high quality protein, amino acids and absorbable dietary minerals (Adeyeye *et al*., 2015b). It contains both important micro- (minerals and vitamins) and macro- (protein, fat) nutrients. Furthermore, fish contains a high level of polyunsaturated fats (PUFA) which helps in lowering cardiovascular diseases in humans (*Mishra, 2020***).**

Good handling of fish from point of harvest to final consumption is very essential for the quality of fish to be maintained. Fish handling involve all the procedure aimed at maintaining the quality. Improving food security requires making better use of fish produced by reducing post-harvest losses and increasing the percentage of fish used for direct human consumption. Converting low-value resources, into products for direct human consumption, rather than reducing them to fishmeal, would also contribute to greater food security (FAO, 2010).

The demand for fish is growing and the post-harvest losses can make a major contribution to satisfy this demand, improving quality and quantity for consumers and increasing income for producers (Bilgin *et al*., 2008). However, fish is highly perishable because it provides favorable medium for the growth of microorganisms after death (Adeyeye *et al*., 2015a; Aliya *et al.*, 2012; Oparaku and Mgbenka, 2012). Different preservation techniques e.g., cold storage, salting, drying, fermentation, and smoking are commonly used in fish preservation technology

(Alcicek and Atar, 2010). The shelf-life of smoked fish product is usually extended primarily due to the reduced water activity (Eyo, 2001).

To achieve this, proper handling and preservation must be carried out if the fish is to have a long shelf life and retain a desirable quality and nutritional value. This is because fish is a highly perishable food which needs proper handling and preservation **(**FAO, 2012**)**.

Smoking is one of the oldest methods of fish preservation developed in pre-historic period. In recent times smoking is used as a method of preservation with the incorporation of smoke flavor and development of color. In underdeveloped countries this method is used as a means of preservation only, while in developed countries this method is used to impart smoke flavor to the product since in these countries there are other sophisticated means of preservation of fish like canning, oven drying and freezing. There is a huge trade in smoked-dried fish as a result of growing demands in increasing number of Africans living in diaspora.

Fish may be re-dried after smoking, depending on the moisture content. Sun drying and smoking are important steps in traditional methods of fish processing in many African countries and in the world. Smoking demands great qualities of firewood and wood has become scarce and expensive thereby eroding the profit of processors who often have to purchase woods. Most types of wood whether hard or soft can be used for smoking fish. Red mangrove wood which is available in tropical countries burns well even immediately after cutting but hard wood been the best burns slowly and produces more smoke than soft wood. Sepp and Mann (2009) define wood fuel as firewood and charcoal. Most-commonly used forms of wood fuel include firewood. Firewood represents the largest share in wood energy fuels production and consumption **(UNEP, 2019).**

According to **Njenga *et al.* (2018**), firewood and charcoal constitute sustainable wood fuel. Methods of drying and smoking fish vary between different countries and within same country and may differ depending on the species of fish used and the product desired **(Obande, 2009)**. The same author found that fuel woods is generally used as the conventional smoking source; however, deforestation and emission of greenhouse gases makes alternatives means of fish smoking other than fuel woods necessary. In Arid zones of Nigeria, fish mongers result to use of cow dung as fuel source for fish smoking which does not impart good flavor on the product **(Ayuba *et al.*, 2015**)

Rice Husk a major by-product of the rice milling industry is one of the most commonly available lignocelluloses materials that can be converted to different type of fuels and chemical feedstock through a variety of thermo-chemical conversion process.

Sawdust or wood dust is a by-product of cutting, grinding, drilling, sanding or otherwise pulverizing wood with a saw or other tools: it is composed of fine and rough particles of wood, such as woodpecker and carpenter ant. Sawdust is the main component of particle board or as a fuel.

There is a drastic increase in the cost of fire wood used for smoking fish, this has eroded or interfere with the interest (profit) of fish processors involved in smoking fish and this calls for alternative heat sources. Using alternative heat source from Agricultural wastes such as saw dust and rice husk will not only solve this problem but prevent indiscriminate cutting down trees to obtain firewood (deforestation). The objective of the study is to determine the effect of two fuel sources on the proximate composition and microbial quality of smoked *Heterobranchus longifillis*.

