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Warm water containing wood ash as a novel sterilant to reduce microbial and aflatoxin B_1 levels in smoked-dried fish

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ABSTRACT

This study evaluated the effects of warm water containing wood ash on the microbial aflatoxin B1, and nutritional contents of smoked dried croaker (*Micropogonius undukiuts*), catfish (*Siluriformes* sp.), and tilapia (*Oreochromis niloticus*) sold in Akure, Nigeria. Raw and treated samples (cold water, warm water, and warm water with wood ash) were analyzed for microbial load using standard cultural methods, aflatoxin B_1 by spectrophotometry, and nutritional composition by chemical methods. Bacterial counts ranged from 20.0×10^3 to 29.0×10^3 CFU/g, and mold counts ranged from 2.0×10^3 to 12.0×10^3 SFU/g across different markets. Pre-treatment with the sterilizing solutions, particularly warm water containing wood ash, significantly reduced microbial and aflatoxin B_1 levels while preserving nutritional quality. Identified microorganisms included pathogenic and spoilage species such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *A. niger*, and *Rhizopus stolonifer*. The results demonstrate that warm water containing wood ash effectively reduces microbial and aflatoxin contamination in smoked fish without compromising nutritional value, suggesting it as a practical, low-cost decontamination method for improving fish safety and quality in local markets.

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1. Introduction

The increasing number of Africans living in the diaspora has significantly contributed to the growth of international trade in smoked-dried fish. Countries such as the United States and various European nations, popular destinations for African migrants have witnessed a rising demand for traditional African food products, particularly smoked-dried varieties of fish like catfish and tilapia. Nigeria alone exports an estimated five tons of dried fish monthly via airfreight, with additional quantities

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shipped by sea (1). Other West African countries, including Senegal, Ghana, Cameroon, and Côte d'Ivoire, are also major contributors to this expanding market.

Despite its economic promise, the export of smoked-dried fish from West Africa is increasingly hindered by food safety concerns. Heightened global awareness about bioterrorism, microbial hazards, and chemical contaminants such as mycotoxins has led to stricter import regulations in destination countries. According to the Food and Agriculture Organization (2), nearly 40% of dried fish exported from Africa is either rejected or destroyed at ports in the United States and Europe due to contamination, inadequate packaging, or improper labeling. Of particular concern is aflatoxin B_1, a potent mutagen and carcinogen, which frequently contaminates fish products due to fungal infestation during post-harvest handling and storage (3). These safety failures not only pose significant public health risks but also undermine the economic viability of fish exports from the region.

Fish remains a critical source of high-quality animal protein worldwide, with global per capita consumption reaching approximately 20 kg annually (4). In West Africa, fish is a dietary staple and a vital complement to carbohydrate-rich meals (5, 6). However, due to its high perishability, preservation is essential to ensure food security. In regions lacking reliable refrigeration and storage infrastructure, traditional methods such as drying, salting, fermentation, and smoking are commonly employed. Among these, smoking is the most widely used technique in Nigeria, where fish is exposed to heat and smoke from smoldering hardwood. This process reduces moisture content, inhibits microbial growth, and imparts distinctive flavor and aroma through the infusion of volatile antimicrobial compounds (7, 8).

Nonetheless, while smoking enhances shelf life and sensory appeal, it does not eliminate microbial contamination or detoxify existing mycotoxins. In open markets, smoked and smoked-dried fish are often displayed without protective packaging, exposing them to environmental contaminants such as dust, pests, and human handling. These products are frequently consumed with minimal heat treatment, such as rinsing in warm or salted water, which is insufficient to significantly reduce microbial or toxin loads.

In light of these challenges, there is an urgent need for affordable and culturally acceptable post-processing interventions that can improve the microbiological and toxicological quality of smoked fish, particularly in resource-limited settings. One such traditional remedy is the use of wood ash solution—a basic alkaline extract derived from burned plant biomass. Previous studies have demonstrated its potential in reducing aflatoxin content in grains (9, 10) and inhibiting bacterial pathogens in environmental samples (11). Despite its established role in indigenous food processing and fermentation, its application in detoxifying smoked-dried fish remains largely unexplored.

The novelty of this study lies in its scientific evaluation of wood ash solution as a post-smoking sterilant for smoked-dried fish. This approach combines traditional knowledge with modern food safety objectives by investigating the efficacy of wood ash in reducing microbial loads and aflatoxin B_1 contamination. To our knowledge, this is among the first studies to apply this

indigenous method to smoked fish preservation, while also assessing its impact on the nutritional composition of the treated product. This integrated perspective provides valuable insights into balancing detoxification with the preservation of dietary quality.

Given the central role of fish in human nutrition and its by-products in animal feed systems, improving its safety profile has wide-reaching implications. Aflatoxin-contaminated fish can enter livestock feed chains, impairing animal growth, reproduction, and immune function. Therefore, effective detoxification strategies not only protect human health and facilitate trade but also enhance animal productivity and food system sustainability.

This study aims to evaluate the effects of traditional sterilants, including wood ash solution, warm water, and saline treatments on the microbial quality, aflatoxin B_1 levels, and nutritional composition of smoked-dried fish sold in markets within Akure Metropolis, Nigeria. In doing so, it offers an indigenous, practical approach to enhancing the safety, nutritional value, and marketability of a vital protein source in both local and international contexts.

2. Materials and Methods

2.1. Sources of dried fish

A total of 90 fish samples were collected from three markets in Akure Metropolis: Oja Oba, Isinkan, and NEPA Market. Thirty croaker fish (*Micropogonius undulatus*), 10 from each market, 30 catfish (*Siluriformes* spp.), and 30 tilapia (*Oreochromis niloticus*) 10 from each market were collected in sterile polythene bags and transported to the laboratory for microbiological analysis within 1 h of collection.

