EFFECTS OF RICE HUSK EXTRACT ON THE QUALITY OF SMOKED FISH STORED AT AMBIENT TEMPERATURE

Gambo, Ahmad[1], **Negbenebor**, A.Charles[2], and **Dauda**, Y.Mustapha[3]
[1]Department of Food Science and Technology, Bayero University Kano, P.M.B. 3011, Kano State, Nigeria
[2]Department of Biological Sciences, Federal University Otuoke, P.M.B 176, Yenagoa Bayelsa State, Nigeria
[3]Department of Food Science and Technology, Federal Polytechnic, KauraNamoda P.M.B 1012, Zamfara State, Nigeria
ahmadgambo@gmail.com

Abstract
Fish is an important source of protein, providing essential amino acids, low in fat and cholesterol; and also rich in calcium, phosphorus, vitamins A and D. Fish is an extremely perishable commodity. Losses as high as up to 50% has been recorded following postharvest, leading to a huge loss in revenue. Five (5.0) kg of African cat fish (Bagrus bajad) and rice bran (Oryza glaberrima) were obtained from Yankura fish market and a local rice mill respectively, both in Kano State. The fish samples were gutted, washed thoroughly, and divided into four groups. The groups were treated separately with0, 0.5, 1.0 and 1.5% (W/V) of the prepared husk extract, smoked in the smoking kiln for 5 hours at a temperature of 56 to 62^{0}C,cooled and evaluated for sensory scores after smoking. The microbial and chemical analysis were carried out on months 0,1,2 and 3 following storage at ambient temperature. The rice husk extract had no effect ($p<0.05$) on microbial growth and sensory scores irrespective of concentration. However the Free Fatty acid (FFA) values of the treated samples ranged from 0.1870 to 0.2585Meq/kg and were lower ($p<0.05$) than the non-treated controls (0.4170Meq/kg) after three months of storage. The sample treated with 1.5% rice husk extract had the least value of 2.8 mg/100g, the one treated with 1.0% extract had 4.2 mg/100g and sample treated with 0.5% rice husk extract had 5.6 mg/100g.The results of the Bacterial count shows that the samples on month 0 had values of 1.26x10^3, 1.20x10^3, 1.30x10^3 and 1.35 x 10^{3}cfu/g for samples 111, 121, 131 and 141 respectively. There was no significant difference ($p>0.05$) between the samples from month 0 to 3rd month of storage irrespective of treatment. The results of the mould count indicated that at month 0, samples 111, 121, 131 and 141 had 1.36x10^3, 1.29x10^3, 1.33x10^3 and 1.41x10^{3}cfu/g respectively, with no significant difference ($p>0.05$) between the samples, regardless of the treatment. The mould increased by the third month of storage when compared to the first month. The findings for this study suggest that the samples are considered to be within the acceptable limits up to the third month of storage. The use of rice husk extracts will be beneficial to the fish processing industry as it would enhance the quality and oxidative stability of the smoked fish products.

Key words: Smoked fish, Rice husk extract, antioxidants, Cat fish, quality, ambient temperature

Received: 15.02.2018 Reviewed: 31.05.2018 Accepted: 06.06.2018

INTRODUCTION

Fish is an important source of protein, providing essential amino acids, low in fat and cholesterol; and also rich in calcium, phosphorus, vitamins A and D (Aremu *et al.*, 2014). Fish is an extremely perishable commodity. Losses as high as up to 50%has been recorded following postharvest, leading to a huge loss in revenue (Negbenebor *et al*., 1999). This spoilage is primarily due to enzymatic and microbial actions, resulting in disagreeable taste, smell and texture, thereby reducing consumer acceptability (Brigette *et al*., 1994: Agbon *et al.,* 2002). Other factors responsible for fish spoilage include fish health status, presence of parasites, bruises and wounds on the skin, methods of handling and preservation (Akinneye *et al.*, 2007).

