American Journal of Food Science and Technology, 2023, Vol. 11, No. 1, 1-7
Available online at http://pubs.sciepub.com/ajfst/11/1/1
Published by Science and Education Publishing
DOI:10.12691/ajfst-11-1-1

Production and Microbial Quality of "*charmout*", a Dried Meat Produced in Chad

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Received November 21, 2022; Revised January 02, 2023; Accepted January 12, 2023

Abstract The *charmout* is a meat product obtained by drying meat and used in the preparation of sauces in some Sahelian countries. However, *charmout* is produced and sold under unhygienic conditions. It is therefore susceptible to contamination by pathogenic and toxigenic microorganisms. The objective of this study was to isolate and identify bacterial and fungal strains contaminating *charmout* produced and sold in six localities in Chad. Thus, 30 samples were collected in 6 production localities. The enumeration of total flora, coliforms, *Staphylococcus* sp, yeasts and molds was done according to standard microbiological methods. The API 20E gallery was used for the identification of isolated *Escherichia coli* and *Salmonella* strains. Fungal strains were identified on the basis of morphological and cultural criteria using specific identification keys. The results of the microbiological analyses revealed the presence of pathogenic and toxigenic microorganisms in the *charmout* produced in Chad. Loads of total coliforms, thermotolerant coliforms, *Staphylococcus* sp, as well as yeasts and molds ranged from 10^2 to $4.32x10^5$; 10^2 to $1.18x10^5$; $2.80x10^3$ to $1.80x10^5$ and $3.05x10^2$ to $1.7x10^5$ CFU/g respectively. All samples were free of *Salmonella* spp the prevalence of contamination of the samples was 100% (30/30) and 36.67% (11/30) by *Staphylococcus* sp and *Escherichia coli* respectively. The mold species identified were *Aspergilus flavus*, *Aspergilus niger* and *Aspergilus lentulus*. Based on these results, the consumption of *charmout* could constitute a health risk for the consumer. Thus, the application of Good Hygienic Practices and the improvement of production techniques are necessary to guarantee the health of the consumers.

Keywords: *charmout, microbial quality, pathogens, toxinogens, Chad*

Cite This Article: Ali Haroun Hissein, François Tapsoba, Himeda Makhlouf, Cisse Hama, Abel Tankoano, Hassan Mahamat Ali, Cheikna Zongo, Abdessalam Tidjani, and Aly Savadogo, "Production and Microbial Quality of "*charmout*", a Dried Meat Produced in Chad." *American Journal of Food Science and Technology*, vol. 11, no. 1 (2023): 1-7. doi: 10.12691/ajfst-11-1-1.

1. Introduction

Meat is a food very rich in nutrients such as proteins, iron, zinc, selenium, phosphorus and vitamin B12 [1]. However, due to its high nutrient content, meat provides an environment for the growth of spoilage microorganisms and common foodborne pathogens [2]. Its conservation after the slaughter of the animal poses a problem due to a lack of adequate conservation techniques and drastic climatic conditions in sub-Saharan African countries such as Chad. Add to this, refrigeration is insufficient for storage, distribution or processing of meat as manufactured form in many parts of the world [3]. However, drying is one of the oldest food preservation methods known to mankind [4]. With fermentation they have been recognized processes to reduce the pathogen and spoilage germs initially provided by the raw material. [5]. Thus, drying is used for meat preserving by nomadic populations in the event of overproduction and during festive periods [6]. Sun drying is the simplest and cheapest method of air-drying process used for foods [7]. Indeed, in Chad and Sudan, drying is used for the production of dried meat called *charmout*. The latter is a dried meat product used in the preparation of various dishes. It is obtained by cutting the beef into strips and then followed by sun drying [1]. Sun-drying exposes the meat to contamination from the air, which may contain aerosolized micro-organisms, particularly airborne molds and spores, that are resistant to the effects of drying [8]. This makes the *charmout* as a product that can be a source of food poisoning. According to WHO, 420 thousand people, including 125 thousand children die each year from food poisoning [9]. In Chad, few verifiable data exist on cases of food poisoning due to pathogenic microorganisms. Out of 2,735 patients consulted in five large hospitals in the capital in 2014, 84 poisonings were confirmed, following the ingestion of contaminated food [10]. Pathogenic germs which can contaminate meat products and which are the cause of food poisoning are:

Salmonella ssp, *Listeria monocytogenes*, *Clostridium botulinum* type E, *Clostridium perfringens*, *E. coli* O157: H7, *Bacillus cereus*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Shigella* [11,12].