2.2. Preparation of dried smoked fish and wood ash for pre-treatment

An average of 40 g of dried smoked fish tissue was mildly pulverized using a sterile mortar and pestle to increase the surface area for effective pre-treatment. Locally sourced powdery residue from the combustion of *Mangifera indica* wood was sieved to remove unwanted materials and obtain a uniform particle size. The pulverized fish tissue and sieved wood ash were then stored in sterile plastic containers until use.

Pre-treatment of dried fish in cold water, warm water, and warm water containing wood ash

The collected fish samples were sorted into three treatment groups. Ten grams of raw dried fish from each group were pre-treated by immersion in 100 ml of different sterilant solutions. One portion was dipped in sterile cold water (27 ± 1 °C) for 30 min, another in warm water at 40 °C for 30 min, and the third in warm water at 40 °C containing wood ash (1% w/v) for 30 min with intermittent agitation.

2.3. Microbial isolation and population from dried fish

The raw, dried, and pre-treated fish samples were aseptically diluted separately in buffered peptone water until the desired dilution factors were achieved for each sample (12). For bacterial and fungal isolation, 100 µl from the dilution factors of 10^{-3} and 10^{-5} for each sample were introduced into the center of sterile petri dishes in triplicate. Molten agar (nutrient agar for bacteria and potato dextrose agar for fungi) was aseptically poured and swirled to evenly distribute the sample within the molten agar, which was then allowed to solidify. Afterward, the plates were appropriately labeled and incubated at 37°C for 18-24 h for bacteria and at 28 ± 2°C for fungi for 72 h (12). The

microbial population of each sample was determined by counting the emerged distinct colonies after incubation with the aid of a colony counter (TT-20, Techmel) and expressed as colony-forming units per gram (CFU/g) for bacterial population and spore-forming units per gram (SFU/g) for the fungal population. The pure cultures obtained after sub-culturing were kept at 4°C for further use.

2.2. Bacterial and fungal identification

Bacterial isolates from the samples were presumptively identified through cultural and morphological examination, supplemented by biochemical tests in accordance with standard protocols (13). The obtained features and biochemical reactions of the bacterial isolates were compared with the documentation from standard bacteriological references. The pure fungal isolates were examined using a binocular microscope and identified based on their cultural, morphological, and microscopic characteristics. These were then compared with the detailed descriptions provided in the standard references of Barnett and Hunter (14) and Yarrow (15).

2.3. Extraction and quantification of aflatoxin B_1

50 g of finely ground smoked-dried fish sample were transferred into a 250 mL conical flask, and 15 mL of distilled water and 200 mL of chloroform were added. The mixture was agitated on a rotary shaker for 1 h to facilitate aflatoxin extraction. The extract was filtered, and the chloroform phase was collected and concentrated. For qualitative confirmation of aflatoxin B_1 (AFB_1), aliquots (5 and 10 µL) of the extract and AFB_1 standards (0.0025 µg/mL) were spotted onto silica gel thin-layer chromatography (TLC) plates using a microsyringe. The plates were developed in a solvent system consisting of toluene:ethyl acetate:formic acid (6:3:1, v/v/v) for approximately 50 min. The presence of AFB_1 was confirmed by comparison of the migration characteristics of sample spots with those of the standard.

For quantitative determination, a separate aliquot of the extract was reconstituted in 10 mL of methanol-water (7:3, v/v), homogenized for 10 min, and centrifuged to obtain a clear supernatant. Subsequently, 100 µL of the extract was mixed with 600 µL phosphate buffer (pH 7.2), 50 µL tetramethylbenzidine, and 50 µL urea peroxide. The reaction mixture was incubated in the dark for 30 min and terminated by the addition of 100 µL stop reagent. Absorbance was measured at 450 nm using a UV-Visible spectrophotometer. Aflatoxin B_1 concentrations were calculated from a calibration curve prepared using known concentrations of AFB_1 standards according to the method of Leszczynska et al. (16).

2.4. Proximate composition of smoked dried fish treated with different sterilants

The proximate composition of smoked dried fish treated with different sterilants was determined using the methods described by AOAC (17). The total ash content in the samples was measured by incinerating each sample at 600°C. The ash percentage was calculated by dividing the weight of the ash by the sample weight and multiplying by 100. Crude fat content was determined using the solvent extraction method. The fat percentage was calculated by dividing the weight of the fat by the weight of the sample and multiplying by 100. Crude fiber content was determined by digesting the sample with sulfuric acid and NaOH, followed by heat treatment. The crude fiber

percentage was calculated by subtracting the weight of the ashed sample from the weight of the digested sample, then dividing by the sample weight and multiplying by 100. Protein and moisture contents were determined according to standard protocols.

3. Results

3.1. Total viable microbial counts from dried fish samples

Table 1 presents the total viable microbial counts (TVMC) from smoked dried fish samples collected from various markets in Akure metropolis. The highest total viable bacterial count (TVBC), 29.0 × 10^3 CFU/g, was recorded in croaker fish purchased from Oja Oba Market. Additionally, both croaker and tilapia fish from Oja Oba and Isinkan markets showed the highest viable mold counts (TVMC) at 12.0 × 10^3 SFU/g. Conversely, the lowest TVBC (20.0 × 10^3 CFU/g) and TVMC (2.0 × 10^3 SFU/g) were observed in croaker fish obtained from the NEPA market.

