ADAN J. Agric. 2026, 7 (1): 37-42

ISSN 2736-0385
DOI: **10.36108/adanja/6202.70.0150**

Effect of *Moringa Oleifera (Moringa*) leaf extract on the shelf life of smoked (*Oreochromis niloticus*) Tilapia fish using an improved traditional kiln

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Abstract

This study evaluated the effect of Moringa oleifera leaf extract on the shelf-life and quality of smoked Oreochromis niloticus (tilapia) processed using an improved traditional smoking kiln. Tilapia samples were treated with varying concentrations of aqueous Moringa oleifera extract (16, 19, and 21 mg/L) prior to smoking. Proximate composition and microbial load analyses were conducted bi-weekly over two months to assess the impact of treatment on moisture, protein, lipid, ash content, and microbial contamination. Results showed a significant decrease in moisture and lipid content and an increase in protein and ash concentration with increasing Moringa extract levels. Microbial analyses indicated dose-dependent reductions in major bacterial populations including Staphylococcus spp., Bacillus spp., Escherichia coli, and fungi such as Aspergillus niger on treated samples compared to controls. Despite these reductions, microbial counts remained relatively high, suggesting the need for improved hygiene and complementary preservation strategies. The findings demonstrate that Moringa oleifera leaf extract effectively enhances smoked tilapia's shelf-life by inhibiting microbial growth and improving nutritional quality when used in conjunction with an improved smoking kiln. This approach offers a sustainable, natural alternative to synthetic preservatives for fish processors, potentially reducing post-harvest losses and supporting food security in resource-limited settings.

Keywords: *Moringa oleifera*, *Oreochromis niloticus*, Improved Traditional Smoking Kiln, Microbial Load.

Introduction

Fish represents one of the most nutritious and widely consumed sources of animal protein globally. It contributes significantly to food security, particularly in developing countries where it provides affordable high-quality protein and essential micronutrients (El-Sayed, 2006). However, fish is highly perishable due to its high-water activity and neutral pH, which support microbial growth and biochemical spoilage (Hussain *et al.*, 2005). In Nigeria, postharvest losses in fishery products have been reported to reach up to 40% (Adeyeye, 2016). To mitigate these losses, effective preservation techniques are essential.

Smoking remains the most accessible and cost-effective preservation method among smallscale fish processors in developing regions (Fapohunda & Ogunkoya, 2006). It combines the effects of heat, dehydration, and antimicrobial compounds from smoke to extend fish shelf life while enhancing sensory qualities such as flavor and color (Souza *et al.*, 2020; Adeyeye *et al.*, 2016). However, traditional smoking kilns often lead to uneven

heat distribution, recontamination, and exposure to harmful smoke compounds, thus reducing the efficiency of preservation (Akinwumi *et al*., 2019).

Moringa oleifera, commonly known as the miracle tree, is a plant rich in bioactive compounds with antimicrobial and antioxidant properties (Dhakad *et al*., 2019). Its extracts contain flavonoids, phenolic acids, tannins, and isothiocyanates capable of inhibiting the growth of bacteria and fungi (Rahman *et al*., 2021). Recent studies have shown that *Moringa* extracts can improve the storage quality of smoked fish and meat products by reducing microbial load and oxidative spoilage (Ademiluyi *et al*., 2021; Sultana *et al*., 2022).

The use of natural preservatives such as *Moringa oleifera* offers a safer alternative to synthetic chemicals while supporting sustainability and consumer health. This study therefore investigates the effect of *Moringa oleifera* leaf extract on the proximate composition and microbial load of smoked *Oreochromis niloticus* processed using an improved traditional smoking kiln.