2.0 Materials and Methods

2.1 Fish Sample Collection

Samples of *Heterobranchus longifillis* comprising of different sizes were purchased from Fishermen at fish-landing site at Wadata in Makurdi, Benue State, Nigeria. The length and weight were taken using a measuring board calibrated in centimeters and digital weighing balance, respectively. The fish were eviscerated and washed thoroughly with clean water. After washing, 20 of the fish samples were divided into two parts (10 each) per fuel source.

2.3 Smoking of Fish

Fish samples were placed in different smoking kiln and smoked using sawdust and rice husk, respectively. The fish were monitored constantly to prevent burning and they were smoked for a period of two to four days. The smoked dried fishes were weighed and packed for further analysis.

2.4. Preservation of Fish

The smoked fish samples were analyzed immediately after smoking to determine the proximate and microbial load at week 0 before storing in the laboratory condition for a period of two months (8 weeks). Samples were taken after 8 weeks for analysis to determine the effects of storage time on the nutritional composition of the smoked fish using different fuel sources.

2.5 Proximate Analysis

The proximate analysis of the smoked fish samples was carried out immediately after smoking at week 0 according to **A.O.A.C (2005)** in the Department of Biological Sciences, Joseph Sarwuan Tarka University, Makurdi. Thereafter, the analysis was carried out after storage of smoked fish at week 8.

2.5.1 Determination of moisture (%)

Moisture or water content was determined using the oven method. 5g of the samples were transferred into crucibles of known weight. The crucibles with the samples were then covered with their respective lids. On placing the crucibles in the oven, the lid was removed and the temperature was set at 105˚C to effect drying. The samples were allowed to remain in the oven for 1 hour to dry to constant weight and then cooled in a **desiccator** before weighing.

The percentage of moisture was calculated thus

$$\%\text{moisture} = \frac{wt\ of\ sample+dish\ before\ drying - wt\ of\ sample+dish\ after\ drying}{weight\ of\ sample\ taken} \times 100$$

2.5.2. Determination of ash

Percentage ash content was determined by incineration. 5g of sample was accurately weighed and transferred into a porcelain dish of known weight. This was placed into a preheated muffle furnace and ignited at 500˚c for 6 hours and a gray ash resulting. This was cooled in a desiccator and weighed.

Calculation;

$$\%\text{Ash} = \frac{\textbf{weight of gray ash}}{\text{weight of sample}} \times 100$$

2.5.3 Determination of Crude protein (%)

The crude protein was determined using Kjeldhal method. 2g of ground sample was weighed and wrapped in filter paper and put in a kjeldhal digestion flask. Catalyst mixture (8g) containing copper sulphate and Potassium sulphat ($CUSO_4$) in ratio 1:2 then take 0.5 g to add and gently swirled unto no particle adheres to the bottom of the flasks. Four piece of anti-bumping granule was added. The flask was then put on kjeldhal digestion apparatus for 3 hours until the liquid turns light green, the digested sample was cooled, diluted with 100ml distilled water, Aliquot (5ML) of the diluted solution with 7ml of 60% of sodium hydroxide was put into markham distillation apparatus and distilled into 5ml boric acid containing 4 drops of methyl red-methylene blue indicator until about 50ml of distillate was collected. The distillate was then titrated with standard 0.01N hydrochloric acid to purple color end point. A blank titration will be carried out using 2g of sucrose in place of the sample and its result will be subtracted from all the titre values obtained for each sample

The percentage nitrogen (%) will be calculated using the formula

$$\%N = \frac{(a-b)\times 0.01\times 0.0014\times v\times 100}{W\times Va}$$

Where;

a = Titre value of the digested sample
b = Titre value of blank sample
c = Volume made
w = Weighted of dried sample in grams
Va = Volume of a liquor used
0.01 = normality of HCI
0.0014 = Relative molar mass of nitrogen
%Crude protein -%N×6.25, where 6.35 is conversion factor.

