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Microbial Quality of "Tchachanga", a Barbecued Mutton Sold in Benin

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Abstract

The microbial quality of Tchachanga, a barbecued mutton sold at Bohicon and Hilla-Condji bus stations in Benin was assessed in accordance with French standards (DGAL, 2000). The analyses revealed that the average total viable counts (TVC) recorded in Bohicon (3.96×10^8 cfu/g) and Hilla-Condji (5.51×10^8 cfu/g) exceeded standard safety limits (3×10^5 cfu/g). Similar observations were made for other parameters such as fecal coliforms count, *Escherichia coli* count, sulphite-reducing anaerobes, *Staphylococcus aureus*, yeasts and molds. *Salmonella* sp were absent in all samples. There was no significant difference ($P > 0.05$) between the microbial loads obtained in Bohicon and Hilla-Condji. This study shows that barbecued mutton sold in these two stations is unsafe for human consumption. It is therefore important for food safety authorities in Benin to take appropriate measures and sensitize sellers on strict observance of hygiene rules in order to preserve public health.

Keywords

Food Safety, Tchachanga, Street-Vended Food, Microorganisms, Public Health

1. Introduction

In recent years, the phenomenon of rapid urbanization in developing countries has not spared Benin, where the urban population is growing steadily. This rapid and intensive urbanization together with unemployment and economic crisis have enhanced the growth of street-vended foods [1]. Street-vended foods are ready-to-eat foods prepared and sold by vendors or hawkers primarily along the

streets and public places [2]. They play an important role in the daily lives of thousands of people whose education and food processing skills are often limited and who initiate this professional activity primarily to escape poverty and provide consumers with low-cost foods [3]. While street-vended foods are a cost-effective solution for nutritional needs in developing countries, the conditions for their preparation, processing, preservation and distribution do not always ensure the quality, safety and hygiene required [4]. The main concern of these foods is their poor microbiological quality, mainly because their production and sale sometimes take place in unhygienic environments. They are often contaminated with pathogenic microorganisms that cause diarrheal diseases to consumers [5]. Among these street-vended foods, meats are of paramount importance. The composition of meat makes it an excellent proliferation medium for many microorganisms, particularly bacteria [6]. Epidemiological studies have identified consumption of meat and meat products as important risk factors for diarrheal diseases such as salmonellosis, foodborne illnesses caused by *E. coli*, *S aureus* and *Clostridium perfringens* [7] [8]. In Benin, mutton is transformed into a number of derived products, including tchachanga, a barbecue generally sold along the streets by vendors with low levels of education [9] [10]. It is an important source of animal proteins for the populations, but unfortunately produced and sold under unhygienic conditions leading to its contamination by pathogenic microorganisms having negative impacts on the health of consumers. It is therefore important to assess the microbiological quality of this meat in order to assess safety risks to consumers. The available literature revealed a lack of data on the quality of this food sold outside the city of Cotonou in Benin. To fill this gap of knowledge, the present study aimed to assess the microbiological quality of barbecued mutton sold at Bohicon and Hilla-Condji bus stations in Benin.

2. Material and Methods

2.1. Study Area

The study was conducted from May to October 2016 at two bus stations in Benin: Hilla-Condji stations at the Benin-Togo border and Bohicon station along the Inter State Benin-Niger road (150 km from Cotonou, the main economic city of Benin).

2.2. Sampling

Barbecued mutton were sampled from vendors in both sites. The samples (**Table 1**) were taken one day per week for 7 weeks as per ISO 17604: 2003 standards. The sampling day varied weekly so that the results were representative of the entire week. On each sampling day, four samples were taken, *i.e.* 28 samples per bus station. Collected samples (around 250 g per sample) were placed in sterile stomacher bags and stored in a container with cooling element. Samples were then transported to the laboratory within 4 hours and immediately analysis.

Table 1. Sampling days and sample size per week.