3.2. Total viable microbial counts from fish samples treated with different sterilants

Table 2 shows the total viable microbial counts from fish samples treated with different sterilants. All sterilant treatments, cold water, warm water, and warm water with wood ash significantly reduced microbial loads in comparison to untreated samples. For instance, treating croaker from Oja Oba Market with warm water and warm water containing wood ash significantly reduced TVBC from 28.0 × 10^3 CFU/g to 1.8 × 10^2 and 0.6 × 10^2 CFU/g, respectively. Similarly, croaker from Isinkan Market treated with warm water and warm water plus wood ash showed TVBC reductions of approximately 93% and 97%, while the same treatments applied to croaker from the NEPA market led to reductions of approximately 94% and 96%, respectively. Cold water had no notable effect on TVBC in croaker from Oja Oba and Isinkan markets.

In terms of mold counts, treatment of croaker from Oja Oba with any of the sterilants significantly reduced TVMC from 12.0 × 10^3 SFU/g to 8.0 × 10^2 (cold water), 6.0 × 10^2 (warm water), and 2.0 × 10^2 SFU/g (warm water with wood ash). In contrast, cold water treatment of croaker from Isinkan and NEPA markets led to increased TVMC levels—from 8.0 × 10^3 and 2.0 × 10^3 SFU/g to 1.2 × 10^3 and 4.0 × 10^2 SFU/g, respectively. Only warm water with wood ash was effective in significantly reducing TVMC in these cases.

For catfish from Oja Oba and NEPA markets, all sterilants reduced TVBC, but warm water with wood ash was the most effective. For example, TVBC in catfish from Oja Oba decreased from 27.0 × 10^3 CFU/g to 2.2 × 10^2 (cold water), 2.1 × 10^2 (warm water), and 0.4 × 10^2 CFU/g (warm water with wood ash). In catfish from the NEPA market, the reductions were from 25.0 × 10^3 CFU/g to 1.7 × 10^2, 1.0 × 10^2, and 0.6 × 10^2 CFU/g, respectively. However, treatment with cold water increased TVMC in catfish from Oja Oba and Isinkan markets by 50% and 100%, respectively. Notably, catfish treated with warm water containing wood ash from both Oja Oba and NEPA markets recorded zero TVMC. Among the treatments applied to tilapia fish, warm water with wood ash was the most effective in reducing both TVBC and TVMC. Specifically, TVBC was reduced by 86%, 73%, and 73% in tilapia from Oja Oba, Isinkan, and NEPA markets, respectively. Overall, warm water with wood ash demonstrated the greatest efficacy in reducing microbial loads across all fish types (croaker, catfish, and tilapia).

Table 1. Total viable microbial counts from smoked dried fish samples from different markets in Akure Metropolis

Samples	Microbial count	A	B	C
Croaker	TVBC	29.0×10^3	23.0×10^3	20.00×10^3
	TVYMC	12.0×10^3	8.0×10^3	2.0×10^3
Cat fish	TVBC	27.0×10^3	21.0×10^3	25.0×10^3
	TVYMC	6.0×10^3	6.0×10^3	6.0×10^3
Tilapia fish	TVBC	28.0×10^3	22.00×10^3	26.00×10^3
	TVYMC	8.0×10^3	12.0×10^3	4.0×10^3

Values are means of triplicate determination (n=3).
Keys: TVBC: Total viable bacterial count, TVYMC: Total yeast and mold count, A- Oja oba market, B- Isinkan market, C- Nepa market.

Table 2. Total viable microbial counts from fish samples treated with different sterilants

Samples	Microbial count	$I^O\times10^3$	$T1^O\times10^2$	$T2^O\times10^2$	$T3^O\times10^2$	$I^I\times10^3$	$T1^I\times10^2$	$T2^I\times10^2$	$T3^I\times10^2$	$I^N\times10^3$	$T1^N\times10^2$	$T2^N\times10^2$	$T3^N\times10^2$
Croaker	TVBC	28.00^f	28.00^e	18.00^c	6.00^a	23.00^d	23.00^d	16.00^{bc}	7.00^a	20.00^c	16.00^{bc}	13.00^b	9.00^{ab}
	TVYMC	12.00^e	8.00^d	6.00^c	2.00^a	8.00^d	12.00^e	8.00^d	3.00^{ab}	2.00^a	4.00^b	3.00^{ab}	2.00^a
Cat fish	TVBC	27.00^g	22.00^d	21.00^d	4.00^a	21.00^d	28.00^f	24.00^e	11.00^b	25.00^e	17.00^c	10.00^b	6.00^a
	TVYMC	6.00^f	9.00^d	6.00^c	0.00^a	6.00^c	12.00^e	9.00^d	5.00^{bc}	6.00^c	3.00^b	0.00^a	0.00^a
Tilapia fish	TVBC	29.00^f	22.00^d	21.00^d	4.00^a	22.00^d	22.00^d	18.00^c	6.00^a	26.00^e	19.00^c	18.00^c	7.00^b
	TVYMC	8.00^f	6.00^{cd}	4.00^c	0.00^a	12.00^e	4.00^c	6.00^{cd}	2.00^b	4.00^c	5.00^c	6.00^{cd}	0.00^a

Values are means $\pm$ S.E of samples (n=3). Values with the same subscript letter (s) along the same rows are not significantly different ($p<0.05$) using Duncan's New Multiple Range test.
TVBC: Total viable bacterial count, TVYMC: Total viable yeast and mold count
I^O-Initial microbial count for fish bought from Oja oba market, I^I-Initial microbial count for fish bought from Isinkan market, I^N-Initial microbial count for fish bought from Nepa market, $T1^O$, $T1^I$, $T1^N$- Microbial count of fish pre-treated with cold water bought from Oja oba, Isinkan and Nepa market, respectively. $T2^O$, $T2^I$, $T2^N$- Microbial count of fish pre-treated with warm water bought from Oja oba, Isinkan and Nepa market, respectively. $T3^O$, $T3^I$, $T3^N$- Microbial count of fish pre-treated with warm water + wood ash bought from Oja oba, Isinkan and Nepa market, respectively.