In addition to this, as in the case of production, the marketing takes place in the open air and on the ground, on small straw mats or on pieces of plastic bags without any protection. Therefore, improving the techniques of production, conservation, and marketing of *charmout* is therefore necessary. Hence the interest of this study, which was to identify the *charmout* production technology, to assess the risky practices of the actors in the *charmout* production chain as well as its microbiological and physicochemical quality.

2. Material and Methods

2.1. Field Survey among Producers and Sampling of *Charmout*

This is a prospective study. A survey was conducted among the 30 identified *charmout* producers. The data of the survey were collected by means of an interview based on a survey form and a dashboard based on the research of information on the quality of the production of *charmout* according to the method of the 5 M. For the analysis, 30 *charmout* samples were collected from these producers chosen on the basis of their production frequency in six localities (Lignia, Dourbali, Massaguet Massakori, Karmé and Mourkou) in Chad. Five samples were collected per locality.

2.2. Microbiological Analysis of *Charmout*

Enumeration of the total aerobic mesophilic flora (FAMT) was carried out according to the ISO 4833 [13], that of the coliforms (total coliforms and thermotolerant coliforms) was carried out according to the ISO 4832 [14], Staphylococci were counted according to ISO 6888-1 [15], yeasts and molds were counted according to ISO 7954 [16] and isolation of *Salmonella* was carried out according to the international standard method ISO 6579 [17].

The API 20E was used for confirmation of suspected strains of *Salmonella* spp and of *E. coli*, after characterization by Gram stain, oxidase test, and the gallery minimal.

2.3. Physicochemical Analysis of *Charmout*

The water, the lipid and the total ash contents were determined according to the standard NBF 01-081 [18], NBF 01-179 [19]. and NF V03 720 [20] respectively.

2.4. Statistical Analysis

The data was processed using SPSS version 20.0.0 and R version 3.2.5 software. QGIS software version 2.18.11 were used for the preparation of the study area map. The analysis of variance (ANOVA) was performed to compare the means of the parameters studied. Pearson's test correlation analysis and principal component analysis (PCA) were performed to assess the relationships between the parameters studied.

3. Results and Discussion

3.1. Field Survey among *Charmout* Producers

3.1.1. Identification and Organization of Charmout Producers

The results (Table 1) obtained from the survey of 30 producers show that the production of *charmout* is an activity dominated by women in rural areas. In fact, 73% and 27% of women and men respectively are involved in this activity. This female predominance corresponds to that found by Doutoum et al. [21] (82.02%). In terms of level of education, 87% of the producers have not attended school, compared to 13% who have a primary level of education. The level of education could be a fundamental asset that would enable them to analyze their practices and identify solutions to their problems. Concerning the organization of work, the results of the questionnaires showed that out of all the producers interviewed, 70% produce individually against 30% who produce in groups. *Charmout* is produced in an artisanal way without any authorization of exploitation.

Table 1. Sociodemographic characteristics of *charmout* producers

Variables	Percentage (%)
Sex	
Male	73
Female	27
Level of study of producers	
Any	87
Primary	13
Secondary or Superior	00
Organization of work	
Individual	70
Group	30

