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Evaluation of The Microbiological Quality of "Charmoute" Dried Meats Sold on Ndjamena/Chad City Markets

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ABSTRACT

Charmoute is a beef-based product widely consumed by the Chadian population. However, its microbiological quality and technological process are almost unknown. The aim of this study was to assess the microbiological quality of charmoute sold on markets in the city of N'Djaména. A total of 30 samples were collected from 6 markets. Loads of Total Aerobic Mesophilic Flora (TAMF), Total Coliforms (TC), Thermotolerant Coliforms (TTC), S. aureus and Yeast and Mould (L&M) were determined using standard microbiological analysis methodology. The results of this study show that the TTMF load ranged from 1.12×10^4 to 5.61×10^7 CFU/g. The average CTT load ranged from 6 to 6.28×10^3 CFU/g. On the other hand, S. aureus were present in samples from the Dembé market, with loads ranging from 10^2 to 8.55×10^3 CFU/g. The average L&M load ranged from 0.81×10^2 CFU/g to 1.83×10^4 CFU/g. Based on FAMT, 27% of samples were deemed unsatisfactory, 57% were deemed satisfactory and 16% were deemed acceptable. For S. aureus, 33% of samples were deemed satisfactory, compared with 50% unsatisfactory and 17% acceptable. While all samples not showing the presence of Salmonella were judged satisfactory (100%). Analysis of variance in FAMT shows that there is a significant difference between samples from the different markets studied (p= 0.0107). Analysis of variance in CT shows that there is no significant difference between the samples from the different markets studied (p= 0.407). The L&M load varies between $9.99.10^4 \pm 1.14.10^5$a and $1.04.10^3 \pm 8.94.10^3$a. Analysis of variance in L&M shows a highly significant difference between samples from the different markets studied (p= 0.0001).

This study indicates the presence of hygiene-indicating germs in charmoute and microbes that could present a health risk for consumers.

Keywords
Charmoute, Meat, Microbiological quality, N'Djaména.

Introduction
Chad covers an area of 1,284,000 km². Its population was estimated at 11.7 million in 2015, concentrated in the southern and central areas of the territory, 80% of whom live in rural areas [1]. In Chad, as in other Sahelian countries, the livestock sector plays an essential role in the national economy. Indeed, the livestock population is numerically significant, since there are 16.3 million cattle, 8 millions small ruminants and 5.2 millions dromedaries. This represents almost one Tropical Bovine Unit (TBU) per inhabitant, whereas the world average is close to one unit per 6 inhabitants. These figures place Chad among the world's livestock-raising countries [2]. Global consumption, all animal production combined, reached 286.2 million tonnes in 2010. It has grown at

a rate of 2.3% per year over the last ten years. Per capita meat consumption worldwide is 41.8kg/capita. In developing countries, meat consumption is 31.5kg/capita.

In developing countries, meat consumption rose from 10kg per capita in 1960 to 26kg per capita in 2000, and could reach 36kg per capita by 2030 [3].

In Chad, despite strong population growth and a large influx of refugees from Darfur, meat remains a staple food for Chadians. According to the Chad Ministry of Livestock, annual meat consumption is estimated at 22 kg/capita between 2009-2016. Beef is also the most widely consumed meat in Africa [4].

It is a major source of infection in humans [5]. Similarly, it has been reported as ideal for the growth of a wide range of spoilage bacteria [6]. Meat has traditionally been considered as a vehicle for foodborne diseases in humans [7]. Food poisoning can pose serious health problems, and market functioning can be severely restricted if food quality and certification are inadequate [8]. Consuming uncontrolled meat can expose consumers to food poisoning and foodborne illness which might results to public health problems in the presence of micro-organism [9]. Also, in N'Djamena, where the use of refrigeration remains costly and not guaranteed, the production of meat products stable at room temperature such as charmoute, using inexpensive and easy-to-implement methods represents an important issue. Most charmoute producers ignore or neglect hygiene rules. This leads to contamination of the meat by micro-organisms during and after preparation, especially during handling at the point of sale. It is with a view to improving the hygienic quality of charmoute, which remains the most widely consumed food in the city of N'Djamena, that we undertook this study, the general aim of which is to assess the hygienic quality of charmoute sold in markets and by dried meat producers in the city of N'Djamena (Chad). Specifically, we wanted to assess the hygienic quality of Charmoute sold in the markets of the city of N'Djamena.