3.3. Identities of microorganisms isolated from dried fish samples

Tables 3a and 3b present the identities of bacterial and fungal isolates obtained from dried fish samples. The bacterial isolates were presumptively identified as *Escherichia coli, Staphylococcus aureus, Klebsiella* sp., *Staphylococcus epidermidis, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Aeromonas* sp., and *Micrococcus* sp. The fungal isolates were identified as *Aspergillus flavus, Aspergillus niger*, and *Rhizopus stolonifer*.

3.4. Distribution and percentage occurrence of bacterial and fungal isolates from dried fish samples

The distribution and percentage occurrence of bacterial and fungal isolates from dried fish samples are presented in Table 4. *Escherichia coli* exhibited the highest percentage occurrence at 28.40% across all samples, croaker (*M. undukiuts*), catfish (*Siluriformes* spp.), and tilapia (*O. niloticus*) purchased from all markets. This was followed by *Bacillus subtilis*, with a 23.27% occurrence, also present in all samples from all markets. *Aeromonas* spp. and *Micrococcus* spp. showed the lowest occurrence, each at 3.44%. Among the fungal isolates, *Aspergillus niger* had the highest occurrence (1.72%) and was isolated from croaker fish samples obtained from Oja Oba and Isinkan markets. *Aspergillus flavus* and *Rhizopus stolonifer* both had the lowest fungal occurrence, each at 0.86%.

3.5. Aflatoxin B_1 content of dried fish treated with different sterilants

The effects of various sterilants on aflatoxin B_1 (AFB_1) content in dried fish from different markets in Akure metropolis are illustrated in Figure 1. All treatments significantly reduced AFB_1 levels in the samples. Among the untreated (raw) samples, dried catfish from the Oja Oba market exhibited the lowest AFB_1 content, while tilapia from the same market showed the highest content at 4.2 µg/kg. The greatest reduction in AFB_1 was achieved using warm water combined with wood ash, followed by warm water alone. The least reduction was observed in samples treated with cold water. Treatment with warm water plus wood ash led to a significant reduction in AFB_1 across all samples by at least 98.5%.

3.6. Proximate composition of dried fish pre-treated with different sterilants

Table 5 presents the effects of different sterilants as pre-treatment agents on the proximate composition of dried fish obtained from various markets. Significant variations were observed between the raw (untreated) samples and those subjected to sterilant treatments. The moisture, protein, ash, fat, and carbohydrate contents of catfish varied notably across samples from different markets. For example, raw catfish from the Isinkan market had protein, fat, and carbohydrate contents of 45.13%, 17.53%, and 18.64%, respectively; those from Oja Oba had 44.45%, 26.55%, and 11.95%; and samples from Nepa market had 46.54%, 25.82%, and 13.69%, respectively.

Pre-treatment with sterilants led to an increase in moisture content across all fish types (catfish, tilapia, and croaker) compared to untreated samples. Notably, samples treated with warm water and wood ash exhibited the highest protein content across all markets. Additionally, pre-treatment of raw catfish from Isinkan and Nepa markets, tilapia from Oja Oba, and croaker from Isinkan, Oja Oba, and Nepa markets resulted in a

Table 3a. Identities of bacteria isolated from dried fish samples

Macroscopic			Biochemical														
Colour	Elevation	Margin	Gram stain	Gram shape	Catalase	Oxidase	Citrate	Nitrate red.	Motility	Indole	Coagulase	Mannitol	Glucose	Sucrose	Lactose	Gas	Suspected bacteria
Metallic sheen	convex	Regular	-	Rod	+	-	-	+	+	+	-	+	+	+	+	+	*Escherichia coli*
Cream	Slightly Raised	Undulate	+	Rod	+	-	+	+	+	-	+	-	+	+	-	+	*Bacillus subtilis*
Cream	Slightly Raised	Entire	+	Cocci	+	-	-	+	-	-	+	-	+	+	+	+	*Staphylococcus epidermidis*
Cream	Slightly Raised	Undulate	+	Rod	+	-	+	+	+	-	+	-	+	+	-	+	*Bacillus cereus*
Light green	Raised	Regular	-	Rod	+	+	+	+	+	-	-	+	-	-	-	+	*Pseudomonas aeruginosa*
Black	Raised	Entire	-	Rod	+	-	-	+	+	-	-	+	+	-	-	-	*Klebsiella sp.*
Cream	Raised	Entire	-	Rod	+	-	+	+	+	+	-	-	+	+	-	+	*Aeromonas* sp.
Cream	Convex	Entire	+	Cocci	+	+	-	+	-	-	-	+	+	+	-	-	*Micrococcus* sp.

Table 3b. Identities of fungi isolated from dried fish samples

Isolate Code	Cultural and morphological characteristics	Microscopic features	Suspected fungi
1	Fast growing tall filamentous colonies with fluffy appearance resembling cotton candy. Apical spores on colonies that are slightly yellow and dark grey upon maturation.	Long apical sporangia with un-branched sporangiophores lacking basal rhizoids. Hyphae are non-septate with branched columellae	*Rhizopus stolonifer*
2	Greyish or olive green fast growing powdery colonies with white edge. Yellow pigmentation bottom or inverse upon maturation.	Bushy septate conidiophore with septate hyphae with a brush shaped conidiophores	*Aspergillus niger*
3	Shiny, smooth and creamy fast growing colonies with fringed magin	Branched hyphae appear creeping resembling fork with slimy conidia.	*Aspergillus flavus*

Table 4. Distribution and percentage occurrence of bacterial and fungal isolates from dried fish samples