In Nigeria, a number of processing techniques such as chilling, freezing, salting, canning, drying and smoking are employed. However, smoking is the most popular method of fish processing in the absence of refrigerated storage facilities (Eyo, 2000). The use of smoke from smouldering wood for the preservation of perishable foods dates back to ancient civilization. The raw materials preserved in this way include fish and fish products which were most vulnerable to deterioration (Clucas, 1992). The bacteriostatic, bactericidal and antioxidant functions of smoke and the dehydration effect of the process were used inadvertently by the early fish processors in the preservation of fish. Although consumers are generally attracted by the flavour of smoked fish, its nutritive value is also of paramount important since every consumer want to obtain good quality protein from fish consumption.

Rice husk is the hard outer layer of rice and consists of combination of aleuron and pericarp along with germ. It is an integral part of whole grains and several thousand tones are produced annually in Nigeria as by-product of milling industry, without any known economic benefit to the local fish processing industry. It is cheap and readily available. Olorusanya *et al.*(2009) reported lower TBA values in pork meat treated with 1.5% rice husk extract stored under refrigerated temperature for 6 days. The anti-oxidants properties of rice husk and rice bran are apparently related to their phenolic contents. However, information on the effects of rice husk extract on quality of smoked fish product is scanty. Therefore the main objective of this study was to investigate the effects of rice husk extract on quality of smoked fish. The use of rice husk extract might actually improve the shelf life of smoked fish products and reduce postharvest fish losses for the fisher folks.

MATERIALS AND METHODS

Collection of Samples

Raw fresh fish samples (5kg) of African catfish (*Bagrus bajad*) were purchased from Yankura Fish Market, Kano State, Nigeria. Samples were washed immediately thoroughly with potable water, packed in ice boxes 90cm x 25cm x 35cm (LXBXH) and transported to Processing Laboratory, Kano University of Science and Technology Wudil for processing. Samples of sawdust (15kg), rice bran (*Oryza glaberrima*) (2kg) were collected from a local rice mill in Kura town, Kano State, Nigeria.

Preparation of the samples

Fish samples

Upon arrival fish samples were re-washed thoroughly with potable water then headed, gutted, and washed thoroughly with clean water. The rice husk was finely ground using a manual grinder to improve extraction and then soaked (macerated) in 90% methanol in the ratio of 1:3 (husk: methanol) for 24 hours (Olorunsanya *et al*., 2009).The miscella was decanted and evaporated using open-dish evaporation to obtain 20 mls of brownish yellow extract. The samples were dried partially under the sun (37- 35^{o}C) for an hour. The rice husk extract was rubbed on the fish samples. The fish samples were divided into four portions, each weighing one kg. The first portion was the untreated control sample, coded (111), the second portion was treated with 0.5% extract (121), the third portion was treated with 1.0% extract (131), and the fourth portion was treated with 1.5% extract (141).

Smoking process

Smoking of the fish samples was carried out using a smoking kiln fabricated by the Department of Food Science and Technology, Kano University of Science and Technology Wudil (Figure 1) to simulate what is practiced by local fish mongers (Salan *et al*., 2006). Smoking was carried out in rectangular kiln (0.6m by 1.6m) as seen in the Appendix. Fish were placed in trays at a distance of 58 cm from the fire point, and smoking was made with addition of small amount of saw dust at regular intervals. Smoking was carried out for 5 hours at a temperature of 56-62^{0}C. After smoking, the fire was extinguished and the samples were allowed to cool down to ambient temperature (28-36^{0}C), kept in transparent polyethylene bags for microbiological, chemical and sensory analysis for a period of four months at predetermined intervals.

Plate 1 : The Smoking kiln used for the experiment

Microbiological Analysis

Aerobic Plate Count

A 10-g portion of the smoked fish product was removed from each test sample and homogenized at high speed for 2 min in a sterile blender with 90 ml of sterile potassium phosphate buffer (0.05 M, pH 7.0). The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 ml aliquots of the diluents were poured into duplicate Petri plates for aerobic plate count (APC) with plate count agar (Difco, Detroit, MI, USA) containing 3% NaCl. Bacterial colonies were counted after the plates were incubated at 32ºC for 48 h. The bacterial counts of the smoked fish products were expressed as colony forming units (cfu)/g (AOAC, 2000).