Materials and Methods

Study framework

The study was first prospective and then experimental. It was conducted from June 15 to August 12, 2023. Samples were taken in N'djaména (Chad), directly at the markets. Analyses were carried out in N'djaména at the Laboratoire de Recherche en Sciences des Aliments et Nutrition (LaRSAN).

Sampling

Samples were collected using the aseptic technique, to prevent an increase in the initial microbial load in the sampled product during collection.

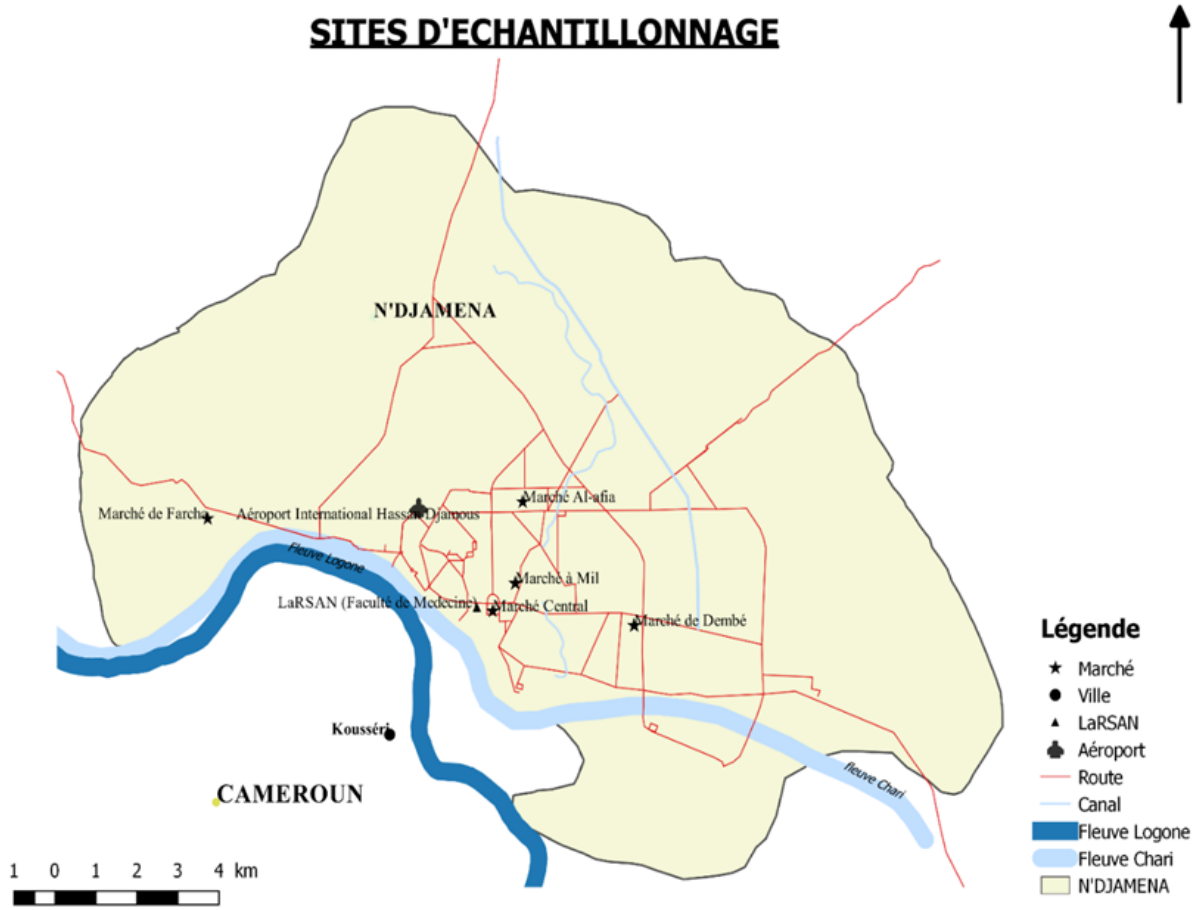


Figure 1: Sampling sites and laboratory location.

A total of 30 samples of Charmoute were collected from 6 markets in the city of N'djaména, represented as follows: marché de Farcha, marché à Mil, marché central, marché al-afia, marché de Dembé and marché al-Adala. The sample size was 50 g each.