Weeks	Days	Sample size	
		Bohicon	Hilla-Condji
1	Monday	4	-
	Friday	-	4
2	Tuesday	4	-
	Saturday	-	4
3	Wednesday	4	-
	Sunday	-	4
4	Thursday	4	4
	Monday	-	
5	Friday	4	-
	Tuesday	-	4
6	Saturday	4	-
	Wednesday	-	4
7	Sunday	4	-
	Thursday	-	4

2.3. Enumeration and Research of Microorganisms

2.3.1. Preparation of Serial Dilutions

Serial dilutions were made from the various samples according to ISO 6887-3: 2003. A stock solution was prepared by crushing 25 g of each sample in 225 ml of Buffered peptone water (BPW). For each sample, 1 ml of the stock solution was aseptically added using a sterile, graduated glass pipette into a sterile tube containing 9 ml of diluent to make the dilution 10^{-1}. Thereafter, 1 ml of the dilution 10^{-1} was aseptically introduced into another sterile tube containing 9 ml of the same diluent to make dilution 10^{-2}. The procedure was repeated until dilution 10^{-6}.

2.3.2. Total Viable Count (TVC)

TVC was performed according to ISO 4833: 2003. 1 ml of the stock suspension and its dilutions were added in sterile petri dishes then 10 - 15 ml of Plate Count Agar (PCA Oxoïd CM 0325), was poured into it, and then the whole was perfectly homogenized. After complete solidification, the plates were turned over and incubated at 30˚C for 72 hours. The assay was done in triplicate for each dilution.

2.3.3. Fecal Coliforms Count

Fecal coliforms were enumerated according to ISO 4832: 2006. 1 ml of the stock solution and its dilutions were placed in sterile Petri dishes. Violet Red Bile Glucose Agar (VRBG LAB 88) was then added. After solidification, a second layer was made. After complete solidification, the plates were turned over and incubated at 44˚C for 24 hours.

2.3.4. *Escherichia coli*

E. coli strains were counted according to AFNOR BRD-07/1-07/93. From the VRBG plates containing about 15 and 150 typical colonies, five colonies were

removed and subcultured onto Eosin Methylene Blue (EMB) agar. After 24 hours of incubation at 37˚C, colonies that appeared in a bright metallic form were considered characteristic for *E. coli.*

2.3.5. *Staphylococcus aureus*

S. aureus was counted according to ISO 6888-1: 1999. We used Baird-Parker media (LAB 85), incorporated in egg yolk and potassium tellurite (Oxoïd SR 0054C). The precast medium was cultured on the surface with 0.1 ml of the stock solution or its decimal dilutions. The plates were incubated at 37˚C for 24 to 48 hours. Colonies appearing black, shiny, bulging, surrounded by an opaque white border and a lightening halo were considered characteristic for *S. aureus*.

2.3.6. Sulphite-Reducing Anaerobes

Sulphite-reducing anaerobes were detected according to ISO 15213: 2003. 5 ml of the stock solution and its dilutions were placed in sterile tubes after heating for 10 min at 80˚C in a water bath to destroy the vegetative forms. Then, trypticase-Sulfite-Neomycin (Biokar) agar kept at 45˚C was added. After complete solidification, the tubes were incubated at 37˚C for 24 hours.

2.3.7. Yeasts and Molds NF ISO 7954: 1998

The counts were made on Sabouraud Dextrose Agar with Chloramphenicol. The medium was cultured on the surface with 0.1 ml of the stock solution and its decimal dilutions. The plates were incubated at 25˚C for 5 days. Colonies that appeared whitish and milky are those characteristic of yeasts while the other forms are molds.

2.3.8. *Salmonella sp*

The search for *Salmonella* sp was done according to ISO 6579: 2002. It was carried out in four successive steps. A pre-enrichment was performed by homogenizing 25 g of the sample in 225 ml of buffered peptone water incubated at 37˚C for 18 h ± 2 h, followed by an enrichment in Rappaport Vassiliadis broth incubated at 41.5˚C for 24 h. Isolation was carried out on XLD agar incubated at 37˚C for 24 h. The biochemical identification of the presumptive isolates was carried using API 20E strips.