Materials and Methods

The study was carried out at the University of Abuja Fish Farm and Animal Science Laboratory, Gwagwalada, Federal Capital Territory, Nigeria. The area has an annual rainfall of 1,145–1,630 mm, with temperatures ranging between 25.8℃ and 31.1℃ (Awowole Brown, 2007). Fresh *Moringa oleifera* leaves were obtained from Voice of Nigeria Area, Lugbe, Abuja. The leaves were shade-dried, ground into powder, and 57.14 g was soaked in 1.05 L of distilled water for 24 hours. The solution was filtered and divided into three concentrations: T1 = 16.32 mg/L, T2 = 19.05 mg/L, and T3 = 21.77 mg/L. Sixty Tilapia (*O. niloticus*) weighing between 300–400 g was procured from the University fish farm. The fish were descaled, eviscerated, and washed thoroughly before treatment. Each group was marinated with *Moringa oleifera* extract according to its designated concentration.

An improved traditional kiln (Gana *et al*., 2021) made from two modified oil drums was used for smoking the fish samples. The design allowed for controlled smoke flow, efficient heat distribution, and reduced exposure to harmful emissions. Fish samples were smoked at 240°F for 12 hours and cooled before analysis.

Experimental design

A complete randomized design (CRD) was adopted where Four (4) treatments was used;

Treatment(C) being the control with no preservative. Treatment (T1) was 300ml of Aqueous

Moringa powder Extract, Treatment (T2) was 350ml of Aqueous Moringa powder Extract, Treatment (T3) was 400ml of Aqueous Moringa powder Extract which was used to marinate the fish. It was kept for some time in order for the Moringa leaf aqueous extract to penetrate through it very well before smoking.

Proximate Analysis

Samples of the fish were analysed on biweekly basis for percentage moisture, crude protein (CP), lipid, crude fibre (CF) and ash, using established protocols (AOAC, 2006)

Microbial Analysis

Determination of Microbial Load.

The samples were prepared by taking 1g representative sample aseptically from the muscle of each of the smoked and dried sample into 9ml of water in a beaker and homogenizing in blender. Four sterile test-tubes were filled with 9ml of sterile water each, then further serial dilution method was carried out using 1ml of the homogenized sample and transferring it into the first test tube. 1ml was then aseptically transferred from the second into the third test tube. These was continuously repeated until the fourth tube (Cheesbrough, 2006). 10 g of grounded sample was measured into a beaker followed by the addition of 10 mL peptone reagent. Sample was covered for 20 minutes stirred at intervals and stored under room temperature until it completely dissolves. 1 mL of dissolved

sample was injected into Rigad® Automatic Micro-analyzer (Model AA-092-088, Netherlands) inlet sample collector and set at an optimal humidity of 66 %, setting accuracy of ≤ 0.2°C, an ionization energy (0-240 eV) according to the manufacturers recommendation in order to obtain accurate readings.

For the enumeration of bacteria population, HD008 reagent was added to the machine to examine the count of the microorganisms on a metallic tray and each result was expressed in coliform forming unit (cfu) while HD 002 was added to another 5 mL of injected into the kit. *Aspergillus niger*, *Mucor spp, Penicillium sp, Neurospora sp* and *Rhizopus nigricans* population on the counting plate were identified according to colour presentation of white, brown, yellow, pink and orange respectively.

Bacteria population was expressed in 10^8 cfu while fungi was 10^5 zoospore forming unit.

- **Preparation of culture media**
  The medium used for this practical was Potato Dextrose Agar and Nutrient Agar. It was prepared according to Manufacturer's instruction. The agar was sterilized in an autoclave at 121° C for 15mins, it was then allowed to cool to about 60°C and then dispensed into petri dishes aseptically and left to cool and solidify.

- **Total bacterial/Fungi count**
  A 0.1 ml of each dilution prepared earlier was pipetted and spread plated in sterile Petri dish containing Sabouraud Dextrose agar (SDA) for fungi and Nutrient Agar for bacteria, inoculated plates was incubated at 37°C for 24hrs and 3-5days for bacteria and fungi respectively.

- **Plate Count**
  The Nutrient Agar culture plates was examined after 24hrs of incubation. The number of bacteria colonies on the plate which can have between 30-300 colonies was counted and the viable number colony forming units was calculated while the colonies that were too numerous to count (INTC) was discarded. The number of fungi colonies on the SDA plate was counted after 3-days and calculated. The formula used to calculate:

  Viable count (CFU/ml) =number of colonies formed on the plate (CFU/g) \ Volume of sample inoculated (ml)\Dilution factor.