Microbiological Analysis

Stock solution preparation and cascade dilution (ISO 6867-1)
Samples were ground with a mortar next to a flame. Ten (10) g of each sample was taken and introduced into a sterile bottle containing 90 ml of physiological water. The mixture was then homogenized by shaking to obtain the stock solution. From this stock suspension, a series of successive decimal dilutions was performed: 1 ml of solution was pipetted into a tube containing 9 ml of physiological water. The dilution is made up to the highest dilution desired.

Inoculation and Incubation

After the incubation period, plates with fewer than 300 colonies were counted in accordance with ISO 7218.

Enumeration of Microorganisms

The formula below was used if the number of colonies on the selected plates was between 15 and 300.

$$N = \frac{\Sigma C}{V(n1 + 0{,}1n2)d1}$$

When all boxes have less than 15 colonies, the evaluation is based on the arithmetic mean M of the colonies counted on the boxes, according to the formula:
N=M/d
The result was considered as follows: N< 1/d when no colonies were counted.

Total aerobic mesophilic flora count

Total aerobic mesophilic flora were counted in accordance with international standard ISO 4833 [10]. Plating was carried out on PCA agar and plates were incubated in an oven at 30°C for 72 h ± 3 h.

Research and Enumeration of Total and Thermotolerant Coliforms

Total and thermotolerant coliforms were enumerated in accordance with international standard ISO 4833 [11]. Inoculation was carried out on EMB agar and plates were incubated at 37°C for total coliforms and 44°C for thermotolerant coliforms, for 24h ± 2h.

S. aureus Detection and Enumeration

0.1 ml of the appropriate dilution of the sample is streaked onto Chapman's mannitol agar, poured into Petri dishes using a sterile loop. Incubation takes place at 37°C for 48 hours. Microorganisms fermenting mannitol give yellow colonies. This characteristic is an orientation criterion for the identification of Staphylococcus aureus.

Catalase Test

A drop of bacterial suspension was transferred using a pasteur pipette into a drop of hydrogen peroxide and placed on a slide. A positive reaction is indicated by the release of an air bubble.

Salmonella Testing

Salmonella testing was carried out in three stages, in accordance with ISO 3565:1975:

- Pre-enrichment with buffered peptone water for 24 hours at 37°C;
- Enrichment on liquid selective media: Rappaport Vassiliadis (RV) and MKTTN (Muller Kauffmann with Tetra thionate and Novobiocin);
- Isolation on solid selective media (Hektoen agar and Xylose Lysine Desoxycholate (XLD)).

Enumeration of Fungal Flora

Fungal flora were counted in accordance with international standard ISO 7954 [12]. SABOURAUD chloramphenicol agar was prepared and poured into Petri dishes, then 0.1 ml of the sample was inoculated onto the dishes. The dishes were incubated at 25°C for 48 to 72 hours. A reading was taken to assess colony types:

- **Yeasts:** colonies had an appearance identical to that of a bacterial colony. They were round with a regular outline, opaque and milky-white in color.
- **Molds:** had a velvety, more or less prominent pigmented appearance.

Yeast and mold colonies observed after enumeration were purified on Mueller Hinton agar. These strains were stored in BCC at -20°C for subsequent analysis.

Interpretation of Results

The assessment criteria used are taken from regulation 2073/2005/EC on meat products and their derivatives.

Table 1: Interpretation of the enumeration results (coliforms, yeasts and molds, and *Staphylococcus aureus*) was as follows.

m	3m	M= 10m	S=103 m	solid medium
	10m	M=30m	S=103 m	liquid medium
Satisfactory	Acceptable if C/n<2/5		Unsatisfactory	Unsatisfactory Toxic product
	Unsatisfactory if C/n > 2/5			

The sampling plan is based on the European Commission's guidance document.

- Results between m and ≤ 3m were considered satisfactory.
- Results between > 3m and ≤ 10m were considered acceptable.
- Results above > 10m were considered unsatisfactory.

Table 2: Microbiological criteria for meat products and derivatives.