3.1.2. Production and Preservation of Charmout

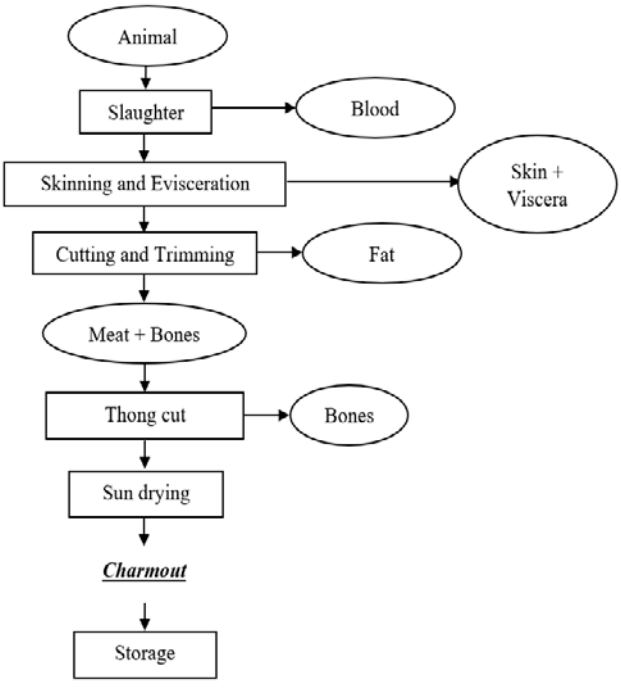

Figure 1. Diagram of artisanal production of *charmout*

The process used for the production of *charmout* corresponds to the one known as "fezzanese" or "Arabic" process [22]. The beef is cut into strips and dried in the sun for 3 to 5 days. However, during the rainy season, the drying time is quite long. Indeed, air drying is very dependent on the aerological conditions of the place [23]. It should be noted that the raw material (meat) comes from domestic slaughter (100%). The animal is slaughtered without any respect for the rules of slaughter. Thus, for the production of *charmout*, four main steps are identified, namely slaughtering, cutting, drying and storage (Figure 1).

3.2. Microbiological Quality of *Charmout*

Table 2 shows the average values by location of the microbiological parameters studied. From this table, it appears that the average load of the total counts (FAMT) is $7.38.10^6$ CFU/g. This average is lower than that of Mbawala et al. [24] who found an average level of contamination in the order of $(2.78 \pm 0.24) \times 10^7$ CFU/g in unpigmented *kilishi*. This is in contrast to the $8.9.10^5$ CFU/g found in biltong by Matsheka et al [8]. These differences could be explained by the fact that the addition of peanut paste and other ingredients in *kilishi* could increase the total flora of the finished product. In contrast, in the case of Biltong, several conditions would limit microbial growth, such as maturation (salts, spices and vinegar).

Regarding the enumeration of total and thermotolerant coliforms, the results obtained show that the average total load of total coliforms is $9.34.10^4$ CFU/g and that of thermotolerant coliforms is $1.74.10^4$ CFU/g. However, Tiendrebéogo et al. [25] found a load of less than 10 CFU/g of CT and CTT in *kilishi* from the traditional process. This lower load is thought to be due to the authors' application of good hygiene and manufacturing practices during the production process. However, the high count of these coliforms in our samples indicates poor hygienic conditions during the processing of this food. Their presence also reflects poor hygiene conditions during slaughtering operations and indicates recent contamination. Although the presence of thermotolerant coliforms can be an indication of the presence of enteropathogenic microorganisms, the risk is more particularly related to the presence of *E. coli* [26].

In terms of staphylococcal counts, the overall average is similar to that found by Shale & Malebo [27] in Biltong which is in the order of $1.01.10^5$ CFU/g. They believe that this excessively high figure indicated sub-optimal hygiene management in all points of sale. However, this value is higher than $0.33.10^4$ CFU/g and $3.01.10^3$ CFU/g found by Ribah & Manga [28] in the analysis of ready-to-eat meat products (*kilishi*) and Iheagwara & Okonkwo [29] in the analysis of traditional *kilishi* respectively. The presence of *Staphylococcus* spp. in all *charmout* samples analyzed could be explained by the fact that staphylococci are widely distributed in nature and are truly ubiquitous. These organisms survive and spread in the environment as saprophytes but are also facultative parasites of humans and animals [30]. As staphylococcal populations are often indigenous to the human nose, throat and skin, high numbers of staphylococci often indicate poor human handling practices [31].

Regarding the enumeration of yeasts and molds, the average value is lower than that enumerated by Iheagwara & Okonkwo [29] in the traditional *kilishi* which was $1.45.10^5$ CFU / g. On the other hand, it is higher than that enumerated in *kitoza* by Pintado et al. [32] which was $2.69.10^2$ CFU/g. The presence of yeasts and molds in *charmout* samples could be explained by the fact that the colonization of foodstuffs by molds and yeasts is a common phenomenon. *Eurutium*, *Penicillium* and *Aspergillus* species are frequently found on the surface of dried meat products [8].

Compared to the detection of *Salmonella*, although the presence of *Escherichia coli*, a reference organism, traditionally highlights the suspected presence of other pathogens, such as *Salmonella*, because they are able to survive in the same environmental niches [31], has here is no *Salmonella* was detected in samples of *charmout* analyzed. This same result was found in the *kitoza* analysis, where no sample showed *Salmonella* [33].

