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Evaluation of the Microbiological Quality of Three Varieties of Kilichi Produced in Niger

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Kilichi is a food based on beef, mutton or goat meat that is processed by slicing, dressing, drying in the sun and applying spice porridge and roasting over a hot fire. Traditional processing conditions and practices for this product may pose risks of microbiological contamination and pose serious public health concerns.

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The objective of this study was to assess the microbiological quality of Niger's kilichis. A total of twenty-two samples of kilichi of three variants (single kilichi "KS", archide kilichi "Kilichi Fari" and peanut paste kilichi with Jawa "Kilichi Ja"). Total Mesophilic Aerobic Flora (TMAF), total coliforms, faecal coliforms, Escherichia coli and Salmonella were determined after the preparation of the stock solutions, decimal dilution and seeding on selective media. The results showed that the samples of the kilichi coated with the peanut paste sauce are more loaded with sought-after sprouts. However, single kilichi is more loaded with faecal coliform, $3{,}52x10^6$ CFU/ml; $2{,}11x10^5$ CFU/ml and $1{,}17x10^6$ CFU/ml for kilichi single, kilichi Fari and kilichi Ja respectively. These results show that the microbial load of kilichi samples is higher than the microbial criteria that cooked deli products must meet.

Keywords: Niger; kilichi; microbiology; charcuterie.

1. INTRODUCTION

Kilichi is a Hausa word that refers to beef, mutton or goat meat, which is processed by slicing, dressing, drying in the sun and applying spice porridge and roasting in a blazing fire [1]. It is a popular product especially in northern Nigeria, Cameroon, Chad, Niger and other countries in the Sahelian region of sub-Saharan Africa [2].

In Niger, it's manufacture is an artisanal activity commonly practiced by butchers [2,3]. Processing of meat into kilichi is an economic activity carried out by meat professionals throughout the national territory, in both rural and urban areas [4]. Its consumption seems does not age, religious or ethnic barriers [2].

In addition, kilichi production is a successful technology that is the pride and even an identity of Nigeriens internationally and is part of the national heritage [4]. It has been produced throughout the national territory for several decades. Meat products have a high nutritional value; they contain carbohydrates, fats and proteins that can be assimilated by the body and easily used by microorganisms too. They provide an ideal environment for microbial growth and sensory deterioration of the food [5]. In the sub-region, numerous studies have highlighted the presence of many microorganisms in kilichi; these include [6,7,8] in Cameroon; [5,8,9] in Nigeria, [4,10] in Niger. These studies conducted in Niger were carried out in the Niamey region. The objective of this work is to assess the microbiological quality of kilichis produced in Niger. This study will take into account all major kilichi production areas in Niger.

2. MATERIALS AND METHODS

2.1 Biological Materials

The present study was conducted mainly in four regions of Niger (Maradi, Niamey, Tahoua and Zinder). The study involved twenty-two samples of kilichi including three variants (single kilichi « KS », archide kilichi « Kilichi Fari » and peanut paste kilichi with Jawa ((Bixa orellana) « Kilichi Ja »).

2.2 Collection, Packaging and Transport of Samples

The production areas of the kilichi were known in advance and the choice was made according to the availability of the producer. Producers were informed of the objective of the study, the levy and its requirements. The samples were taken directly from the total quantity produced just after the grill, introduced into a sterile plastic bag and then wrapped in kraft paper envelopes and packed in cardboard before transportation to the microbiology laboratory of the Department of Biology of the Faculty of Science and Technology of the Abdou Moumouni University of Niamey.

2.3 Microbiological Analysis Methods

A 25 g serving of each kilichi sample was, cut into small pieces using a sterile chisel, crushed and poured into a vial. After grinding, a volume of 225 mL of the diluent (tryptone-salt) was added. The resulting filtrate was homogenized for 45 minutes under magnetic stirring. Finally, this solution serving as the stock solution was used to achieve a series of decimal dilutions, according to ISO 6887-V08-010-6 (2013).

The seeding was carried out with the different decimal dilutions of the order of 10^{-6}. The microorganisms sought are the indicators of food hygiene, namely: total mesophilic aerobic flora, coliforms, *E. coli* and *Salmonella*.

- Total Mesophilic Aerobic Flora (TMAF) determination was carried out according to ISO V08-051(1992)/ ISO 4833. The

enumeration of this flora was carried out on PCA agar (Plate Count Agar). Seeding was carried out with dilutions 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}; 0.1 mL of each dilution and introduced into a petri dish containing PCA agar; then incubated at 37º C for 24 hours.