Designation	*FAMT*	*CT et CTT*	*S.aureus*	*Salmonelle*
Satisfactory UFC/g	$\leq 3.10^6$	$\leq 10^3$	$\leq 5.10^2$	Absence
Acceptable UFC/g	9.10^6	3.10^3	15.10^2	Absence
Unsatisfactory UFC/g	3.10^7	10^4	5.10^3	Presence

Data Processing

The data collected on the basis of the questionnaire and the results of the various analyses were entered and processed using Microsoft WORD, EXCEL and XLSTAT software. For statistical processing, these data were subjected to an analysis of variance at a significance level of p = 0.05. Means were compared using the CHI^2 test. The map was produced using QGIS software.

Table 3: Results of ANOVA statistical test of microbiological parameters according to markets.

Area	TAMF	Coliforms		*S. aureus*	LM
		Totaux	Thermo		
Market Mil	$1,54.10^{6} \pm 2,23.10^{6a}$	$1,00.10^{2} \pm 0,00^{a}$	$1,00.10^{2} \pm 0,00^{b}$	$1,00.10^{2} \pm 0,00^{a}$	$1,28.10^{4} \pm 0,00^{a}$
Market Dembe	$4,68.10^{5} \pm 3,29.10^{5a}$	$1,14.10^{3} \pm 1,60.10^{3a}$	$3,44.10^{2} \pm 5,46.10^{2a}$	$1,79.10^{3} \pm 3,78.10^{3a}$	$1,22.10^{3} \pm 2,80.10^{3b}$
Market Farcha	$1,93.10^{7} \pm 2,32.10^{7a}$	$1,00.10^{2} \pm 0,00.10^{a}$	$0,64.10^{2} \pm 0,48^{2a}$	$2,38.10^{2} \pm 3,09.10^{2a}$	$2,67.10^{3} \pm 1,16.10^{2c}$
Market al-afia	$4,96.10^{6} \pm 4,42.10^{6a}$	$4,09.10^{3} \pm 3,70.10^{3a}$	$2,47.10^{3} \pm 2,60.10^{3ab}$	$2,57.10^{3} \pm 2,40.10^{3a}$	$9,91.10^{2} \pm 4,92.10^{2a}$
Market Center	$9,99.10^{4} \pm 1,14.10^{5a}$	$1,00.10^{2} \pm 0,00^{a}$	$1,00.10^{2} \pm 0,00^{a}$	$1,00.10^{2} \pm 0,00^{a}$	$1,04.10^{3} \pm 8,94.10^{3a}$
Market al-Adala	$1,62.10^{7} \pm 4,15.10^{6a}$	$2,64.10^{6} \pm 5,90.10^{6a}$	$1,00.10^{2} \pm 0,00^{a}$	$1,66.10^{3} \pm 3,48.10^{3a}$	$1,00.10^{3} \pm 3,23.10^{3a}$
P Value	0,0125 *	0,439	0,0107	0,407	0,0001

N.B. For the same column, values with the same superscript letter are not significantly different according to the CHI^2 test.

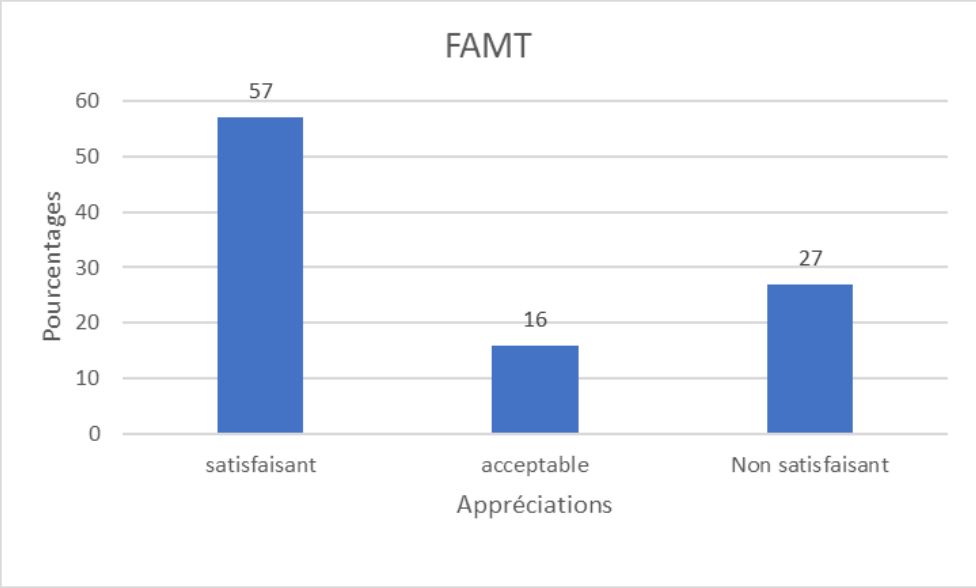


Figure 2: Microbiological quality according to hygiene indicator germs.