Mould Count

For Mould count,3g of the fish sample was weighed out into 27ml of diluent to make the 10^{-1}, 10^{-3} and 10^{-5}serialdilutions of the fish stock mixture which were inoculated into potato dextrose agar (PDA) in duplicates to which antibiotic mixture was added to discourage bacteria growth. Plates were incubated at ambient temperature for 3-5 days. Developing colonies were counted using a Geiger Colony counter (AOAC, 2000).

Chemical Analysis

Free Fatty Acid (FFA)

Five grams of the comminuted smoked fish sample were mixed with exactly 50 ml of neutral ethanol in a flask and the FFA was determined by methods as described by AOAC (1984).

Trimethylamine (TMA) value

One gram of sample was measured into a conical flask containing 4g of magnesium oxide and 24ml of distilled water, the flask connected to a distillation unit and steam distilled into a flask containing 2% boric acid into which 2drops of methyl red indicator was added. The trimethylamine (TMA) values were carried out by AOCS method (1994).

Sensory Evaluation

Semi trained panelists were made to evaluate the smoked fish samples on taste, flavour, colour and general acceptability, using the 7-point hedonic scale at 0.05 level of significance. With like extremely (7), like much (6), like moderately (5), neither like nor dislike (4), dislike moderately (3) dislike much (2) and dislike extremely (1) respectively (Ihekoronye and Ngoddy 1995)

Statistical Analyses

Data were subjected to statistical analyses using SPSS v.9.0 for Windows. Analysis of variance (ANOVA) was used and statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Trimethylamine (TMA) values

The results of the TMA analysis (Table 1) shows that samples treated with 0.5%, 1% rice husk extract and the control samples had similar values of 1.4 mg/100g while the sample treated with 1.5% rice husk extract had a lower ($p<0.05$) value of 0.75 mg/100g in month 0 of storage. These values increased significantly for all the samples irrespective of treatment up to the third month of storage at ambient temperature. In month 3, the untreated control sample had the highest TMA value of 7.0 mg/100g and was significantly higher ($p<0.05$) than all the treated samples. (Table 1)' The sample treated with 1.5% rice husk extract had the least value of 2.8 mg/100g, the one treated with 1.0% extract had 4.2 mg/100g and sample treated with 0.5% rice husk extract had 5.6 mg/100g. Similar trend was observed by Krisen *et al.*, (2014) AndIbrahim *et al.*,(2008) when samples treated with liquid smoke of coconut shell were compared with untreated controls. The initial values of TMA in traditional-smoked whole and gutted fish were 1.68 and 0.98 mg\100g flesh respectively (Ibrahim *et al.*, 2008). The presence of TMA in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO), which is naturally present in the living tissue of many marine fish species (Huss, 1995).Rice bran and oil have been shown to be rich in potent antioxidants such as oryzanol and tocopherol (Lloyd *et al.*, 2000; Gopala-Krishna 2002).

These may have reacted with TMAO and therefore responsible for effectively controlling the TMA production during storage of samples treated with rice husks at ambient temperature. Fish with TMA concentrations less than1.5 mg TMAN/100 g is considered to have good quality while between 10-15 mg TMAN/100 g is considered as an acceptable limit (Huss, 1995). Fish is considered unacceptable or spoiled if they have TMA values above 30 mg N /100 g (Bonnel, 1994). The findings for this study suggest that the samples are considered to be within the acceptable limits up to the third month of storage. From the results, increase in treatment lowers the level of TMA in the smoked fish samples which indicates that the rice husk extract retards the formation of Trimethylamine oxide.