2.5.4. Determination of ether extracts (%)

The ether extract was determined using the soxhelt method. 3 grams each of dried samples was weighed, put into label thimbles of known weight and plugged with cotton wool. The thimbles were placed in the extractor connector connected to a weighed flask containing 100ml of petroleum ether (40-60^{0}C) and a reflux condenser. It was extracted thereafter for 4 hours under reflux. The residue was transferred to a small mortar, grind lightly, returned into the thimble and extracted for further 1 hour. Thimble were removed and most of the solvent from the flask was distilled into extractor, recovering each fraction. After extraction, the solvent and residual water contents in the thimble was evaporated by drying in the oven for 2 hours at 70 ^{0}C after which the thimble were removed, cooled in a desiccator and weighed.

Calculation:

$$\%\text{ crude fat (ether extract)} = \frac{W3-W2}{W1}\times 100$$

Where;

W1 = weight (g) of sample before extraction
W2 = weight (g) of flask without fat
W3 = weight (g) of flask with fat

2.5.5. Determination of crude fibre (%)

The crude fibre was determined using the acid-alkali hydrolysis method. 3g of the dried, fat-free sample was weighed into conical flask. 20ml of hot sulphuric acid was added; the flask was placed under the condenser and brought to boiling within a minute. It was boiled gently for about 30minutes. Using distilled water to maintain volume and wash down the particles adhering to the sides of the flask, it was filtered thereafter through whatman No.541 paper in a Buchner funnel and the funnel washed well with boiled water.

The residue was transferred back into the flask and 200ml hot sodium hydroxide solution was added. It was made to boil within a minute and then allowed boil for about 30 minutes. It was filtered as done before and washed with boiled water, 1% hydrochloric acid and with boiled water again; washed twice with alcohol trice with ether/acetone and the insoluble matter transferred into the crucible. It will be dried at 100^o$_C$ to constant weight, cooled in a desiccator and weighed. The insoluble matter was then put in a muffle furnace at 550^{o}C for an hour to ash, cooled in a desiccator and weighed again.

Calculation:

$$\%\text{ CF} = \frac{weight\ of\ insoluble\ matter - weight\ of\ ash}{weight\ of\ sample} \times 100$$

2.5.6. Determination of nitrogen free extract (%)

The nitrogen free extract will be determined by difference.

%NFE =100- (%moisture +%ash+%CP +% EE +%CF)

2.6 Microbial Analysis

The samples of the smoked *Heterobranchus longifilis* were assessed for total viable count and coliform count before storage (week 0) and 8 weeks duration of storage, respectively. 1.0g of fish sample was ground into powder and mixed with 9 ml peptone water to make a stock solution from where serial dilutions were made. 1ml from the stock solution was put into 9ml peptone water till dilution factor 10-2. TVC will be placed on nutrient agar and coliform on Eosine Methylene blue agar.

2.6.1. Identification of Microbes

One gram of fish sample was weighed using digital weighing balance and serially diluted in distilled water to 10^{-4} as described by Onyeagba (2004). One Ml aliquot from 10-3 to 10-5 was inoculated on sterile media plates using the pour plate method; the plates were incubated at 37˚C for 24 hours. Physical observation was done after the incubation period to check for development of colonies (Cheesebrough, 2005). Visible colonies on the plate were counted, plate that have between 30-300 colonies were recorded, those with over 300 colonies were recorded as too numerous to be counted (TNTC) while those with fewer than 30 will be recorded as too few to be reliable Viable count was counted using the formula;

$$\text{Number of organisms} = \frac{\text{Number of Colonies}}{\text{Dilution factor}} = \frac{}{\text{Volume of inoculation}}$$

2.7 Statistic Analysis

Data obtained was subjected to one way analysis of variance (ANOVA) and multi variance at 5% level of significance using SPSS version 21. Means were separated using Duncan multiple range tests.