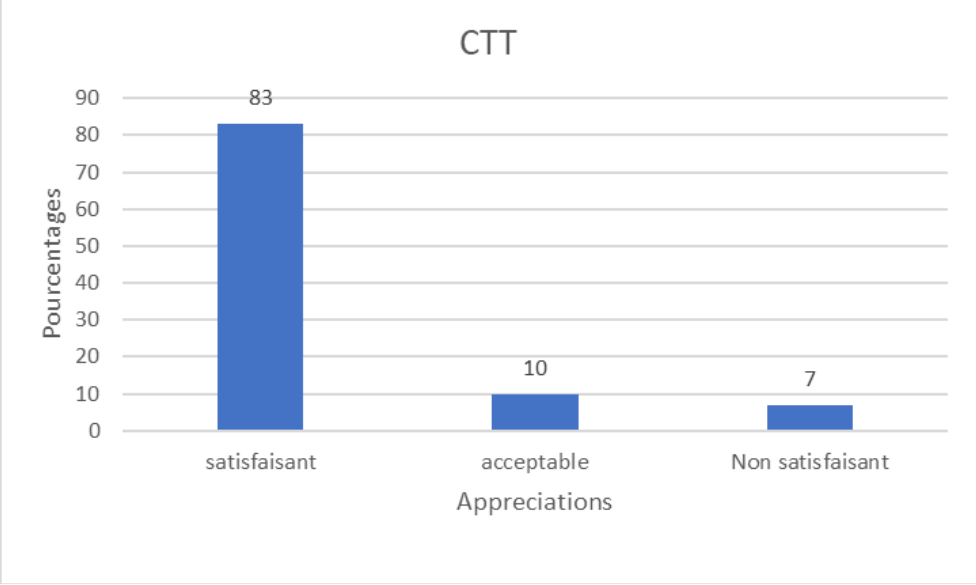


Figure 3: Microbiological quality by fecal contamination index flora.

Legend: TTC: thermotolerant coliforms

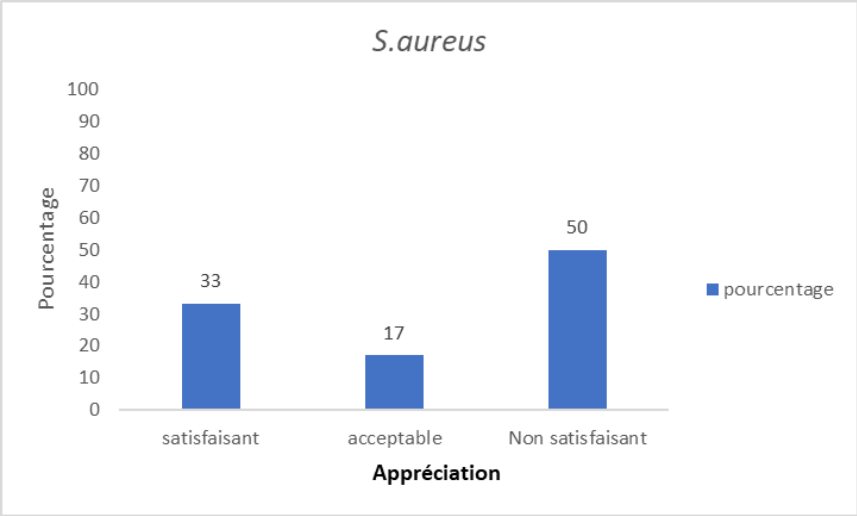


Figure 4: Microbiological quality according to *S. aureus* pathogens.

