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Microbiological Quality and Artisanal Manufacturing Practices for "Kilishi" Dried and Spiced Meat in Niamey (Niger)

Hadiza Bawa Ibrahim[1,2*], Mamadou Abdoulaye Konare[3], Mahamadou Bello Issa Adam[4], Mohamed Sanoussi[2], Alio Mahamadou Fody[5,6], Nicolas Barro[2]

[1]Department of Life and Earth Sciences, Lédéa Bernard Ouedraogo University, Ouahigouya, Burkina Faso
[2]Molecular Biology Laboratory for Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food and Water, University of Joseph Ki-Zerbo, Ouagadougou, Burkina Faso
[3]Biosciences and Applications Laboratory (LBA), University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali
[4]Laboratory of Biochemistry, Biotechnology, Food Technology and Nutrition (LABIOTAN), Ouagadougou, Burkina Faso
[5]Higher Normal School, Niamey, Niger
[6]Department of Life and Earth Sciences, Abdou Moumouni University, Niamey, Niger
Email: *hadizabawa@yahoo.fr

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Abstract

Kilishi, Niger's iconic dried and spiced meat, is a crucial source of protein and an essential socio-economic asset. However, its artisanal production and informal marketing raise significant concerns about quality and safety. This study examined the Kilishi value chain in Niamey by documenting traditional manufacturing methods and evaluating the product's microbiological quality. Manufacturing practices were characterized through semi-structured interviews and direct observations. Sixty samples of three Kilishi types (red, white, plain) were analyzed using standard culture techniques, multiplex PCR for identifying *Escherichia coli* (*E. coli*), and serotyping for *Salmonella*. The findings showed that traditional practices such as open-air drying, the use of cement bag paper for packaging, and manual handling pose multiple vulnerabilities. Microbiological testing revealed notable contamination: 41.7% (25/60) of samples contained *Escherichia coli*, with pathogenic strains identified (Enterotoxinogen *Escherichia coli* (ETEC): 6.67%; Enterotoxinogenic *Escherichia coli*-Enteropathogenic *Escherichia coli* (ETEC-EPEC): 5%). *Salmonella* spp. were found in 6.7% (4/60) of samples, including one strain of *Salmonella typhi* (1.67%). The average fecal coliform level (3.40×10^4 CFU/g) consistently exceeded the local standard of 10^3

CFU/g. Traditional Kilishi practices pose significant health risks to consumers. It is crucial to implement hygiene and safety measures throughout the entire production process to preserve Kilishi's cultural value while ensuring its safety.

Keywords

Kilishi, Food Safety, Artisanal Practices, Microbial Contamination, Health Risk, Niamey/Niger

1. Introduction

Meat plays an essential role in human nutrition, being a rich source of protein, iron, zinc, selenium, and B vitamins [1]. In Niger and the Sahel, Kilishi, a traditional specialty made from thin strips of meat (primarily beef), is widely popular for its distinctive flavor and long shelf life [2]. It represents a vital protein source and holds significant economic and cultural importance for many households [3]. However, despite its importance, the production and marketing of Kilishi primarily occur in artisanal, unregulated settings, raising serious concerns about food safety [4]. Traditional techniques often lack hygiene standards, monitoring, and awareness of good practices [5]. The product's exposure to the external environment (dust, insects, stray animals) during drying, along with post-cooking handling practices, compromises its integrity, leading to risks of microbiological, physical, or chemical contamination [6]. The final cooking, while beneficial, is insufficient when application conditions vary or subsequent recontamination occurs. This issue is a significant public health concern, as Kilishi is widely consumed, including by at-risk groups. In this context, this study aims to analyze the microbiological quality and artisanal manufacturing practices of the dried and spiced meat (Kilishi) in Niamey (Niger).

2. Methodology

2.1. Study Type, Sites, and Period

This study, conducted in Niamey, Niger, combined qualitative methods (observations and interviews) and quantitative methods (laboratory analyses) to assess the microbiological quality of Kilishi (the finished product). Sample collection took place from June to September 2024, and analyses ran from October 2024 to February 2025.