Bacterial And Fungi Identification

Bacterial isolates were identified based on colony morphology including and biochemical tests: Color, form, and colony surface properties was noted in each isolate. Cellular morphology of isolates observed including Gram reaction. Citrate test, methyl red test, and catalase test are the biochemical tests that was performed. For fungi identification cultural morphology and lectophenol staining was used.

Statistical Analysis: Data were analysed using SPSS version 27.0. Duncan's Multiple Range Test was used to compare means at $p<0.05$.

Results and Discussion

The proximate composition of smoked Tilapia is as presented in Table 1. The protein values ranged from 59.95 – 66.01%, Lipid ranges from 9.45 – 11.79%, Crude fiber ranges from 2.56 – 3.64%, while ash values ranged from 8.10 - 9.51%. The result indicated that as the level of *Moringa oleifera* extract increases there was significant change in the values of the parameters. Therefore, *Moringa oleifera* extract significantly reduced moisture and lipid levels while increasing protein and ash contents. Increased protein and ash concentration resulted from dehydration and concentration of solids (Ayeloja *et al*., 2020). These findings agreed with Souza *et al*. (2020) and Adeyeye (2016), who reported similar proximate trends in smoked fish products.

Table 1: Proximate Composition of Smoked Tilapia (*Oreochromis niloticus*).

Parameters	Treatment				
	C	T1 (300ml/16mgL^{-1})	T2 (350ml/19mgL^{-1})	T3 (400ml/21mgL^{-1})	S.E.M
Moisture	13.5367 ^{d}	11.7283 ^{c}	11.1583 ^{b}	10.2467 ^{a}	± 0.19
Ash	8.1033 ^{a}	8.3625 ^{b}	8.6375 ^{c}	9.5100 ^{d}	± 0.09
Crude Protein	59.9533 ^{a}	60.9942 ^{a}	62.3200 ^{b}	66.0067 ^{c}	± 0.39
Crude Fiber	2.5650 ^{a}	2.5867 ^{a}	3.5892 ^{b}	3.6475 ^{b}	± 0.11
Lipid	11.7942 ^{d}	11.3058 ^{c}	10.5392 ^{b}	9.4517 ^{a}	± 0.15

Values in the same row having different superscript are significantly different from one another $p<0.05$.

Table 2 showed that *Staphylococcus spp* ranges from 3.62 - 2.26, *Bacillus spp* ranges from 0. 23 -0.54, *Escherichia coli* ranges from 4.23 - 3.21, *Streptococcus spp* ranges from 0.99 - 0.41, *Pseudomonas spp* ranges from 1.99 - 1.80, *Corynbacterium spp* ranges from 0.19 - 0.1, *Micrococcus spp* ranges from 4.78 - 3.33, *Klebsiella spp* ranges from 0.73 - 0.01.

Bacterial counts remained above the acceptable limits ($<10^5$ CFU/g) set by ICMSF (2005), suggesting possible recontamination during post-processing. This highlights the need for improved hygiene, packaging, and temperature control to ensure consumer safety (Abdullahi *et al*., 2020)

Table 2: Bacterial Load of Smoked Tilapia (*Oreochromis niloticus*) (CFU/g × 10^8).