2.4. Statistical Analyses

Data were recorded in a designed Excel database. The SAS 9.2 software [11] was used for statistical analysis. The mean microbial loads of each of the microbiological parameters were calculated per location and the comparisons between these values were made two by two using the student t-test after an analysis of variance to determine the zone effect.

3. Results and Discussion

3.1. Results

The microbiological characteristics of barbecued mutton samples collected at

Bohicon and Hilla-Condji bus stations are shown in Table 2. The average microbial loads of TVC are 3.96×10^8 cfu/g in Bohicon and 5.51×10^8 cfu/g in Hilla-Condji. These values are higher than the critical limits (3×10^5 cfu/g) set by French regulations (2000) [12], which indicates the unsatisfactory nature of the different samples analyzed. Fecal coliforms are present in the samples investigated at high levels (3.81×10^3 to 4.28×10^3 cfu/g respectively in both sites) compared to the standard limit (10 cfu/g). Similar observations were made for *E. coli*. Likewise, the counts of *S. aureus* and sulphite-reducing anaerobes, in Hilla-Condji and Bohicon exceeded the tolerance thresholds required by the same regulations. For yeasts and molds, the average microbial loads in Bohicon and Hilla-Condji were 9.61×10^2 cfu/g and 12.14×10^2 cfu/g, respectively. In addition, all the samples analyzed are free of *Salmonella* sp. The microbial loads of the samples of the two sampling sites revealed that there was no significant difference between the various parameters investigated in Bohicon and Hilla-Condji.

3.2. Discussion

The microbiological analysis of barbecued mutton sold at Bohicon and Hilla-Condji bus stations revealed high levels for total viable counts, fecal coliforms and *E. coli*, sulphite-reducing anaerobes, *S. aureus*, yeasts and molds. Samples of the two sites were contaminated beyond the tolerable limits required by the French regulations. The high total viable count confirms that barbecued mutton commonly known as "tchachanga", sold at Bohicon and Hilla-Condji bus stations, is processed and sold in poor hygienic conditions. The product is poorly covered or not covered at all making it prone to pollution by the ambient air generated by cars, motorcycles and insects in these bus stations. This exposure environment was mentioned in the diagnostic study carried out by Agli *et al.* [1]. The vendors of these stations are generally installed at the edge and along the roads, which bring them even closer to aerosols produced by mobile gears. This

Table 2. Variation of the microbial loads in barbecued mutton per sampling sites.

Variable	Bohicon		HillaCondji		Significance	Standards (cfu/g)
	M	SE	M	SE		
TVC (10^8 cfu/g)	3.96	3.19	5.51	1.97	NS	3×10^5
FC (10^3 cfu/g)	3.81	1.41	10.3	4.28	NS	10
E. coli (10^3 cfu/g)	0.952	0.352	2.57	1.07	NS	-
SRA (10^0 cfu/g)	194	106	817	729	NS	30
S. aureus (10^3 cfu/g)	3.12	1.57	3.57	2.71	NS	10^2
Salmonella (cfu/25g)	absence	absence	Absence	absence	-	Absence
YM (10^2 cfu/g)	9.61	5.6	12.14	5.63	NS	-

M: mean; SE: standard error; NS: not significant ($p > 0.05$); TVC: Total viable count; FC: fecal coliforms; SRA: Sulphite-reducing anaerobes; YM: Yeats and Molds; Standards: DGAL (2000).

total flora, which include pathogenic microorganisms and spoilage germs, are higher in this study than those reported (1.43×10^6 and 1.75×10^7 cfu/g) by Baba Moussa *et al.* [10] and Ahouansou [13] on the tchachanga sold in Cotonou. The high total viable count in the samples have a double effect. From the technological point of view, this implies that the process of microbial spoilage of the samples is strongly involved and, on the hygienic level, suspects the presence of pathogenic microorganisms in the products.