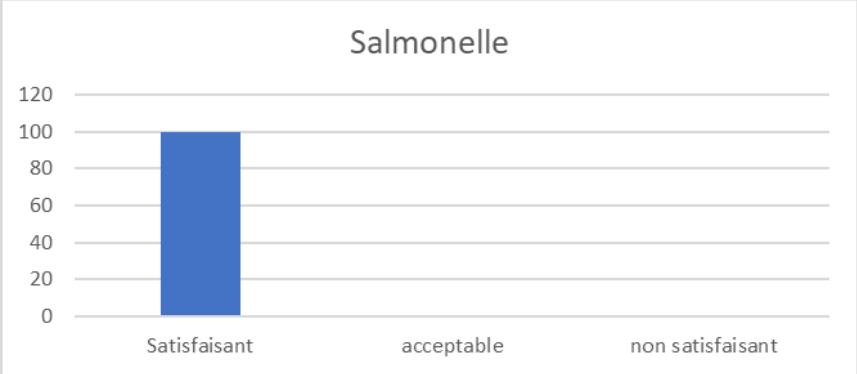
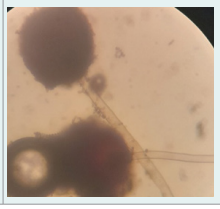


Figure 5: Microbiological quality as a function of Salmonella.

Table 4: Macroscopic examination of molds on Sabouraud medium.

Srain	Sabouraud medium	Image
E15	Color Revers : beige claire Mycélium : blanc Spore : noire Diamètre : 5,4 Cm Croissance : rapide Aspect : granuleux	

Table 5: Microscopic examination of molds.

Souches	Description	Image	Espèce
E15	Filament cloisoneux Sporocyste globuleux Spore ronde		Aspergillus sp

Results

Microbiological Quality of Charmoute

Results of analysis s of microbiological parameters

Microbiological Quality as a Function of Total Aerobic Mesophilic Flora

Figure 2 shows the percentage of microbiological quality according to hygiene indicator germs. These results have been compared with the standards of the European Union regulation [13].

The diagram below shows the microbiological quality of the 30 charmoute samples according to fecal contamination index flora.

Microbiological quality with regard to pathogens

The results of microbiological analyses of pathogenic germs compared with the threshold set by European Commission 2073/2015/CE are illustrated in the diagram below:

Referring to the standards of European regulations (2073/2015/CE), 33% of samples are satisfactory, 50% of samples unsatisfactory and 17% of samples acceptable.

Microbiological Quality By Salmonella

Fungal Strain Identification

Purified molds are identified by microscopic examination after 5 days' incubation on Sabouraud medium at 25°C. Microscopic examination on slides using the Methylene Blue ribbon technique gives the results shown in the tables below.

On the basis of the results obtained and in our study, we obtained that Aspergillus sp in our samples charmoute which presents the following character:
Cloisonous filaments, round (black) spore, globular sporocyst ...

Principal Component Analysis (PCA) And Classification of Study Parameters for All Samples

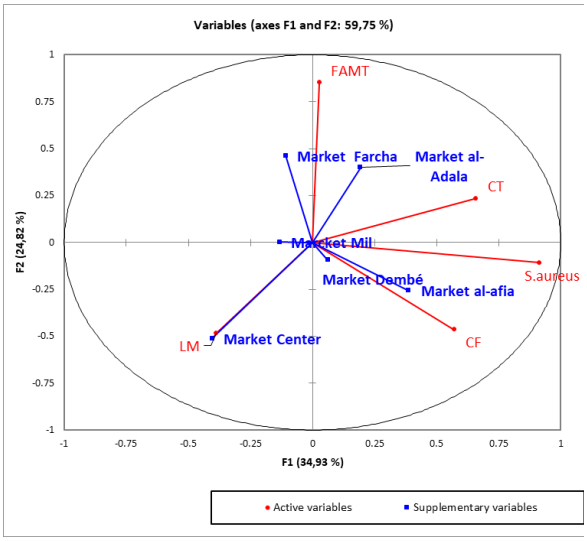


Figure 6: Principal Component Analysis (PCA).

Contribution of the variables (%):					
	F1	**F2**	**F3**	**F4**	**F5**
TAMF	0,0430	58,3964	1,3377	40,2228	0,0001
CT	24,8778	4,4001	37,4948	2,4556	30,7717
CF	18,6548	17,4002	27,4230	15,4892	21,0328
S.aureus	47,8834	0,9439	1,6446	1,3676	48,1604
LM	8,5409	18,8593	32,0999	40,4649	0,0350

Discussion

The microbiological quality of charmoute was determined on the basis of counts of certain hygiene indicator germs (FAMT, LM, CF and CT), pathogenic germs (*S. aureus*) and salmonella.