Microbes (Cfu/g)	Treatment				
	C	T1 (300ml/16mgL^{-1})	T2 (350ml/19mgL^{-1})	T3 (400ml/21mgL^{-1})	S.E.M
Bacteria					
Staphylococcus spp	3.6208 x $10^{8\,b}$	2.6567 x 108 **a**	2.3400 x $10^{8\,a}$	2.2625 x $10^{8\,a}$	± 0.11
Bacillus spp	0.5392 x $10^{8\,c}$	0.3508 x $10^{8\,b}$	0.2800 x $10^{8\,b}$	0.2283 x $10^{8\,a}$	± 0.02
Escherichia coli	4.2258 x $10^{8\,b}$	3.2900 x $10^{8\,a}$	3.1842 x $10^{8\,a}$	3.2142 x $10^{8\,a}$	± 0.09
Streptococcus spp	0.7700 x $10^{8\,c}$	0.6342 x $10^{8\,b}$	0.4142 x $10^{8\,a}$	0.9975 x $10^{8\,d}$	± 0.04
Pseudomonas spp	1.9733 x $10^{8\,a}$	1.9858 x $10^{8\,a}$	1.8075 x $10^{8\,a}$	1.8075 x $10^{8\,a}$	± 0.05
Corynbacterium spp	0.4025 x $10^{8\,b}$	0.1883 x $10^{8\,a}$	0.1675 x $10^{8\,a}$	0.1008 x $10^{8\,a}$	± 0.02
Micrococcus spp	4.7833 x $10^{8\,b}$	3.7125 x $10^{8\,a}$	3.5225 x $10^{8\,a}$	3.3292 x $10^{8\,a}$	± 0.16
Klebsiella spp	0.7333 x $10^{8\,b}$	0.0267 x $10^{8\,a}$	0.0125 x $10^{8\,a}$	0.0067 x $10^{8\,a}$	± 0.01

Values on the same row having different superscript are significantly different from one another $p<0.05$.

The limited response of *Pseudomonas spp*. (roughly constant across treatments) was notable. *Pseudomonas* spp. is psychrotrophic and possess diverse resistance mechanisms; their persistence indicated that the treatment(s) used were less effective against this genus or that surviving cells were deeply embedded in tissue microenvironments (Gram & Huss, 2000).

Results from the Table 3 shows *Aspergillus niger* ranges from 1.47 - 0.38, *Mucor spp* ranges from 0.29 - 0.12, *Penicellium spp* ranges from 0.53 - 0.16, and *Neurospora spp* ranges from 2.29 - 0.86. Microbial counts generally decreased with increasing *Moringa* extract concentration. The antimicrobial properties of *Moringa oleifera* are attributed to its flavonoids, tannins, and

phenolic compounds (Rahman *et al*., 2021; Leone *et al*., 2016). The results align with Akinwumi *et al*. (2019) and Obodai *et al*. (2018), who reported that improved kilns and natural antimicrobials reduce microbial contamination in smoked fish.

Table 3: Fungal Load of Smoked Tilapia (*Oreochromis niloticus*) (CFU/g × 10^5).

Microbes (Cfu/g)	Treatment				
	C	T1 (300ml/16mgL^{-1})	T2 (350ml/19mgL^{-1})	T3 (400ml/21mgL^{-1})	S.E.M
Fungi					
Aspergillus niger	1.4658 x $10^{5\,a}$	0.6325 x $10^{5\,a}$	7.3767 x $10^{5\,a}$	0.3767 x $10^{5\,a}$	± 1.67
Mucor spp	0.2933 x $10^{5\,b}$	0.2483 x $10^{5\,a}$	0.1892 x $10^{5\,a}$	0.1192 x $10^{5\,a}$	± 0.02
Penicillium spp	0.5383 x $10^{5\,c}$	0.3808 x $10^{5\,b}$	0.3000 x $10^{5\,b}$	0.1633 x $10^{5\,a}$	± 0.03
Neurospora spp	2.2983 x $10^{5\,b}$	1.1500 x $10^{5\,a}$	1.1175 x $10^{5\,a}$	0.8642 x $10^{5\,a}$	± 0.09

Values on the same row having different superscript are significantly different from one another $p<0.05$.

Conclusion

The application of *Moringa oleifera* leaf extract during smoking significantly enhanced the nutritional quality and reduced microbial load of smoked *Oreochromis niloticus*. The extract's antimicrobial and antioxidant properties contributed to lower moisture and lipid contents while improving protein and ash values. Although microbial loads decreased with increasing Moringa concentration, counts remained higher than recommended safety limits, indicating that improved sanitation and storage practices are still essential.

Recommendation

Fish processors should maintain hygienic conditions to prevent recontamination during smoking and storage and incorporate natural antimicrobial coatings and vacuum packaging to extend shelf life. The improved kiln design should be promoted for rural fish processors to reduce smoke exposure and improve heat efficiency. Regular quality assessments are also necessary to ensure that the products meet food safety standards.

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