Free Fatty Acid (FFA) Values

The results of the Free Fatty acid (Table 2) shows that the samples in month 0 of production had values of 0.1259, 0.1860, 0.0945 and 0.0945 meq/kg for samples 111, 121, 131 and 141 respectively. There was significant difference ($p<0.05$) in FFA values between the samples from month 0 to the third month of storage, with the untreated control having higher values than the treated samples. The higher the concentration of the husk extract, the lower the FFA values after 3 months of Storage. Rice bran and oil is rich in phenolic compounds such as oryzanol and tocopherol (Lloyd *et al.*, 2000; Gopala-Krishna 2002). These may have been responsible for lowering the free fatty acid content of the smoked fish samples during storage. El-Oudiani and Missaoui (2013) reported that when samples of smoked fish, kept under freezing (-20ºC) storage for a period of six months were evaluated for Free Fatty acid content, the initial value was 0.95% oleic and the value at the end of six month storage was found to be 2.03% oleic acid. The results of this study also agrees with the findings of Idris *et al.* (2010) where samples of smoked dried catfish treated with different ginger concentrations, stored for eight weeks, increased in FFA values irrespective of treatment. However the control samples had higher FFA values and was significantly different ($P<0.05$) when compared with the treated samples. It is well known that free fatty acid (FFA) values are as a result of enzymatic hydrolysis of lipids. Eyo (2001) stated that most oil rancidity is noticeable when the FFA calculated as oleic acid is in the region of 0.5 – 15%. All the smoked products for this study are below the stated value.

Microbial Count

The results of the Bacterial count (Table 3) shows that the samples on month 0 had values of $1.26x10^3$, $1.20x10^3$, $1.30x10^3$ and $1.35 x 10^3$cfu/g for samples 111, 121, 131 and 141 respectively. There was no significant difference ($p>0.05$) between the samples from the month 0 to 3 of storage irrespective of treatment. The values were $1.82x10^3$, $1.87x10^3$, $1.84x10^3$ and $2.14x10^3$cfu/g for samples 111, 121, 131 and 141 respectively after 3 months of storage. Result indicate that the use of husk extract had no significant effect ($p>0.05$) on total aerobic plate count. The findings of this study is in line with the findings of Min *et al.*, (2009) where the bacterial counts of catfish patties samples treated with rice bran extract did not differ significantly ($p>0.05$) with the bacterial count of the untreated control sample. The values increased significantly ($p<0.05$) in all the patties during 8 days of storage. The values for this study did not reach unacceptable limit of 10^6cfu/g as stated by ICMSF (1986) for cooked fish samples. It was also observed that microbial growth was not inhibited by the Rice extract and rice bran extract.

TABLE 1. Effect of rice husk extract on Trimethylamine values of the fish samples during storage at ambient temperature (mg/100g)

SAMPLE	111	121	131	141
Month 0	1.4 ±0.10[a]	1.4 ±0.12[a]	1.4 ±0.03[a]	0.75 ±0.07[b]
Month 1	2.8 ±0.03[c]	2.1 ±0.07[d]	2.1 ±0.06[d]	1.4 ±0.02[a]
Month 2	2.8 ±0.08[c]	2.1 ±0.11[d]	2.1 ±0.10[d]	2.1 ±0.14[d]
Month 3	7.0 ±0.15[g]	5.6 ±0.05[f]	4.2 ±0.00[e]	2.8 ±0.06[c]

[1]Values are means of three determinations ±Standard deviation; [2]Sample with similar superscript in the same row or column are not significantly different at 5% level significance; Key:111 = Untreated control sample; 121 = Sample treated with 0.5% rice husk extract; 131 = Sample treated with 1.0% rice husk extract; 141 =Sample treated with 1.5% rice husk extract

TABLE 2: Effect of rice husk extract on free fatty acid (FFA) values of the smoked fish during storage at ambient temperature (Meq/kg)

SAMPLE	111	121	131	141
Month 0	0.1259 ± 0.007[b]	0.1860 ± 0.008[a]	0.0945 ± 0.002[c]	0.0945 ± 0.002[c]
Month 1	0.1860 ± 0.008[a]	0.1975 ± 0.004[a]	0.1055 ± 0.005[bc]	0.1065 ± 0.004[bc]
Month 2	0.2585 ± 0.013[d]	0.2005 ± 0.012[ae]	0.1180 ± 0.000[bc]	0.1150 ± 0.004[bc]
Month 3	0.4170 ± 0.016[f]	0.2585 ± 0.013[d]	0.2330 ± 0.008[de]	0.1870 ± 0.007[a]