The microbial load of FAMT in the charmoute samples ranged from $1.12{,}10^4$ to $5.61{,}10^7$ CFU/g, and that of CT averaged $0.065{,}10^2$ to $7.1{,}10^3$ CFU/g. The average CF load was $0.11 .10^2$ to $6.28.10^3$ CFU/g. This result is higher than that obtained by Ali Haroun et al. [14], who found an average of $7.38\ 10^6$ CFU/g in charmoute. On the other hand, in our case, several conditions would limit microbial growth, such as ripening (salts, spices and vinegar). Results showing a high microbial coliform load (1.98 105 CFU/g) were obtained by Mbawala et al. for kilishi. The thermotolerant coliform load in this study was lower than that obtained by Ali H.H [15], which ranged from 10^2 CFU/g to $5.32\ 10^5$ CFU/g.

The presence of coliforms in the proportions indicated above may be due to the unsanitary conditions under which charmoute was produced. For *S. aureus*, average contamination levels are higher, at around $8.55.10^3$ to $3.12\ 10^3$ versus 10^2 CFU/g of *S. aureus* obtained by Njongmeta et al. [16]. However, they are lower than those obtained by Ali H.H [15], which are $2.80\ 10^3$ CFU/g and $3.10\ 10^5$ CFU/g.

The presence of *S. aureus* was revealed in charmoute samples from the Démbé market. The presence of *S. aureus* in foodstuffs is indicative of their insalubrity. Their presence in food can cause nausea, vomiting and diarrhoea, with even more serious consequences for young children and vulnerable individuals. A total of 30 charmoute samples were analyzed. In line with European Commission microbiological standards (2073/2005/EC) of 106 CFU/g for FAMT, 27% were unsatisfactory, 57% satisfactory and 16% acceptable. FAMT is an indicator of food microbiological quality, reflecting the sample's exposure to contamination and, in general, the existence of conditions favorable to the growth of microorganisms.

The enumeration of this flora is useful to indicate whether cleaning and disinfection during preparation have been sufficiently carried out. This concentration of FAMT does not necessarily indicate the process of deterioration in the sanitary quality of these producers' charmoute, as it is a general indicator of poor hygiene and sanitation. The FAMT loads recorded in Table 7 range from the lowest load found in samples from the central market ($9.99.10^4 \pm 1.14.10^5$a) to the highest load found in the Al-adala market ($1.62.10^7 \pm 4.15.10^6$a). The analysis of variance in FAMT shows that there is a significant difference between the samples from the different markets studied, as (p= 0.0125).

The TC loads recorded in the table range from the lowest load found in the central market samples and the Mil market samples, which are $1.00.10^2 \pm 0.00$a, to the highest load found in the Al-adala market samples, which is $2.64.10^6 \pm 5.90.10^6$a. Analysis of variance in CT shows that there is no significant difference between the different markets studied, as the (p= 0.439). This difference may be due to the authors' application of good hygiene and manufacturing practices, the authors' application of good hygiene and manufacturing practices during the production process. However, the high number of these coliforms in our samples indicates poor hygienic conditions during the processing of these foods. Their presence also reflects poor hygienic conditions.

Thermotolerant coliforms are considered to be indicative of faecal contamination of foodstuffs following handling. According to Campos et al., the presence of coliforms in food indicates contamination often caused by poor hygiene following food handling. However, many faecal coliforms can also come from the surrounding air and from packaging bags [17].

The CTT loads recorded in the table range from the lowest load found in samples from the Farcha market ($0.64.10^2 \pm 0.482$a) to the highest load found in the Al-afia market ($2.47.10^3 \pm 2.60.10^3$ab). CTT analysis of variance shows that there is a significant difference between samples from the different markets studied, as the (p= 0.0107).

A total of 30 charmoute samples were analyzed. In line with the microbiological standards set by the European Commission (2073/2005/CE), which is the absence of Salmonella, 0% are unsatisfactory, 100% are deemed satisfactory and 0% are acceptable. It is also important to note the marked absence of Salmonella in the 30 samples analyzed.