2.2. Qualitative Data Collection

Data were collected through semi-structured interviews and direct observations with 60 actors in the Kilishi value chain (producers and sellers). Interviews focused on production stages (meat selection, drying/cooking techniques, preservation, and packaging) (**Figure 1**). Special attention was paid to hygiene conditions and the production environment (exposure to dust, insects, and stray animals),

with visual documentation produced.

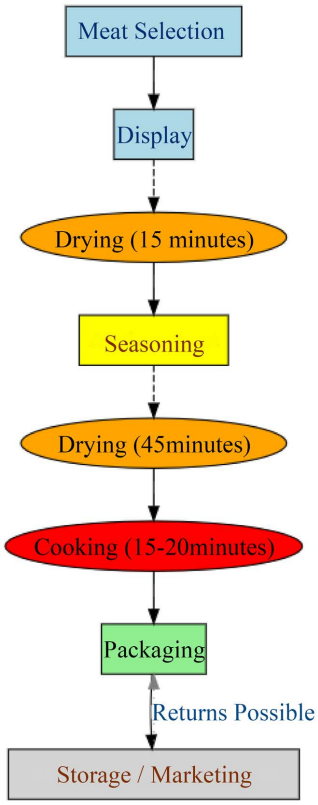

Figure 1. Kilishi production diagram.

2.3. Sample Collection

Sixty (60) Kilishi samples were collected from vendors, following their standard cutting and packaging procedures. Samples were placed in sterile freezer bags, labeled, and transported promptly in a cooler with cold packs to the LaBESTA Laboratory in Ouagadougou, Burkina Faso, for microbiological analysis.

2.4. Microbiological Quality Analysis

Microorganisms were detected using the standard solid culture technique.

2.4.1. Solution and Dilution Preparation

A stock solution (pre-enrichment) was prepared by placing 25 g of Kilishi in 225 ml of Buffered Peptone Water (BPW) (Liofilchem), incubated at 37˚C for 24 h. Decimal dilutions were performed up to 10^{-3} for *Salmonella* and 10^{-6} for *Escherichia coli* (*E. coli*).

2.4.2. Detection and Identification of *Salmonella* spp.

Detection followed standard 6579-1: Selective Enrichment: 0.1 ml of pre-enriched broth was plated on Rappaport Vassiliadis (RV) broth at 42˚C, and 1 ml was added to Muller-Kauffman Tetrathionate (MKTT) broth at 37˚C, both incubated 18 to 24 h.

Isolation: Enriched broth was streaked onto selective SS and XLD agar (Liofilchem).

Identification: Suspect strains were identified using the API 20E biochemical profile (BioMérieux).

2.4.3. Serotyping of *Salmonella* spp.

Serotyping, following biochemical identification, was performed by direct slide agglutination with specific antisera.

2.4.4. Enumeration of Thermotolerant Coliforms and *E. Coli* Identification

Coliforms were enumerated by surface plating on Violet Red Bile Lactose (VRBL) medium, incubated at 44˚C for 24 h (Standard V08-060, AFNOR, 2009) [7]. Colonies between 15 and 150 were counted to calculate the bacterial load (CFU/gram) using the standard formula. *E. coli* suspects were confirmed using the API 20 E biochemical gallery (BioMérieux).

$$N = \frac{\sum C}{(n_1 + 0,1n_2) * d * V}$$

N = Number of colony-forming units (CFU)/gram of food;

V = Volume of solution deposited;

ΣC = Total number of colonies counted in boxes with colonies between 15 and 150;

n_1 = Number of boxes counted from the first dilution;

n_2 = Number of boxes counted from the second dilution;

d = Dilution factor from which the first counts were made.

2.4.5. Multiplex Polymerase Chain Reaction (16-Plex PCR)

This study employed a 16-plex PCR for the simultaneous detection of 16 virulence genes belonging to the five main *E. coli* pathovars (Enterohemoragic *Escherichia coli* (EHEC), Enteropathogenic *Escherichia coli* (EPEC), Enteroaggregative *Escherichia coli* (EAEC), Enteroinvasive *Escherichia coli* (EIEC), Enterotoxinogenic *Escherichia coli* (ETEC) [8].