[1]Values are means of three determinations ±Standard deviation
[2]All Samples with similar superscript in both row and column are not significantly different at 5% level of significance
Key: 111 = Untreated control sample; 121 = Sample treated with 0.5% rice husk extract; 131 = Sample treated with 1.0% rice husk extract; 141 = Sample treated with 1.5% rice husk extract

TABLE 3: Effects of rice husk extract on the Total aerobic plate count of the smoked fish during storage at ambient temperature (cfu/g)

SAMPLE	111	121	131	141
Month 0	$1.26 \times 10^3 \pm 0.08$[a]	$1.20 \times 10^3 \pm 0.14$[a]	$1.30 \times 10^3 \pm 0.07$[a]	$1.30 \times 10^3 \pm 0.07$[a]
Month 1	$1.90 \times 10^3 \pm 0.01$[a]	$1.97 \times 10^3 \pm 0.07$[a]	$1.90 \times 10^3 \pm 0.05$[a]	$1.96 \times 10^3 \pm 0.05$[a]
Month 2	$1.96 \times 10^3 \pm 0.05$[a]	$2.02 \times 10^3 \pm 0.05$[a]	$2.00 \times 10^3 \pm 0.02$[a]	$2.28 \times 10^3 \pm 0.04$[a]
Month 3	$1.82 \times 10^3 \pm 0.12$[a]	$1.82 \times 10^3 \pm 0.12$[a]	$1.84 \times 10^3 \pm 0.08$[a]	$2.14 \times 10^3 \pm 0.01$[a]

[1]Values are means of three determinations ±Standard deviation
[2]All Samples with similar superscript in the same row are not significantly different at 5% level of significance
Key: 111 = Untreated control sample; 121 = Sample treated with 0.5% rice husk extract; 131 = Sample treated with 1.0% rice husk extract; 141 = Sample treated with 1.5% rice husk extract

Ibrahim *et al.*, (2008) reported values of traditional-smoked whole and gutted fish to be 5.0×10^3 and 8.9×10^3cfu/g flesh, respectively at the initial stage. The corresponding counts of liquid–smoked whole and gutted fish were 6.0×10^4 and 6.6×10^3cfu/g flesh, respectively. After two weeks, TVC reduced in all products and then gradually increased up to 8 weeks. A reverse trend was observed in this study where the bacterial counts increased in the first and second month of storage and reduced in the third month of storage. The differences in result may have been as a result of species differences, method of processing and humidity of the environment. Akinwumi *et al.*, (2015) evaluated three samples of smoked tilapia fish obtained from Ondo State, among the tilapia species, the highest total count (2.9×10^5cfu/g) was found in the samples obtained from Akure market while the lowest count (1.3×10^4cfu/g) was observed in the samples hawked in Akungba-Akoko. The findings in this study suggest that Rice husk extract has no effect on the total bacterial count of the smoked fish samples.

The results of the mould count (Table 4) indicated that at month 0, samples 111, 121, 131 and 141 had 1.36×10^3, 1.29×10^3, 1.33×10^3 and 1.41×10^3cfu/g respectively, with no significant difference (p>0.05) between the samples, regardless of the treatment. The mould increased by the third month of storage when compared to the first month. At the end of the third month of storage samples 111, 121, 131 and 141 had 2.05×10^3, 2.02×10^3, 2.14×10^3 and 2.14×10^3cfu/g respectively, with no significant difference (P >0.05) between the samples. Result indicate that the use of husk extract had no significant effect (p>0.05) on mould count. Idris *et al.* (2010) reported that the higher the concentration of ginger the higher the antifungal effects. Ibrahim *et al.,* (2008) reported initial values for traditional-smoked whole and gutted fish as $2.4x10^3$ and $6.0x10^2$cfu/g flesh, respectively. The corresponding values of liquid–smoked whole and gutted fish were $1.4x10^3$ and $4.46x10^2$cfu/g flesh, respectively. The mould counts in this study were within the same range; however, results suggest that Rice husk extract has no effect on the mould count of the smoked fish samples.