Table 2. Microbiological characteristics of *charmout* dried meat according to localities

Localities	Microbiological parameters (germs/g)				
	FAMT	**CT**	**CTT**	**Staphylococci**	**L & M**
Lignia	$9.99.10^6 \pm 5.22.10^{6a}$	$1.39.10^5 \pm 1.82.10^{5a}$	$2.15.10^4 \pm 2.07.10^{4a}$	$1.83.10^4 \pm 6.65.10^{3a}$	$1.71.10^3 \pm 3.24.10^{3a}$
Dourbali	$3.47.10^6 \pm 2.15.10^{6a}$	$1.16.10^5 \pm 2.33.10^{5a}$	$2.52.10^4 \pm 5.21.10^{4a}$	$2.75.10^4 \pm 2.60.10^{4a}$	$4.34.10^4 \pm 2.29.10^{4a}$
Mourkou	$1.25.10^7 \pm 1.40.10^{7a}$	$1.01.10^5 \pm 8.59.10^{4a}$	$1.69.10^4 \pm 1.84.10^{4a}$	$5.98.10^4 \pm 3.58.10^{4a}$	$3.46.10^4 \pm 4.68.10^{4a}$
Massakory	$3.98.10^6 \pm 5.08.10^{6a}$	$4.46.10^4 \pm 4.51.10^{4a}$	$5.15.10^3 \pm 7.80.10^{3a}$	$2.95.10^4 \pm 3.30.10^{4a}$	$7.72.10^4 \pm 6.54.10^{4a}$
Massaguet	$9.16.10^6 \pm 1.08.10^{7a}$	$1.21.10^5 \pm 7.94.10^{4a}$	$3.15.10^4 \pm 4{,}00.10^{4a}$	$1.02.10^5 \pm 1.23.10^{5a}$	$4.46.10^4 \pm 5.16.10^{4a}$
Karmé	$5.16.10^6 \pm 2.89.10^{6a}$	$3.96.10^4 \pm 3.43.10^{4a}$	$4.18.10^3 \pm 5.71.10^{3a}$	$7.43.10^4 \pm 7.11.10^{4a}$	$4.65.10^4 \pm 5.36.10^{4a}$
Overall average	$7.38.10^6$	$9.34.10^4$	$1.74.10^4$	$5.18.10^4$	$4.12.10^4$
P-value	0.3943	0.7737	0.6287	0.2743	0.2568
Standard values*	$< 1.10^4$	$< 1.10^2$	$< 1.10^1$	$< 1.10^2$	$< 1.10^2$

The identical letter a in the same column indicates that there is no statistical difference (p = 0.05) between the values of the germs according to the localities.
FAMT: Total counts; **CT**: Total coliforms; **CTT**: Thermo-tolerant coliforms; **Staph**: *Staphylococcus*.
* Burkinabe standard NBF 01-208 applicable to *kilichi* (dried meat) (in number of germs per gram of *kilichi*) [34].

As regarding to the microbiological standards applicable (Table 2) to *kilichi*, dried meat (NBF 01-208 [34]), the *charmout* produced in the six localities of Chad concerned by this study are all 100% unsatisfactory for all the germs enumerated, except for *Salmonella* that no strain was detected.

3.2.1. Identification of *Escherichia coli* Strains and Fungal Strains

In addition to the enumeration, to assess the hygienic quality of the *charmout* samples analyzed, bacterial strains such as *Escherichia coli* and also fungal strains were characterized. The presumptive *Escherichia coli* strains isolated were confirmed by the API20E gallery and the reading was done using the spreadsheet for microbial identification. Thus, 11 strains of *Escherichia coli* were confirmed. The presence of *E. coli* in dried meat has been reported by several authors [33,35,36,37,38].

Relative to fungal strains, the mold species characterized include: *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus lentulus*. The isolation of the genus *Aspergillus* in our samples is consistent with the characterization done in some studies [39,40] where these microorganisms were isolated. The contamination of dried meat by molds could be the result of the meat's contact with the ground [39]. Alternatively, the fungal species which colonize the samples of sun-dried meats must have been present in the atmosphere as spores and have been in contact with the product during processing or during the storage period due to inadequate storage facilities as well as in the market and also during transportation [40]. It is noted ability to produce mycotoxins [8]. The Fungal species present on dried meat must therefore be correctly identified if we want to recognize the possible presence of mycotoxigenic species and the risks associated with their mycotoxins [41].

3.3. Physicochemical Analysis of *Charmout*

The average results by locality of the physicochemical parameters obtained on the charmout samples are presented in Table 3.

The physicochemical parameters studied are the water content, the fat content, and the ash content. In addition to this, the results of the statistical test for the comparison of the data recorded in this same table show that there is no significant difference at the 5% threshold between the means of the samples of the six localities for all the physical parameters. chemicals studied.