DNA Extraction: Bacterial DNA was extracted from pure *E. coli* strains using the heating method (boiling for 10 min, followed by centrifugation at 11,337 rpm for 10 min). The supernatant was stored for PCR.

Amplification: The 20 µl reaction included a premix (Taq polymerase, dNTPs, buffers, $MgCl_2$) and specific mixtures of 16 primers (Mix 1 and Mix 2). The thermocycler program involved 35 cycles with a hybridization step at 62.5˚C for 60 seconds.

Electrophoresis: PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and visualized under a UV lamp. A 100 bp molecular weight marker was used.

Interpretation: Pathovar determination was based on the presence of specific genes (elt for ECET; stx1/stx2 for ECST; uidA as the general *E. coli* marker) (**Table 1**). Reference *E. coli* strains from THL (Helsinki, Finland) served as controls.

Table 1. Primer sequences for multiplex PCR.

Pathovars	Targeted Gene	Primer Sequences (5' to 3')	T in pb	[C] in µM	Ref.
	eaeA	eae-F: TCAATGCAGTTCCGTTATCAGTT			2
		eae-R: GTAAAGTCCGTTACCCCAACCTG	482	0.1	1
STEC-ETEC	escV	MP3-escV-F: ATTCTGGCTCTCTTCTTCTTTATGGCTG			
		MP3-escV-R: CGTCCCCTTTTACAAACTTCATCGC	544	0.4	1
	ent	ent-F: TGGGCTAAAAGAAGACACACTG			
		ent-R: CAAGCATCCTGATTATCTCACC	629	0.4	1
	stx1	MP4-stx1A-F: CGATGTTACGGTTTGTTACTGTGACAGC			
STEC		MP4-stx1A-R: AATGCCACGCTTCCCAGAATTG	284	0.2	1
	stx2	MP3-stx2A-F: GTTTTGACCATCTTCGTCTGATTATTGAG			
		MP3-stx2A-R: AGCGTAAGGCTTCTGCTGTGAC	324	0.4	1
	ipaH	ipaH-F: GAAAACCCTCCTGGTCCATCAGG			2
		ipaH-R: GCCGGTCAGCCACCCTCTGAGAGTAC	437	0.1	2
EIEC	invE	MP2-invE-F: CGATAGATGGCGAGAAATTATATCCCG			
		MP2-invE-R: CGATCAAGAATCCCTAACAGAAGAATCAC	766	0.2	1
	aggR	MP2-aggR-F: ACGCAGAGTTGCCTGATAAAG			
EAEC		MP2-aggR-R: AATACAGAATCGTCAGCATCAGC	400	0.2	1
	pic	MP2-pic-F: AGCCGTTTCCGCAGAAGCC			
		MP2-pic-R: AAATGTCAGTGAACCGACGATTGG	1111	0.2	1
	astA	MP2-astA-F: TGCCATCAACACAGTATATCCG			
		MP2-astA-R: ACGGCTTTGTAGTCCTTCCAT	102	0.4	1
	elt	MP2-LT-F: GAACAGGAGGTTTCTGCGTTAGGTG			
		MP2-LT-R: CTTTCAATGGCTTTTTTTTGGGAGTC	655	0.1	1
ETEC	estIa	MP4-STIa F: CCTCTTTTAGYCAGACARCTGAATCASTTG			
		MP4-STIa-R: CAGGCAGGATTACAACAAAGTTCACAG	157	0.4	1
	estIb	MP2-STI-F: TGTCTTTTTCACCTTTCGCTC			
		MP2-STI-R: CGGTACAAGCAGGATTACAACAC	171	0.2	1
E. coli	uidA	MP2-uidA-F: ATGCCAGTCCAGCGTTTTTGC			
		MP2-uidA-R: AAAGTGTGGGTCAATAATCAGGAAGTG	1487	0.2	1

STEC: Shigalike Toxin *E. coli*; ETEC: Enterotoxinogenic *E. coli*; EIEC: Enteroinvasive *E. coli*; EAEC: Enteroaggregative *E. coli*; Ref.: Reference; T: Expected amplicon size in base pairs (bp); [C]: Concentration; 1: [9]; 2: [8].