A- Oja oba market, B- Isinkan market, C- Nepa market

Microbial isolate	Location	Croaker	Cat fish	Tilapia fish	N	% occurrence
Escherichia coli	A,B,C	+	+	+	33	28.40
Bacillus subtilis	A,B,C	+	+	+	27	23.27
S. epidermidis	A,B	+	+	-	22	18.96
Bacillus cereus	A,B	+	+	-	9	7.78
Pseudomonas aeruginosa	A,C	_	+	+	7	6.03
Klebsiella sp.	A,B,C	_	+	+	6	5.17
Aeromonas spp	A,B,C	-	+	+	4	3.44
Micrococcus spp	A,B,C	+	-	+	4	3.44
Aspergillus niger	A,B	+	-	-	2	1.72
Rhozopus stolonifer	A,B	-	+	+	1	0.86
Aspergillus flavuss	B	-	-	+	1	0.86

A- Oja oba market, B- Isinkan market, C- Nepa market

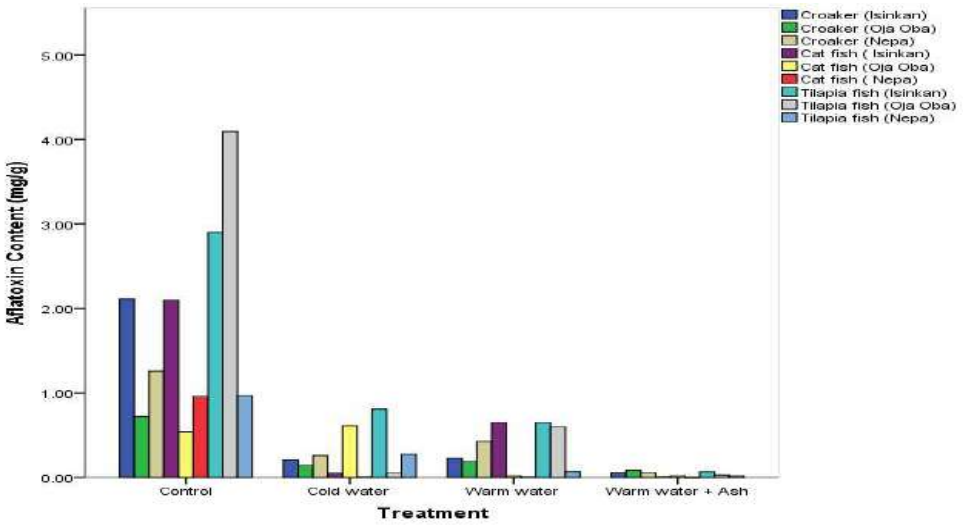

Figure 1. Aflatoxin B_1 contents of dried fish treated with different organic sterilant

Table 5. Proximate compositions of dried fish pre-treated with different organic sterilants (%)