The average fecal coliforms loads in the samples are one hundred times higher than that prescribed by the standards, which is 10 cfu/g. These high levels of fecal coliforms that are indicators of fecal contamination are evidence of the inadequate processing of this food, which is prepared in low hygienic conditions with high risks of cross-contaminations [14]. Moreover, the contamination of samples by fecal coliforms and particularly *E. coli* also indicates a lack of personal hygiene and mainly testifies a lack of hands washing among handlers. Unwashed hands usually carry fecal microorganisms (*E. coli*, other heat-resistant bacteria), which are often responsible for diarrheal diseases and gastroenteritis [15] [16].

The average microbial loads in *S. aureus* (3.12×10^3 cfu/g and 3.57×10^3 cfu/g respectively at Bohicon and Hilla-Condji stations) are higher than those reported (0 cfu/g and 130 cfu/g) by Anihouvi [9] and Baba Moussa *et al.* [10] in the same product in Cotonou. They are; however, lower than that obtained (1.5×10^5 cfu/g) by Ahouansou [13] in Hausa barbecues sold in Cotonou. The presence of *S. aureus* in the samples represents a serious health risk to consumers. *S. aureus* produces enterotoxins (thermostable) whose ingestion causes food poisoning that can lead to sudden death by shock.

The SRA count showed that "tchachanga" samples contained average microbial loads of 194 cfu/g and 817 cfu/g respectively at Bohicon and Hilla-Condji bus stations. These values are considerably higher than the 2 cfu/g reported by Baba Moussa *et al.* [10]. The presence of SRAs in "tchachanga" could be explained by an insufficient heat treatment or a cross-contamination of the samples by these telluric strains which are carried by the dust. There is therefore a risk of food poisoning to consumers because these microorganisms might be strains of *Clostridium perfringens* or *Clostridium botulinum* producing toxins detrimental to human health. Botulinum toxins and tetanus toxins are the most active poisons known, and 100 grams of these toxins is enough to suppress all human life on the surface of the globe. They are 15,000 times more active than the most toxic chemical, aconitine at equal mass [17].

Our study revealed a significant contamination of tchachanga by yeasts and molds (9.61×10^2 cfu/g in Bohicon and 12.14×10^2 cfu/g in Hilla-Condji). These values are higher than that (53 cfu/g) obtained by Baba Moussa *et al.* [10]. This non-negligible presence of yeasts and molds in this commodity is ascribed to the insufficient cooking of the meat for the destruction of mold spores. They could also be due to cross-contamination of the product by these spores or also to re-

cycling products. The presence of yeasts and molds can strongly influence the hygienic quality of this meat because some species of mold synthesize toxic metabolites, mycotoxins under certain conditions, making them potentially injurious for human health [18].

The absence of *Salmonella* sp in all samples analyzed is a guarantee of food safety against salmonellosis. This result is consistent with that obtained by Baba Moussa *et al.* [10].

In sum, braised sheep meat investigated microbiologically in both target areas is of unsatisfactory quality and presents health risks to consumers.

4. Conclusion

The present study evaluated the microbiological quality of "tchachanga" sold at Bohicon and Hilla-Condji bus stations. Results show that "tchachanga" sold in these two sites is of low microbiological quality and therefore does not guarantee safety of consumers. Moreover, results obtained show that there is no significant difference between the microbial loads obtained in the two sites. It is necessary to raise awareness in all stakeholders in this sector to respect good hygienic practices during preparation, handling and sale, to intensify checks on these products and to propose ways of improving the quality of this ready-to-use food in order to preserve public health. In order that this work may arouse people attention to food safety, it is better to focus on communication near authorities, stakeholders and consumers regarding risks associated with these products.

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