2.5. Data Analysis

2.5.1. Qualitative Analysis

Data from interviews and observations were transcribed and subjected to qualitative thematic analysis to identify key manufacturing stages and potential health risks.

2.5.2. Quantitative and Statistical Analysis

Quantitative microbiological data underwent descriptive statistical analysis to

determine the prevalence of the different microorganisms (expressed as frequencies and percentages). Software: Excel and Epi-Info version 3.5.1 were used for prevalence calculations. Statistical Significance: Med Calc 11.0.1.0 software was used to determine p-values. Differences were considered statistically significant at a p-value less than or equal to 0.05.

3. Results

3.1. Kilishi Manufacturing Processes

Artisanal Kilishi in Niamey is the result of a traditional process, involving specific steps to guarantee its texture, taste, and preservation.

3.1.1. Meat Selection and Preparation

Kilishi is mainly prepared from beef, with the hind leg being preferred for its yield. The meat is first scraped clean of bone fragments, then meticulously flattened into thin strips to ensure optimal and uniform drying.

3.1.2. Initial Drying

Meat slices are spread out for intense sun drying, usually in the open air. Observations have shown that this phase lasts about 3 hours per side, after which the meat is turned over. The drying process lasts approximately 24 hours. During this stage, the meat is exposed to environmental elements, which is a critical point for product hygiene and safety.

3.1.3. Preparation, Seasoning, and Final Cooking

After initial drying, the meat is coated with peanut oil and seasoned. Salt and peanut oil are essential ingredients found in all varieties. Three main varieties of Kilishi exist. Red Kilishi: Distinguished by the addition of red chili pepper (Tattassé), White Kilishi: Includes classic condiments (groundnuts, garlic, ginger, chilies) but without red pepper, and Unseasoned Kilishi: Contains only peanut oil and salt.

The seasoning mixture is evenly spread. The meat is then grilled over a wood fire at a low temperature (about 15 to 20 minutes per side. This final cooking provides the Kilishi its unique flavor and appearance while potentially reducing the existing microbial load.

3.2. Marketing and Storage Practices

3.2.1. Shelf Life and Storage

Kilishi is known for its long shelf life, ranging from three months to one year at ambient temperature without refrigeration, attributed to low water activity resulting from intensive drying and cooking.

3.2.2. Packaging

The product is typically wrapped in paper (including paper from empty cement bags) or aluminum foil. Plastic bags are avoided. The use of recycled paper raises hygiene concerns.

3.2.3. Display Conditions and Sales Points

Sales occur in various locations (markets, unofficial stalls) with varied exposure. Observations highlighted: Frequent open-air display, sometimes only covered by a piece of cloth. Critical Factors: Direct exposure to dust, high ambient temperatures, constant presence of insects (flies), and, in some cases, proximity to stray animals. Handling: Frequent direct hand handling by vendors without gloves poses a potential source of cross-contamination post-cooking.

3.3. Microbiological quality of Kilishi

The collected Kilishi samples showed contamination primarily with *Salmonella* and *E. coli*, with frequencies of 4 (6.7%) and 25 (41.7%), respectively (Figure 2). The microbial loads of these strains were quantified and are presented in Table 2. Regarding faecal coliform counts, the bacterial load ranged from 2×10^2 CFU/g to 1.20×10^6 CFU/g, with an average of 3.40×10^4 CFU/g.