Samples	Moisture content	Crude Protein	Ash content	Crude Fat	Carbohydrate
Cat fish (IR)	15.75± 0.3^{a}	45.13± 0.4^{b}	2.94± 0.5^{a}	17.53± 0.3^{c}	18.64± 0.3^{b}
Cat fish (ICW)	27.36± 0.6^{c}	47.21± 0.4^{c}	2.88± 0.7^{a}	13.55± 0.7^{a}	8.98± 0.7^{a}
Cat fish (IWW)	27.36± 0.3^{c}	44.18± 0.3^{a}	2.92± 0.6^{a}	16.59± 0.5^{b}	8.94± 0.6^{a}
Catfish (IWWA)	20.29± 0.4^{b}	47.61± 0.4^{c}	3.87± 0.4^{b}	18.99± 0.6^{d}	9.24± 0.8^{a}
Catfish (OR)	13.09± 0.2^{a}	44.45± 0.3^{b}	3.95± 0.4^{b}	26.55± 0.2^{d}	11.95± 0.4^{b}
Catfish (OCW)	24.70± 0.6^{c}	46.54± 0.6^{c}	3.89± 0.8^{b}	22.57± 0.8^{b}	2.28± 0.7^{a}
Catfish (OWW)	24.70± 0.4^{c}	43.51± 0.4^{a}	3.93± 0.7^{b}	25.61± 0.6^{c}	2.24± 0.5^{a}
Catfish (OWWA)	22.95± 0.5^{b}	48.28± 0.5^{d}	2.86± 0.5^{a}	9.97± 0.7^{a}	15.93± 0.7^{c}
Cat fish (NR)	11.05± 0.2^{a}	46.54± 0.2^{b}	2.89± 0.5^{a}	25.82± 0.3^{d}	13.69± 0.3^{c}
Catfish (NCW)	22.66± 0.7^{c}	48.63± 0.2^{c}	2.83± 0.9^{a}	21.84± 0.9^{b}	4.02± 0.4^{a}
Catfish (NWW)	22.66± 0.5^{c}	45.60± 0.6^{a}	2.87± 0.4^{a}	24.88± 0.3^{c}	3.99± 0.7^{a}
Catfish (NWWA)	18.25± 0.6^{b}	49.70± 0.6^{d}	2.81± 0.5^{a}	18.26± 0.6^{a}	10.98± 0.6^{b}
Tilapia (OR)	19.05± 0.4^{a}	41.27± 0.4^{b}	1.89± 0.6^{b}	21.11± 0.4^{c}	16.67± 0.4^{c}
Tilapia (OCW)	30.66± 0.5^{c}	43.36± 0.7^{c}	1.83± 0.7^{b}	17.13± 0.6^{a}	7.00± 0.2^{b}
Tilapia (OWW)	30.66± 0.2^{c}	40.34± 0.2^{a}	1.87± 0.6^{b}	20.17± 0.6^{b}	6.97± 0.7^{b}
Tilapia (OWWA)	25.69± 0.4^{b}	46.24± 0.5^{d}	1.41± 0.7^{a}	21.94± 0.4^{d}	4.73± 0.3^{a}
Tilapia (IR)	18.49± 0.3^{a}	43.09± 0.5^{b}	1.49± 0.4^{a}	29.49± 0.4^{d}	7.44± 0.3^{b}
Tilapia (ICW)	30.10± 0.3^{c}	44.17± 0.7^{c}	1.43± 0.6^{a}	22.51± 0.5^{b}	1.77± 0.4^{a}
Tilapia (IWW)	30.10± 0.7^{c}	42.15± 0.6^{a}	1.47± 0.2^{a}	24.55± 0.7^{c}	1.74± 0.8^{a}
Tilapia (IWWA)	26.59± 0.8^{b}	45.71± 0.2^{d}	1.86± 0.7^{b}	14.76± 0.3^{a}	11.08± 0.4^{c}
Tilapia (NR)	19.39± 0.4^{a}	42.57± 0.6^{b}	1.94± 0.3^{a}	22.31± 0.4^{d}	13.79± 0.4^{b}
Tilapia (NCW)	31.32± 0.4^{c}	44.65± 0.8^{c}	1.88± 0.5^{a}	18.33± 0.4^{b}	4.12± 0.8^{a}
Tilapia (NWW)	31.00± 0.6^{c}	41.62± 0.4^{a}	1.92± 0.3^{a}	21.37± 0.8^{c}	4.09± 0.5^{a}
Tilapia (NWWA)	26.25± 0.7^{b}	44.43± 0.3^{c}	1.81± 0.6^{a}	13.56± 0.5^{a}	13.96± 0.5^{b}
Croaker (IR)	12.92± 0.3^{a}	51.12± 0.5^{b}	1.68± 0.3^{a}	17.64± 0.4^{d}	16.63± 0.4^{c}
Croaker (ICW)	24.53± 0.2^{c}	53.20± 0.6^{c}	1.62± 0.3^{a}	13.66± 0.9^{b}	6.97± 0.5^{a}
Croaker (IWW)	24.53± 0.3^{c}	50.18± 0.6^{a}	1.66± 0.6^{a}	16.70± 0.3^{c}	6.93± 0.5^{a}
Croaker (IWWA)	20.12± 0.4^{b}	54.27± 0.8^{d}	1.60± 0.2^{a}	10.09± 0.2^{a}	13.92± 0.2^{b}
Croaker (OR)	12.45± 0.6^{a}	49.48± 0.4^{b}	1.92± 0.4^{a}	18.93± 0.2^{d}	17.21± 0.2^{c}
Croaker (OCW)	24.06± 0.8^{c}	51.56± 0.3^{c}	1.86± 0.8^{a}	14.95± 0.2^{b}	7.55± 0.9^{a}
Croaker (OWW)	24.06± 0.7^{c}	48.54± 0.4^{a}	1.95± 0.4^{a}	17.99± 0.4^{c}	7.51± 0.5^{a}
Croaker (OWWA)	19.65± 0.3^{b}	52.63± 0.7^{d}	1.84± 0.4^{a}	11.38± 0.8^{a}	14.50± 0.7^{b}
Croaker (NR)	18.31± 0.5^{a}	47.46± 0.4^{c}	1.92± 0.2^{a}	23.68± 0.3^{d}	8.62± 0.3^{c}
Croaker (NCW)	29.92± 0.7^{c}	45.55± 0.5^{b}	1.86± 0.4^{a}	19.70± 0.8^{b}	2.96± 0.9^{a}
Croaker (NWW)	29.92± 0.1^{c}	42.52± 0.3^{a}	1.94± 0.5^{a}	22.74± 0.5^{c}	2.92± 0.6^{a}
Croaker (NWWA)	25.51± 0.2^{b}	50.61± 0.8^{d}	1.84± 0.8^{a}	16.13± 0.8^{a}	5.91± 0.6^{b}

Values are means ± S.E of Samples (n=3). Values with the same subscript letter (s) along the same column (within the same treatment) are not significantly different (p<0.05) using Duncan's New Multiple Range test

IR-Raw dried fish from Isinkan market, ICW-Dried fish from Isinkan market treated with cold water, IWW-Dried fish from Isinkan market treated with warm water, IWWA-Dried fish from Isinkan market treated with warm water containing wood ash, OR- Raw dried fish from Oja-oba market, OCW- Dried fish from Oja-oba market treated with cold water, OWW- Dried fish from Oja-oba market treated with warm water, OWWA- Dried fish from Oja-oba market treated with warm water containing wood ash, NR- Raw dried fish from Nepa market, NCW-Dried fish from Isinkan market treated with cold water, NWW- Dried fish from Nepa market treated with warm water, NWWA-Dried fish from Nepa market treated with warm water containing wood ash.

Significant reduction in carbohydrate content. Conversely, catfish from Oja Oba and tilapia from Isinkan treated with warm water and ash showed increased carbohydrate content. No significant change was observed in the carbohydrate content of tilapia from the Nepa market treated with warm water and ash compared to the raw samples.

4. Discussion

Scientific investigations are essential to safeguard consumers from the health risks associated with the consumption of contaminated foods, which are often linked to foodborne illnesses. Smoked dried fish is a widely consumed processed fishery product in the Niger Delta region of Nigeria. Smoking is commonly practiced using organic additives, not only to enhance flavor and color but also primarily to facilitate drying (18). In Nigeria, dried fish is often sold in open markets and is sometimes consumed without proper consideration of the risks posed by microbial contamination. Although it plays a significant role in human nutrition globally, smoked dried fish can serve as a vehicle for foodborne pathogens (19).

Microbial contamination of smoked dried fish can occur at various stages from processing to retail posing significant public health concerns when microbial loads exceed acceptable limits. Such contamination may lead to mild or severe illnesses. The microbial safety of smoked dried fish depends largely on the quality of the harvested fish, the type of wood used for smoking, and the intensity and consistency of the heat applied (18). Therefore, to extend shelf life and ensure safety for human consumption, it is imperative to minimize every potential source of microbial contamination (20).