S. aureus loads ranged from the lowest found on the Mil market ($1.00.10^3 \pm 0.00$a) to the highest found on samples from the Al-afia market ($2.57.10^3 \pm 2.40.103$a). Analysis of variance for *S. aureus* showed that there was no significant difference between samples from the different markets studied, as the (p= 0.407) according to the CHI^2 test. The presence of Staphylococcus spp. In all the charmoute samples analyzed could be explained by the fact that staphylococci are widespread in nature and truly ubiquitous in the world, widespread in nature and truly ubiquitous. These organisms survive and spread in the environment as saprophytes, but are also facultative parasites of humans and animals [18]. As staphylococci populations are often nose, throat and skin, high numbers of staphylococci high numbers of staphylococci are often a sign of poor human [19]. The high level of Staphylococcus in beef and camel meat samples indicates the presence of staphylococci in beef and camel meat foods indicates the presence of cross-contamination, which is usually linked to the materials and clothing used [20].

The LM loads recorded in the table range from the lowest load found in central market samples of $9.99.10^4 \pm 1.14.10^5$a to the highest load found in central market samples of $1.04.10^5 \pm 8.94.10^3$a. Analysis of variance in LM shows that there is a significant difference between the different markets studied, as the (p= 0.0001). The results show a high microbial load in LM higher than that obtained by Jones et al., and lower than that obtained by Ahmat [21], which is $9.76.10^4$.

The presence of yeasts and molds in charmoute samples could be explained by the fact that colonization of foodstuffs by molds and yeasts is a is a common phenomenon. Eurutium, Penicillium and Penicillium and Aspergillus species are frequently found on the surface of dried meat products [22]. Contamination of food and feedstuffs by fungi that may be aflatoxigenic constitutes a major public health risk, with long-term health implications. The low water activity resulting from sun-drying reduces the competitive effects of most bacteria. Several physical factors, including humidity, ambient temperature, storage time, pH and oxygen, affect fungal growth and mycotoxin production in sun-dried meat [23]. The majority of fungal organisms isolated and identified are widespread in nature, and their habitat is mainly in wetlands. These food industry products were processed or introduced during the storage period due to inadequate storage facilities, as well as on the market and during transport to the market, as well as during transport. The majority of these sun-dried meat samples (charmoute) are stored close to agricultural products, which are more susceptible to fungal contamination. The majority of these sun-dried meat samples (charmoute) are stored close to agricultural products, which are more susceptible to fungal contamination and mycotoxin production. Fungal contamination and mycotoxin production, leading to cross-contamination.

The result allows us to illustrate the overall correlation of the 5 germs for the 30 charmoute samples collected in the 6 markets of N'Djaména. The table shows a strong and statistically significant correlation between the levels and between the different markets of the FAMT load, a medium and significant correlation with the steps, a medium and significant correlation with the bacterial load, a medium and significant correlation with the coliform load, a weak and non-significant correlation with the staphylococcal load and a strong inverse and statistically significant correlation with the yeast and mould load. The results of the statistical analyses show that, in general, there is a highly significant correlation between FAMT and CTT in all 30 samples. Similarly, there was a highly significant correlation in terms of contamination between FAMT and LM, and a highly significant correlation between *S. aureus* and CT in the 30 samples analyzed.

Principal component analysis shows that the parameters taken into account in our study can be represented on two axes according to sample collection sites. The two axes summarize 59.75% of the information in Figure 10. Parameters such as CT, CF and *S. aureus* loads presented on axis F1 (34.93%) contribute strongly to the formation of axis 1, while FAMT and LM loads (24.82%) contribute to the formation of axis 2 (Figure 10).

Reading axis 1, we can say that all the samples collected at the Farcha market and Al-Adala market sites are highly loaded with

FAMT and CT, but low in CF, *S. aureus* and LM. However, the correlation here is very weak between FAMT, CT and CF, *S. aureus* and LM. Reading axis 2, we can say that all the samples collected at the central market, Démbé market and Al-afia market sites are highly loaded with CF, LM and *S. aureus*, but low in FAMT and CT.

Conclusion

This study has enabled us to familiarize ourselves with analytical techniques, to acquire and master basic microbiological analysis techniques for food quality control, and to learn about production techniques.

In addition, the microbiological quality of finished products was assessed on 30 samples taken from various markets in the city of N'Djamena/Chad. The samples were highly contaminated with FAMT, CT, CTT, yeast and mould, *S. aureus* and salmonella were absent in all samples. This presence of microbes could present a risk for consumers.

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