3.0 Results

3.1 Proximate Composition of *H. longifilis*

Table 1 presents the results of proximate analysis of *Heterobranchus longifilis* immediately after smoking and storage using rice husk. Crude protein value was recorded as (62.25±0.49), crude fats (8.07±0.01), ash (7.57±0.05) and fibre (3.13±0.01) were all observe to be higher for initial (immediately after smoking) of *H. longifilis* with rice husk compared to the final (after storage) which recorded the following proximate values; crude protein (60.29±0.01), crude fats (8.03±0.01), Ash (4.25±0.03) and fiber (1.20±0.00) using the same heat source. However, moisture was reported to be higher at final (9.45±0.02) (after smoking and storage) than initial (7.24±0.01). There was a significant difference in crude protein, crude fats, ash, moisture and fibre between initial and final smoked *H. longifilis* using rice husk as a heat source.

Results of proximate composition of *H. longifilis* using sawdust as heat source is presented in Table 2. The crude protein (60.94±0.34), crude fats (7.05±0.01), ash (7.44±0.06) and fibre (3.19±0.02) were higher at initial (immediately after smoking) for *H. longifilis* smoked with sawdust compared to final (after storage) crude protein (56.68±0.30), crude fats (7.01±0.01), ash (4.09±0.10) and fiber (1.09±0.01) for *H.longifilis* smoked with sawdust respectively. However, the moisture at initial (9.71±0.06) for sawdust was less than final (12.64±0.09). There was significance difference in crude protein, crude fats, ash, moisture and fiber between initial and final smoked fish with sawdust.

Table 1: Proximate Composition of *H. longifilis* immediately after Smoking and Storage using Rice Husk (% as dry basis).

Parameters	Initial Week 0	Final Week 8	P-value
Moisture	7.24 ± 0.01	9.47 ± 0.02	0.00
Crude protein	62.25 ± 0.49	60.29 ± 0.06	0.00
Crude fat	8.07 ± 0.01	8.03 ± 0.01	0.00
Ash	7.57 ± 0.05	4.25 ± 0.03	0.00
Fiber	3.13 ± 0.01	1.20 ± 0.00	0.00

Table 2: **Proximate Composition of *H.longifilis* immediately after Smoking and Storage using Sawdust (% as dry basis).**

Parameters	Initial Week 0	Final Week 8	P-value
Moisture	9.71 ± 0.06	12.64 ± 0.09	0.00
Crude protein	60.94 ± 0.34	56.68 ± 0.30	0.00
Crude fat	7.05 ± 0.01	7.01 ± 0.01	0.02
Ash	7.44 ± 0.06	4.09 ± 0.10	0.00
Fiber	3.19 ± 0.02	1.09 ± 0.01	0.00

3.2 Microbial Quality of *H. longifilis*

Results of the microbial load (TVC and TCC) of *H. longifilis* immediately after smoking (initial) using different fuel sources are shown in table 3, whereas the results of the microbial load of *H. longifilis* after storage (final) using different fuel sources are shown in table 4 respectively.

From table 3, Rice husk recorded $9.51x10^4$±0.01 TVC while sawdust recorded $6.56x10^4$±0.00 TVC. Also, in addition while rice husk recorded $2.94x10^3$±0.01 TCC, sawdust recorded $2.97x10^3$±0.02 TCC. However, there was no significance difference between TVC and TCC between the two heat sources immediately after smoking. From table 4, rice husk recorded $8.26x10^6$±0.00 TVC, sawdust recorded $5.37x10^5$±0.01 TVC. And also rice husk recorded $4.63x10^6$±0.10 TCC, sawdust recorded $3.10x10^5$±0.01 TCC. Significant difference was observed between TVC and TCC between the two heat sources immediately after smoking.