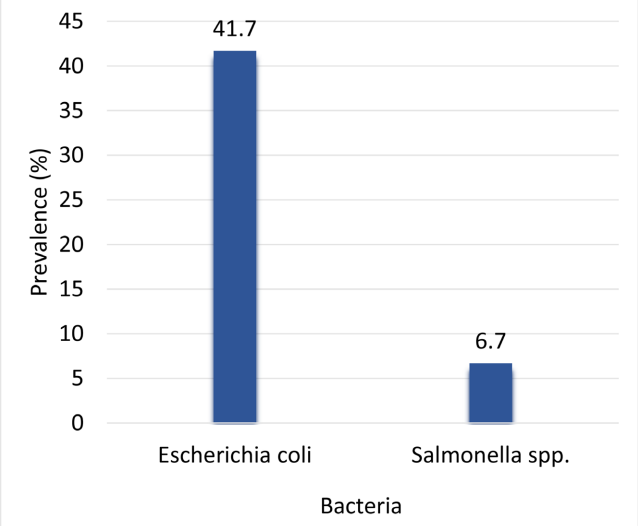

Figure 2. Prevalence of bacteria in kilishi samples

Table 2. Microbial load of thermotolerant coliforms and *Salmonella* in samples.

Study Sites	Code	Thermotolerant Coliforms (UFC/g)	Absence or Presence of *Salmonella* spp. in 25 g
Study 1 (N = 10)	ES1	$2.06 \times 10^4 \pm 2.91 \times 10^{4a}$	Presence
Study 2 (N = 10)	ES2	$3.39 \times 10^4 \pm 3.72 \times 10^{4a}$	Presence
Study 3 (N = 10)	ES3	$2.77 \times 10^4 \pm 4.44 \times 10^{4a}$	Absence
Study 4 (N = 10)	ES4	$9.88 \times 10^4 \pm 3.06 \times 10^{5a}$	Presence
Study 5 (N = 10)	ES5	$1.85 \times 10^4 \pm 3.34 \times 10^{4a}$	Absence
Study 6 (N = 10)	ES6	$2.64 \times 10^4 \pm 4.26 \times 10^{4a}$	Presence
Moyenne ± SD		$3.40 \times 10^4 \pm 1.29 \times 10^5$	4/60

N = Number of sample, $p = 0.40 > 0.05$. The 'a' in bold indicates that there is no significant difference between the results for the different sectors.

3.3.1. Prevalence of *Salmonella* Serovars

The results show a variable prevalence of *Salmonella* in the different kilishi meats collected from the sales sites in the city of Niamey. It appears that 6.7% (4/60) of the samples are contaminated with *Salmonella* spp. The study also shows that 1.67% are infected with *Salmonella typhi* (Table 3).

Table 3. Prevalence of *Salmonella* serovars.

Salmonella Serotypes	Numbers of *Salmonella* (N = 4)	Percentage (%)
S. enteritidis	0	0
***S. paratyphi* A, B, C**	0	0
S. typhi	1	1.67
***S.* spp.**	3	5.03
Total	4	6.7

N = Total number of *Salmonella* isolated; % = Percentage.

3.3.2. Prevalence of *E. coli* Pathovars

Table 4. Prevalence of *E. coli* pathovars.

Pathotypes of *E. coli*	Number of Pathotypes (N = 25)	Percentages (%)
ECST	4	6.67
ECST-ECET	3	5.03
ECEA	0	0
ECEI	0	0
ECET	Traces	1

N = Total number of *Salmonella* isolated; ECST = Shiga-like toxine *Escherichia coli*; ECEA = Enteroaggregative *Escherichia coli*; ECEI = Enteroinvasive *Escherichia coli*; ECET = Entero-toxinogenic *Escherichia coli*.