- The determination of total coliforms was carried out according to ISO V 08-015 (1991)/ ISO 4832. Seeding was carried out on Mac Conkey agar with purple crystal, with dilutions of 10^{-2} to 10^{-5} (10 -^{2}, 10-^{3}, 10^{-4} and 10^{-5}). The boxes were incubated at 37°C for 24 hours.
- The search for E. coli was carried out on the EMB medium (Eosin Methylene Blue) according to ISO 3811 method. The incubation of the petri dishes was done at 37° C for 24 hours.
- The search for Salmonella was carried out in two stages: enrichment on the liquid selective medium (Rappaport Vassiliadis), and isolation on the solid selective medium Salmonella-Shigella (SS), according to ISO 3565: 1975 method.

According to the French Standard V 08-011, each box retained must contain no more than 300 colonies and at least 15 colonies. The number of microorganisms per gram of the sample was calculated from the boxes selected at the level of two successive dilutions by applying the formula below:

$$N = \frac{\Sigma c}{V.(n1+n2\ x0,1).d}$$

Where,

- Σc = Total number of colonies counted in boxes with colonies between 15 and 300.
- n1 = number of boxes counted from the first dilution;
- n2 = number of boxes counted from the second dilution;
- d = dilution factor from which the1st counts were made.

2.4 Statistical Analysis

For the results obtained, IBM SPSS statistics 20 software was used for the calculations of averages and standard deviations.

3. RESULTS AND DISCUSSION

3.1 Results

Several germs indicative of faecal contamination were sought for in this study, on samples of three variants of kilichi (kilichi simple, kilichi Fari and Kilichi JA) taken from several localities in Niger. The germs or group of germs sought are TMAF , coliforms (total and faecal) and *E. coli*.

Table 1. Average bacterial loads (in CFU/g) of simple kilichi samples (n= 9)

	Kilichi single			
	Moyenne ± ET	**Min**	**Max**	**Standard(*)**
TMAF	$1,29x10^{7}$±2,19^{a}	0,00E+00	$6,80x10^{7}$	$5x10^{5}$
TC	$2,88x10^{6}$±7,93^{b}	$6,85x10^{2}$	$2,24x10^{7}$	10^{3}
FC	$3,52x10^{6}$±1,01^{b}	$3,00x10^{3}$	$3,04x10^{7}$	$5x10^{1}$
E.C	$4,59x10^{5}$±1,26^{a}	00	$3,80x10^{6}$	10^{2}
SL	Abs/25g	Abs	Abs	00/25g

Values that do not have the same letter when superscripted on the same column are significantly different (p< 0.05). Kilichi Ja: Peanut paste kilichi with Java; TMAF: Total Mesophilic Aerobic Flora; TC: Total coliforms; FC: Fecal coliforms; E.C: Escheri chia coli; SL: Salmonella; (*) standard for cooked deli products [11]

Table 2. Mean bacterial loads (in CFU/g) of Kilichi Fari samples (n=7)

	Kilichi Fari			
	Moyenne ± ET	**Min**	**Max**	**Standard (*)**
TMAF	$9,43x10^{7}$ ±1,56^{a}	0,00E+00	$4,00x10^{8}$	$5x10^{5}$
TC	$3,60x10^{6}$ ±8,05^{b}	$1,42x10^{3}$	$2,00x10^{7}$	10^{3}
FC	$2,11x10^{5}$ ±4,94^{b}	$2,00x10^{3}$	$1,22x10^{6}$	$5x10^{1}$
E.C	$1,29x10^{7}$ ±2,90^{a}	00	$7,20x10^{7}$	10^{2}
SL	Abs/25g	Abs	Abs	00/25g

Values that do not have the same letter when superscripted on the same column are significantly different (p< 0.05). Kilichi Fari: Kilichi with peanut paste; TMAF: Total Mesophilic Aerobic Flora; TC: Total coliforms; FC: Fecal coliforms; E.C: Escherichia coli; SL: Salmonella; (*) standard for cooked delicatessen products [11]

Table 3. Mean bacterial loads (in CFU/g) of kilichi Ja samples (n=6)

	Kilichi Ja			
	Moyenne ± ET	**Min**	**Max**	**Standard (*)**
TMAF	$1,27x10^{8}±3,32^{a}$	0,00E+00	$8,80x10^{8}$	$5x10^{5}$
TC	$7,06x10^{6}±1,19^{b}$	$2,17x10^{3}$	$2,50x10^{7}$	10^{3}
FC	$1,17x10^{6}±1,51^{b}$	$7,90x10^{3}$	$4,08x10^{6}$	$5x10^{1}$
E.C	$6,88x10^{5}±1,46^{b}$	$2,40x10^{4}$	$4,00x10^{6}$	10^{2}
SL	Abs/25g	Abs	Abs	00/25g