In the present study, microbial loads from different species of dried fish collected from various markets in Akure metropolis, Southwestern Nigeria, were relatively high and showed variation among samples. These findings contrast with those of Anihouvi et al. (21), who reported lower microbial levels in smoked dried fish samples from markets in the Benin Republic. However, similar findings were reported by Ariyawansa et al. (22), who observed higher microbial loads in fish samples from markets in Negombo, Western Province of Sri Lanka.

Yakubu and Ngueku (19) reported high bacterial loads ranging from 109.00×10^5 CFU/g to 136.67×10^5 CFU/g in smoked dried *Clarias* species from markets in Lafia, Nigeria. Similarly, Nyarko et al. (23) documented total bacterial and fungal loads ranging from 6.2×10^4 to 3.3×10^5 CFU/g and 1.1×10^2 to 9.3×10^4 SFU/g, respectively, in *Sardinella aurita* from three smoking sites. Even higher loads—7.2×10^4 to 4.1×10^7 CFU/g (bacteria) and 5.0×10^2 to 8.0×10^4 SFU/g (fungi) were reported from similar samples obtained from markets in Tema, Ghana. Ibrahim et al. (24) reported a bacterial load of 2.06×10^6 CFU/g in smoked dried *Clarias gariepinus*, while Oku and Amakoromo (25) observed fungal loads ranging from 1.0×10^4 to 4.0×10^5 SFU/g in samples from markets in Yenagoa, Nigeria.

Importantly, none of the dried fish samples examined in this study complied with the recommended microbial limits for seafood as stipulated by the Health Protection Agency (26). According to the HPA, the acceptable limit for aerobic mesophilic bacteria in processed seafood (including smoked dried fish) is fewer than 7 CFU/g. No official limits were provided for yeasts and molds. The elevated microbial loads and diversity of organisms observed in this study may be attributed to several factors, including variations in moisture content, exposure to airborne microbes,

unsanitary market conditions, and poor adherence to hygiene practices during post-processing, handling, and storage (12, 27). Furthermore, it is possible that the smoking process does not completely eliminate all contaminating microorganisms (28).

The present study reveals the presence of several pathogenic and spoilage bacterial isolates in the dried fish samples. Many of the bacterial species identified are likely transient contaminants, as their growth and metabolic activities typically require higher moisture levels. The detection of *Escherichia coli* suggests fecal contamination of the samples, likely introduced during post-smoking handling by either humans or animals. This raises significant public health concern, as *E. coli* is associated with foodborne disease outbreaks (21, 26). Notably, certain strains such as Enterohemorrhagic *E. coli* (EHEC) are known to produce Shiga-like toxins (SLTs), which resemble those produced by *Shigella dysenteriae*. These cytotoxins inhibit cell secretion and damage colonic epithelial cells, leading to severe gastrointestinal illness (29). *Pseudomonas aeruginosa*, another bacterium isolated from the samples, is widely recognized as a spoilage organism. Although *Pseudomonas* species are natural inhabitants of soil and aquatic environments, they frequently contaminate a variety of food products. Carrascosa et al. (30, 31) identified *Pseudomonas* spp. as common spoilage organisms in fish harvested from the Mediterranean Sea and temperate regions. The presence of *Bacillus cereus* is also of significant concern, as it is a known foodborne pathogen responsible for gastroenteritis, particularly in developed countries (21, 32). *Klebsiella* species possess decarboxylase activity, which allows them to produce histamine in fish products (33). Histamine accumulation is linked to a variety of adverse health effects in humans (34). Similarly, Abidemi-Iromini et al. (35) reported the isolation of *P. aeruginosa*, *Micrococcus* sp., *B. cereus*, *Aerobacter* sp., and *E. coli* from smoked dried *Clarias gariepinus* in Oyo State, Nigeria, corroborating the present findings.

The isolation of fungi from the dried fish samples is not surprising, given that many fungal species can thrive under low-moisture conditions. The detection of *Aspergillus flavus* is particularly concerning, as certain strains are known producers of aflatoxins, potent carcinogenic compounds affecting both humans and animals (36, 37). *Aspergillus niger* and *Rhizopus stolonifer* are also well-documented spoilage fungi commonly found in post-harvested fish (38). In agreement with this, Abidemi-Iromini et al. (35) also reported the presence of *A. niger* and *Rhizopus* sp. in smoked dried fish samples.

In this study, lower microbial counts were observed in samples pre-treated with warm water alone, while the lowest counts were recorded in samples pre-treated with warm water containing wood ash, compared to untreated samples. The reduction in microbial content following treatment with wood ash aligns with previously published findings on the disinfection of biosolids, urban wastewater, and landfill leachate using wood ash. For instance, Paludan-Müller et al. (39) reported a significant reduction in bacteriological indicator populations in biosolids treated with wood ash. Similarly, Brewster et al. (40) documented the complete inactivation of *Clostridium perfringens* in anaerobically digested sludge following wood ash treatment.

According to Ivanković et al. (41), the pH of wastewater increased to 12.7 after several hours of treatment with wood ash, and this highly alkaline environment exhibited strong antibacterial and bactericidal effects on the bacterial populations studied. Furthermore, Blinova et al. (42) demonstrated the toxic effects of highly alkaline wood ash solutions on *Vibrio fischeri*, the microalga *Pseudokirchneriella subcapitata*, and the crustacean *Daphnia magna*. In general, most bacteria thrive within a pH range of 5 to 8, with optimal growth occurring near neutral pH (43). Only a few bacterial species, such as *Bacillus firmus* and *Nitrosomonas halophila*, are capable of surviving at pH values above 10 (41, 44).