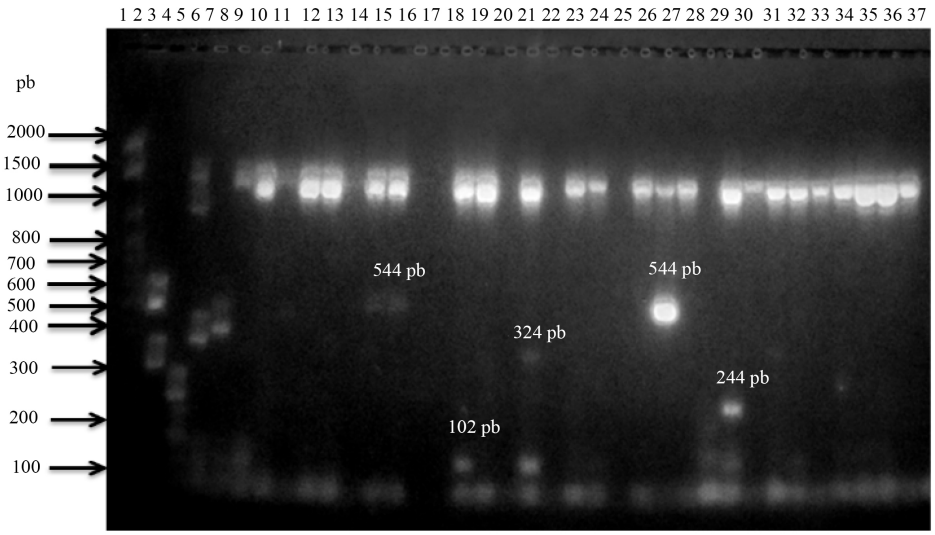

Figure 3. Agarose gel image showing multiplex PCR amplicons.

Molecular analyses showed that 41.7% of Kilishi intended for human consumption contained *E. coli* strains associated with diarrhoea. Five *E. coli* pathovars were identified. The study showed a prevalence of 6.67% ECST, 5% ECST-ECET, 0% ECEA, 0% ECEI, and 1% ECET. The highest prevalence rates were observed for ECST and ECST-ECET (**Table 4**). The uidA gene confirms that the *E. coli* strain was identified in all strains tested. These strains, which only carry the uidA gene, are classified as non-diarrhoeic *E. coli* (**Figure 3**).

4. Discussion

According to the results of surveys, microbiological analyses, and field observations, this research shows that contamination of Kilishi is closely linked to traditional production processes, creating pathovars that allow pathogens to enter the human population, with few preventive measures in place, representing a critical public health problem. Although the production process relies on complex and well-known traditional artisanal expertise, it exposes the product to various sources of contamination. Prolonged exposure to open air, the continuous presence of insects, direct contact with the meat, and the use of inappropriate containers, such as recycled cement bags, are common practices that facilitate the entry of harmful microorganisms. The fecal coliform count ranged from 2×10^2 to 1.20×10^6 CFU/g, with an estimated mean of 3.40×10^4 CFU/g, well above the threshold of 10^3 CFU/g prescribed by Decree No. 624-04 [10]. This high load indicates poor hygiene during preparation and suggests recent fecal contamination [11], primarily due to poor hand hygiene, handling both money and food, and the use of ingredients grown with potentially contaminated manure [12]. The high incidence of fecal contamination and opportunistic pathogens in Kilishi is not accidental but a direct consequence of critical stages in the artisanal process, notably prolonged environmental exposure and post-cooking recontamination, which erode the sanitary barrier and render a nutritious food a potential vector for foodborne illness. The detection of thermotolerant coliforms served as an indirect indicator of enteropathogenic microorganisms [13]. PCR analysis confirmed the presence of several diarrheagenic *E. coli* variants, including ETEC (6.7%) and ETEC-EPEC (5.03%). Although these prevalences are lower than those reported in some regional studies [14], they confirm a significant health threat and the spread of these pathogens.

Contamination by *Salmonella typhi* was confirmed at 6.7%, with one serotype identified at 1.67%. While lower than rates reported in neighboring countries [14], these figures still indicate a considerable risk. *Salmonella* presence is attributed to inadequate formal sanitary controls on meat during slaughter and transport, as well as to cross-contamination during handling and preparation [15]. Regional studies suggest non-typhoidal serotypes linked to animal/environmental reservoirs are common in the area [16].

The final grilling stage (15 - 20 minutes per side) helps reduce the microbial load. However, efficacy relies on sustained temperature and adequate time. The

risk of post-cooking recontamination is significant due to: Handling the product without gloves. Exposure to dust, flies, and dirty surfaces; ambient-temperature storage; unsuitable packaging [17].