Table 3: **Microbial load of *Heterobranchus longifilis* using different Fuel Sources immediately after Smoking.**

Heat source	TVC Week 0	TCC Week 0
Rice husk	$9.51 \times 10^4 \pm 0.01x10^4$	$2.94 \times 10^3 \pm 0.01 \times 10^3$
Sawdust	$6.56 \times 10^4 \pm 0.00$	$2.97 \times 10^3 \pm 0.02 \times 10^3$
p-value	0.10	0.30

Table 4: **Microbial load of *Heterobranchus longifilis* using different Fuel Sources after Storage.**

Heat source	TVC Week 8	TCC Week 8
Rice husk	$8.26 \times 10^6 \pm 0.00$	$4.63 \times 10^6 \pm 0.10x10^6$
Sawdust	$5.37 \times 10^5 \pm 0.01$	$3.10 \times 10^5 \pm 0.01$
p-value	0.00	0.00

4.0 Discussion, Conclusion and Recommendation.

4.1 Discussion

4.1.1 Nutritional Composition

The percentage crude protein was higher in rice husk as a fuel source when compared to sawdust. For each of the fuel sources the crude protein values dropped after storage (final), the decrease in crude protein value could be attributed to increase in re-absorption of moisture, pest and microbial attack. There was relatively high level of fats in *H. longifilis* smoked with rice husk (8.07±0.01) than sawdust (7.05±0.01).

The *H. longifilis* smoked with rice husk significantly recorded high ash content as compare to the one smoked with sawdust. After storage the ash content value of the experimental fish dropped for both heat sources (rice husk and sawdust respectively) which may be attributed to the temperature during storage. The moisture content of *H. longifilis* smoked with sawdust was more than that of rice husk. During storage for a period of 8wks, there was increase in moisture of the fish samples for both fuel sources and this could be as a result of re-absorption of moisture from the environment or improper drying of the fish. The percentage crude fibre was higher in sawdust as a heat source as compare to rice husk.

4.1.2 Microbial Quality

About one-third of the worlds food productions is lost annually as a result of microbial spoilage. In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods (**Lund *et. al.*, 2000).** Smoked fish and

shellfish products can be a source of microbial hazards including *Listeria monocytogenes, Salmonella spp* and *Clostridium botilinum* (Heintz and Johnson, 1998**).** The samples of *H. longifilis* smoked with rice husk were having a TVC of 9.51 x 10^4cfu/g and 8.26 x 10^6 initial and final respectively. The samples smoked with sawdust were having a TVC of 6.56 x 10^3cfu/g and 5.37 x 10^5cfu/g initial and final respectively. Also, samples smoked with rice husk shows a TCC of 2.94 x 10^3cfu/g and 4.63 x 10^6cfu/g initial and final respectively and those smoked with sawdust fuel source shows 2.94 x 10^3 cfu/g initial and 3.10 x 10^5cfu/g final (after storage). The high values of microbes recorded in the fish smoked with rice husk as compared to sawdust is attributed to the high nutrition value of the fish. This implies that the fish smoked with rice husk will have a long shelf life under storage because of its high nutritional value.

4.2 Conclusion

Based on the results of findings it was observed that fuel source has direct implication on the nutritional and microbial quality of smoked fishes. Alternative fuel source to replace firewood will not only abate financial problems associated with buying of firewood's but also environmental problems associated with deforestation. The continuous cutting down of trees for fuel source is causing devastating effects to our environments, therefore the results of the present findings as provided alternative sources to firewood with improved or better results as compared to the former. The high values of microbes recorded in the fish smoked with rice husk as compared to sawdust is attributed to the high nutrition value of the fish. This implies that the fish smoked with rice husk will have a long shelf life under storage because of its high nutritional value. Further research should be carried out on other fish species using alternative heat sources like coconut husk and palm kernel to reduce the cost of fish smoking in other to maximize profit for fishmongers.

Acknowledgements:

Authors are grateful to the Department of Fisheries and Aquaculture, College of Forestry and Fisheries, Federal University of Agriculture, Makurdi, Benue State, for creating and enabling environment to carry out this work.

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11/25/2023