The observed microbial reduction in the treated fish samples is likely attributable to the alkaline conditions induced by wood ash (45), in combination with the physical washing and thermal effects of warm water, which may have helped dislodge or inactivate contaminating microorganisms (46). Brief soaking of the fish in warm water with wood ash likely facilitated the removal of susceptible microorganisms. It is well-established that microbial life is highly temperature-dependent; temperature influences microbial growth and survival as well as biological processes such as sporulation, germination, and motility (47).

An interesting observation in the present study was the apparent increase in fungal counts in some fish samples following cold-water treatment. This phenomenon may be attributed to the detachment of fungal spores from fish surfaces and their redistribution during washing. Washing processes may dislodge surface-associated microorganisms, thereby increasing their recovery during microbiological enumeration without necessarily increasing their actual population density (48). Unlike warm water and wood ash treatments, cold water does not provide sufficient thermal or antimicrobial effects to inactivate fungal propagules. Consequently, fungal spores that were previously attached to fish tissues may have become more readily recoverable during microbiological analysis, resulting in higher enumerated counts.

In this study, aflatoxin B_1 was detected in all the fish samples, and the sterilant significantly reduced its levels in all samples. The reduction of aflatoxin B_1 in the fish samples treated with wood ash solution aligns with findings from other *in vitro* studies that reported the detoxifying capabilities of wood ash (49-51). The results of this study are consistent with Ayo et al. (51), who used an *in vitro* technique to evaluate the aflatoxin-adsorbing capacity of rice husk ash, reporting a 94.6% reduction of aflatoxin B_1. Among the detoxifying or binding materials evaluated by Ayo et al. (51), rice husk ash demonstrated a binding capacity similar to that of the reference binder, especially in reducing aflatoxin B_1, the most toxic fungal metabolite. This novel aflatoxin-binding ability of rice husk ash was attributed to its cation exchange capacity, influenced by metal ions such as calcium (Ca^{2+}) and potassium (K^{+}), which promote its aflatoxin-binding capacity (52). Additionally, the treatment of maize kernels with maize cob ash solution resulted in a significant 91.6% reduction in aflatoxins (10, 53). In this study, the reduction in aflatoxin B_1 in dried fish samples may have resulted from alkaline degradation and the subsequent leaching of the toxin into wash water (53, 54-56).

In several countries, wood ash is used in food processing, for instance, in nixtamalization, a corn

tenderization process where dietary aflatoxins are reduced due to hydrolysis of the lactone ring and disruption of non-covalent hydrogen bonds by the alkalinity of ash. This reaction produces a water-soluble β-keto acid that can be easily removed (57,58).

The European Union (EU) sets maximum permissible levels for aflatoxin B_1 at 2 µg/kg and for total aflatoxins at 4 µg/kg (59,60), while the United States allows up to 20 µg/kg for total aflatoxins (61,62). In the current study, aflatoxin B_1 levels in the raw fish samples exceeded EU regulatory limits. Average concentrations ranged from 0.5 to 4.2 µg/kg, which are lower than the values reported in *Petrocephalus* sp. (20.4 µg/kg) and *Limnothrissa* sp. (17.2 µg/kg) (3).

According to the U.S. Food and Drug Administration (63), the lethal dose (LD_{50}) for total aflatoxins in rabbits is 0.3 µg/kg. Based on this, the levels detected in untreated samples could pose health risks to humans. Variations between aflatoxin B_1 content in this study and previous reports may be due to differences in fish species, environmental conditions during processing and storage, or the specific strains of toxigenic *Aspergillus* spp. involved (64,65).

Notably, aflatoxin B_1 levels in fish samples pre-treated with warm water and wood ash were below the maximum limits set by food safety authorities. The substantial reduction observed suggests that a significant portion of the toxin was detoxified and removed during the treatment process. While high temperatures between 237 and 306 °C are typically recommended for aflatoxin detoxification in agricultural products (10), this study demonstrates the novel potential of warm water at just 40 °C combined with wood ash to reduce aflatoxin B_1 to nearly undetectable levels.

Pre-treatment with the sterilants also resulted in increased crude protein and fat content, alongside reduced carbohydrate levels. The higher moisture content in samples treated with cold or warm water compared to those treated with warm water and wood ash suggests improved tissue hydration. The enhanced protein content in warm water-wood ash treated samples indicates that 40 °C is an effective sterilization temperature that does not denature proteins. This is supported by Abidemi-Iromini et al. (35), who reported that protein denaturation in fish occurs at temperatures above 80 °C.

A limitation of the present study is the absence of physicochemical characterization of the Mangifera indica wood ash used for treatment. Future investigations should determine pH, elemental composition, mineral profile, and particle size distribution to facilitate mechanistic understanding and improve reproducibility of the treatment process.

5. Conclusion

The findings of this study have significant implications for animal health and production, particularly in relation to food safety and quality in animal-derived products. The high microbial load and aflatoxin B_1 contamination observed in smoked dried fish present serious risks not only to human consumers but also to animals when such products are used in feed formulation. These contaminants can compromise animal health, reduce productivity, and increase susceptibility to disease. However, the effective reduction of microbial and aflatoxin levels through pre-treatment with warm water containing wood ash offers a simple, affordable, and locally accessible method for

improving feed safety in low-resource settings. By integrating this method into post-processing practices, we can promote safer animal nutrition, enhance animal welfare, and ultimately support more sustainable and productive livestock systems.

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Author contributions

Oladipo Oladiti Olaniyi conceived and designed the study, performed the formal analysis, curated the data, supervised the research, and prepared the original manuscript draft. Oladipo Oladiti Olaniyi and Folakemi Akoyiola Blessing jointly developed the methodology and conducted the investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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