Values that do not have the same letter when superscripted on the same column are significantly different (p< 0.05). Kilichi JA: Peanut paste kilichi with Jawa ; TMAF: Total Mesophilic Aerobic Flora ; TC: Total coliforms ; FC: Fecal coliforms ; E.C: Escherichia coli ; SL: Salmonella ; (*) standard for cooked deli products [11]

The results of Table 1 show that the contamination of the simple kilichi variant by total mesophilic aerobic flora is very high (i.e. an average load of $1.29x10^{7}$ CFU/g of product) followed by coliform loads which vary from $2.88x10^{6}$ to $3.52x10^{6}$ CFU/g respectively for total coliforms and faecal coliforms. In addition, the contamination of this variant by *Escherichia coli* is lower compared to other germs ($4.59x10^{5}$ CFU/g).

As for the Kilichi fari variant, contamination by total mesophilic aerobic flora is still high with an average load of $9.43x10^{7}$ CFU/g. *E. coli* represent the second group of germs counted at the kilichi fari about $1.29x10^{7}$ CFU/g. Fecal coliforms are the least enumerated, with a load of $2.11x10^{5}$ CFU/g (Table 2).

As for the kilichi "Ja", *E coli* and fecal coliforms are the least found, with with values ranging from $2.40x10^{4}$ to $4.00x10^{6}$ CFU / g and $7.90x10^{3}$ to $4.08x10^{6}$ CFU / g of product respectively. Like the other two variants, the contamination of kilichi by the total mesophilic aerobic flora is high, with an average of $1.27x10^{8}$ CFU/g. However, the level of contamination by total coliforms is lower than that of total mesophilic aerobic flora (Table 3).

For all kilichi variants analyzed, the contamination of this product by total mesophilic aerobic flora remains high (Tables 1,2,3). In addition, the bacterial loads obtained are all above the microbiological criteria that cooked deli products must meet, except for *salmonella* where a total absence has been observed (Tables 1,2,3).

3.2 Discussion

Microbiologically, twenty-two samples of the simple « Fari » and « Ja » kilichis, from Niger were analyzed. The germs sought were FAMT, Total Coliforms, Faecal Coliforms*, Escherichia coli* and *Salmonella.*

The results obtained during the analyses revealed very high FAMT loads, in the order of $1.29\ 10^{7}$ CFU/g, $9.43\ 10^{7}$ CFU/g and $1.23\ 10^{8}$ CFU/g respectively for kilichi simple, kilichi « Fari » and kilichi « Ja ». These results are close to those reported by [6] on twenty-four samples of spicy and non-spicy kilichi, taken from eight different producers in Northern Cameroon. Indeed, the values obtained by these authors are of the order of $1.51\ 10^{7}$ CFU/g and $1.00\ 10^{5}$ CFU/g for spicy and non-spicy kilichis. These microbial loads would be related to the drying of kilichi which is done in the open air thereby exposing the product to various contaminations in the atmosphere. A study carried out by [4] evaluated the microbiological quality of three kilichi variants produced using the traditional and modern methods. The number of sprouts obtained for the modern method where drying was carried out using a closed solar dryer is significantly lower than the number of germs obtained for the traditional method where drying was done in the open air.

These results also show a high rate of coliformes (total and faecal) contamination in the kilichi samples studied. The average loads of total coliforms obtained in this study are of the order of $2,88\ 10^{6}$ CFU/g , $3,60\ 10^{6}$ CFU/g and $7,06\ 10^{6}$ CFU/g and in faecal coliforms around $3,52\ 10^{6}$ UFC/g, $2,11\ 10^{5}$ UFC/g and $1,17\ 10^{6}$ UFC/g respectively for simple kilichi, « Fari » kilichi and « Ja » kilichi. These results confirm the studies made by [4] where the coliform levels (total and faecal) are higher than the Nigerien standards for the traditional method [4]. Thus, in Cameroon [6] high coliform loads were reported and were in the, order of $1.00\ 10^{5}$ UFC/g for unspiced. However, [12] noted a total absence of coliforms in samples of kilichi produced by traditional techniques in the urban community of Madaoua

in Niger. This high coliform load could be attributable to the overload of the atmosphere in the vicinity of the slaughter house or contamination by staff who were ill and allowed to handle the meat product. Unskilled labor and traditional and unsatisfactory slaughtering methods do not allow staff to work in good hygienic conditions [5].

Concerning *E. coli*, the results obtained from microbiological analyses indicate loads above the standards [11], except for two (2) samples that do not contain any germs. The values obtained for kilichi simple, kilichi « Fari » and kilichi « Ja » are respectively of the order of $4.59x10^5$ CFU/g, $1.29x10^7$ CFU/g and $6.88x10^5$ CFU/g. These results are similar to those obtained by Raji (2006) who obtained very high loads of *E. coli* on kilichi samples sold in the metropolis of Ilorin (Nigeria). It is important to note that *E. coli* is a subgroup of coliforms.