TABLE 4: Effects of rice husk extract on the Mould count of the smoked fish product during storage at ambient temperature (cfu/g)

SAMPLE	111	121	131	141
Month 0	$1.36 x10^3 ±0.19^a$	$1.29 x10^3 ±0.01^a$	$1.33 x10^3 ±0.04^a$	$1.41 x10^3 ±0.01^a$
Month 1	$1.88 x10^3 ±0.08^a$	$1.83 x10^3 ±0.11^a$	$1.81 x10^3 ±0.12^a$	$1.87 x10^3 ±0.08^a$
Month 2	$1.22 x10^3 ±0.02^a$	$1.24 x10^3 ±0.05^a$	$1.20 x10^3 ±0.14^a$	$1.38 x10^3 ±0.02^a$
Month 3	$2.05 x10^3 ±0.08^a$	$2.02 x10^3 ±0.12^a$	$2.14 x10^3 ±0.19^a$	$2.14 x10^3 ±0.01^a$

[1]Values are means of three determinations ±Standard deviation
[2]All Samples with similar superscript in the same row are not significantly different at 5% level of significance
Key: 111 = Untreated control sample; 121 = Sample treated with 0.5% rice husk extract; 131 = Sample treated with 1.0% rice husk extract; 141 = Sample treated with 1.5% rice husk extract

TABLE 5. Effects of Rice husk extract on the Sensory qualities of the smoked fish in month 0

SAMPLE	111	121	131	141
Flavour	4.9 ± 1.6^a	5.5 ± 0.9^a	5.1 ± 1.1^a	6.2 ± 0.4^a
Colour	5.4 ± 1.3^a	6.1 ± 1.0^a	5.4 ± 1.0^a	5.6 ± 1.1^a
Taste	4.5 ± 1.7^a	5.1 ± 1.1^a	4.8 ± 1.2^a	5.5 ± 0.7^a
General Acceptability	4.4 ± 1.6^a	5.0 ± 1.6^a	4.9 ± 1.2^a	5.6 ± 1.0^a

[1]Samples are means of 10 determinations ± standard deviation
[2]Sensory result is based on 7 Point Hedonic Scale with (7) = Like extremely and (1) = dislike extremely
[3]Samples with different superscript in the same row are significantly different at 5% level of significance
Key: 111 = Untreated control sample; 121 = Sample treated with 0.5% rice husk extract; 131 = Sample treated with 1.0% rice husk extract; 141 = Sample treated with 1.5% rice husk extract

Sensory Analyses

The results of the sensory evaluation (Table 5) shows that the samples were not significantly different (P > 0.05) for all the sensory characteristics evaluated, regardless of treatment. The sample treated with 1.5% rice husk extract had slightly higher values, though not significantly different (p>0.05) for all the sensory characteristics with exception of colour. Swastawati *et al.,* (2012) evaluated samples of smoked stingray fish treated with Corncob liquid smoke against the sample treated with Coconut shells liquid smoke where it was found that Corncob liquid smoke treated sample received slightly higher sensory scores than samples treated with Coconut shells liquid smoke. According to Karim *et al.,* (2007), flavor is an important factor in consumer's acceptability. Based on the findings of these results the rice husk extract has no negative impact on the sensory characteristics of the fish samples.

CONCLUSION

Rice husk extract has no effect on the microbial quality of the smoked fish. However, treatment of fish samples with rice husk extract before smoking has beneficial effect especially in retarding oxidation on the product without adverse effects on the sensory quality. This will help in the keeping quality of the product. The use of rice husk will also be beneficial to the rice processing industry that would find new use for their by-product and in this manner turn waste to wealth.

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