Analysis of the production and marketing steps revealed several critical issues: the absence of an initial meat health inspection, open-air sun drying, and exposure to insects and stray animals [18]. Insanitary storage of ingredients (peanut oilcake and oil) promotes the growth of mycotoxin-producing molds [19]. Use of empty cement bags for packaging, which may introduce microbiological and chemical contaminants [20].

Kilishi has an inherently high nutritional value (proteins, heme iron, B vitamins, zinc, selenium) enhanced by the addition of peanut meal and spices (unsaturated fatty acids, antioxidants). However, Prolonged exposure to sunlight and air can cause lipid oxidation and a decrease in heat-sensitive vitamins (B and E) [21]. Microbial or chemical contamination (particularly by mycotoxins from mold on peanuts or poorly preserved meat) compromises the product's value, potentially rendering it unfit for consumption and reducing nutrient absorption [22].

The safety of Kilishi is compromised by artisanal practices that facilitate microbiological and chemical contamination, notwithstanding its inherent high nutritional value. Although this study provides crucial data on the health safety of Kilishi, certain limitations must be highlighted. First, the study focused specifically on the city of Niamey. While representative of urban practices in Niger, the results could vary in other regions or neighboring countries depending on microclimates and local variations in artisanal processes. Second, the sample size, although statistically significant for a preliminary assessment, would benefit from being expanded in future studies to capture a greater diversity of producers and points of sale. Cross-sectional nature: Finally, the cross-sectional nature of the sampling provides a snapshot of the situation at a given moment. A longitudinal study incorporating seasonal variations (temperature and humidity) would allow a better understanding of the dynamics of microbiological and chemical contamination over the course of the year. These considerations do not call into question the validity of our results, but pave the way for broader future research for a comprehensive mapping of health risks related to Kilishi in West Africa.

5. Conclusions

This study provided an in-depth assessment of the artisanal value chain of Kilishi in Niamey, combining an analysis of production and marketing practices with an evaluation of the microbiological quality of the finished product. The results highlighted the richness of traditional know-how while revealing critical points of contamination that severely compromise consumer health and safety. The high prevalence of hygiene-indicator microorganisms, such as various pathotypes of *E. coli*, alongside the presence of more severe pathogens, including *Salmonella* serovars, clearly demonstrates a link between field manufacturing/marketing conditions and product contamination. Open-air drying, frequent manual handling, the use of recycled packaging, and exposure to environmental contaminants are identified as

the primary sources of these risks. Beyond microbiological hazards, these practices also affect the product's physicochemical quality, notably degrading its nutritional value.

The artisanal practices and the lack of hygiene observed in Kilishi production directly compromise consumer food safety in Niamey by favoring contamination with major pathogens such as *Salmonella* and *E. coli*.

In conclusion, the safety of Kilishi can only be ensured through targeted interventions. The identified challenges require integrating Good Manufacturing and Hygiene Practices (GMP) adapted to the local context while preserving the product's artisanal character and cultural significance. In order to translate these principles into concrete actions for artisans, we primarily recommend:

Modernization of drying infrastructures: Moving away from drying directly on the ground or on precarious supports in favor of elevated and covered dryers. This setup not only helps reduce cross-contamination from dust and animals but also protects the product from chemical atmospheric pollutants.

Improvement of final packaging: Promoting and generalizing the use of single-use food packaging (plastic wrap or sealed bags) instead of recycled paper or non-sterile containers. This measure is crucial for maintaining the microbiological integrity of the product until consumption.

Training on chemical risks: Raising producers' awareness about managing smoking times and the quality of the wood used to reduce the formation of toxic compounds.

By adopting these simple and inexpensive measures, the Kilishi industry can guarantee a product that combines tradition, high nutritional value, and food safety, thus meeting modern consumption standards.

Ethical Approval

Vendor-informed consent is required in accordance with local regulations, and all participants provided verbal consent.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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