A complete absence of *Salmonella* was obtained in this study for all samples analysed, all variants combined. These results are consistent with those obtained by [4,10,12]. But disagree with those of [6].

For all the germs sought, the microbial loads of the coated kilichi samples (kilichi « Fari » and kilichi « Ja ») are the highest compared to those of the single kilichi samples, except for fecal coliforms where the simple kilichi is more loaded. This increase in the level of coliforms would be related to the coating of these kilichis by peanut sauce and spices. This is consistent with what was reported by [10], who, for all the germs sought, found a higher microbial load for coated kilichi than uncoated kilichi. In addition, [13] reported that coated kilichi is more contaminated than simple kilichi. Shamsuddeen [9] reported that some spices do not have antimicrobial activity, so meat treated with these spices may have a high microbial load. Ogunsola et Omojola [14] noted that unless spices are used to reduce microbial load, they can also be a source of a large number of germs in the product to which they are added. On the other hand [6] obtained a higher microbiological load for untreated kilichis than spicy kilichis. These authors explained this observation by the conservative virtues of chili pepper.

However, all the bacterial loads obtained in this study are higher than the maximum values accepted by the microbiological criteria that cooked deli products must meet to be considered fit for human consumption. This could be explained by the lack of hygiene observed throughout the processing (from slaughter to the finished product). It should also be noted that during the manufacture of kilichi, several instruments and containers are used, making it difficult for unidentified producers to comply with hygiene rules. In addition, it must be considered that the drying of kilichi is a step and if the rules of hygiene are not carefully respected, there is a huge risk of contamination by microorganism in the air. The high level of TMAF can be explained by the exposure of the product to air during drying. This finding is consistent with that of [6], who reported that the presence of many microorganisms on kilichi samples can be explained by the fact that kilichi is produced on street sidewalks or next to former landfills, and thus are exposed to dust and flies, vectors of spores of bacteria and fungi. It is also important to note that sick individuals or healthy carriers can participate in the manufacturing process of kilichi, thereby leading to contamination of the product This could explain the high level of coliforms obtained in this study on all samples analyzed. In addition, coliforms are not only a normal flora of the digestive tract of humans and animals, but also a better indicator of hygiene. Sabo [15] linked the high level of kilichi microorganisms obtained in their study to inadequate hygiene measures taken during production, by making an analysis of critical points. They claimed that critical control points limit the contamination of kilichi by microorganisms. For example [16], also stated that critical point analysis significantly improves the microbiological quality, sensory attributes, and storage stability of kilichi. Finally [8], suggests a possibility of post-production contamination, since the high temperature during roasting and the drying process of the meat should have reduced the bacterial load.

In Nigeria [17], studied the effects of storage methods on the shelf life of dried products (kilichi) under ambient conditions for forty-two (42) days to compare the production and packaging system with potassium sorbate treatment and the modern packaging system. The average bacterial loads obtained were of the order of $7.0\ 10^4$ CFU/g at the beginning of the test, $6.4x10^5$ CFU/g on the seventh day, $8.2x10^5$ CFU/g on the fourteenth day, and finally $1.2x10^5$ CFU/g on the twenty-eighth (28) day. Regarding the effects of potassium sorbate use, $2.5x10^3$ CFU/g and $7.6x10^3$ CFU/g were reported for kilichi treated with 10% potassium sorbate and

kilichi packaged without potassium sorbate respectively. The growth rate is higher in the control than the treated kilichi. These results suggest that treatment of kilichi with potassium sorbate and polyethylene packaging provides a certain degree of storage stability and protection against microbial contamination. In addition [7], tested the effect of processing techniques on the microbiological quality of traditional kilichi and sausage-type kilichi with a variable percentage of ingredients over 150 days. These analyses showed that the microbiological properties of kilichi are influenced by the duration of storage and the rate of ingredients used.

4. CONCLUSION

This study has revealed that the microbiological quality of kilichi produced in Niger, is unsatisfactory. Indeed, their loads of microorganisms, indicative of fecal contamination (total and faecal coliforms, *Escherichia coli*) are higher than the recommended limits allowed for cooked deli products. This may be due to the lack of hygiene observed throughout the production chain and also to the lack of mastery of modern production methods. The improvement of the microbiological quality of this product is possible, not only by strict compliance with good hygiene and sanitation practices throughout the production chain, but also by the use of the modern process by replacing open air drying with the solar dryer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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