Table 3. Physicochemical parameters of *charmout* according to the localities

	Settings		
Localities	**Average water content (%)**	**Average lipid content (%)**	**Average Ash Content (%)**
Lignia	16.92 ± 0.62[a]	13.78 ± 6.31[a]	6.06 ± 1.98[a]
Dourbali	13.32 ± 1.43[a]	8.09 ± 4.68[a]	7.46 ± 2.16[a]
Mourkou	15.75 ± 2.91[a]	16.45 ± 5.09[a]	6.45 ± 0.25[a]
Massakory	12.85 ± 2.36[a]	17.06 ± 7.88[a]	7.71 ± 1.77[a]
Massaguet	13.49 ± 4.52[a]	15.17 ± 11.46[a]	6.23 ± 1.78[a]
Karmé	13.63 ± 2.07[a]	18.55 ± 12.50[a]	7.20 ± 2.74[a]
Overall average	14.33 ± 2.82	15.11 ± 8,61	6.85 ± 1.87
P-value	0.129	0.429	0.667
***Standard**	≤ 13	-	-

From these results, it appears that the average water content of the *charmout* samples analyzed is 14.33±2.82%. This average result is significantly lower than that in "Kundi" which is 30-40% [42]. On the other hand, it is higher than the average determined in *kilishi* by Okorie [43] which was 10.33%. The difference between the average water contents in these different dried meat products could be explained by the influence that could have on the drying time and method, the size of the strips, and the lipid content of the meat as well as a light passage through the *Kilishi* fire. The Burkinabe standard [34] on *kilishi* stipulates a water content not exceeding 13%. In fact, for a stable product, the water content must be lower than the value set by the standard (≤ 13).

The average lipid content is 15.11±8.61%. This result is similar to the average of 15.21% found by Dashu et al. [44] on *kilishi* analysis. On the other hand, Okorie [43] determined a higher average than our result which was 22.33%. The difference in fat content of these meat products and the large variation in these rates between our results could be explained by the difference in trimming methods practiced by the producers and also by the influence of the fat composition of the carcass used for production.

Regarding the ash content, the overall average is 6.85±1.87%. This result is higher than that of Engez et al. [45] who found in biltong an average ash content that varied between 5.30 and 6.06%. However, the average obtained in this study is slightly lower than that of Dashu et al. [44] which is 8.5% in *kilishi*. The difference in these results could be explained by the contribution of ingredients in the processing of products such as *kilishi*, or it could be due to the presence of sand in our samples given the drying in the open air and without any protection of the *charmout*.

3.4. Correlation of the Various Parameters Studied

Regarding the study of the relationship between the water content and the different microbial loads of the *charmout* samples analyzed, the results reported in Table 4 show that there is a statistically significant link ($p < 0.05$) between the water content and these different loads except for the case of *Staphylococcus* sp. ($p > 0.05$). Indeed, the table shows a strong and statistically significant correlation between the water content and the load of FAMT, an average and significant correlation with the load of coliforms, a weak and non-significant correlation with regard to the load in Staphylococci and a strong inverse and statistically significant correlation with the load of yeasts and molds.

These correlations recorded between the water content and the different flora counted during this study could be explained by the fact that the water content plays a primordial role in bacterial growth. Indeed, any food substance with high water activity would promote the growth of microorganisms at a high growth rate [39], as shown here in the case of FAMT, CT and CTT. Matsheka et al. [8] also found that the water content has a significant effect (Pearson's correlation coefficient of 0.29) on the total count of viable bacteria at a threshold of 5%. On the other hand, the inverse correlation between the water content and the load of yeasts and molds, could be explained by the fact that the molds prefer a lower humidity level of the substrate and higher temperatures, and are therefore the most frequently isolated. stored products [41].

Table 4. Correlation test between water content and microbiological parameters

Settings		TE	FAMT	CT	CTT	Staph	L & M
TE	R	1	0.756 **	0.390 *	0.457 *	0.033	-0.820 **
	P		0.000	0.033	0.011	0.861	0.000
FAMT	R	0.756 **	1	0.265	0.360	0.067	-0.542 **
	P	0.000		0.158	0.051	0.727	0.002
CT	R	0.390 *	0.265	1	0.869 **	-0.012	-0.390 *
	P	0.033	0.158		0.000	0.950	0.033
CTT	R	0.457 *	0.360	0.869 **	1	0.253	-0.411 *
	P	0.011	0.051	0.000		0.177	0.024
Staph	R	0.033	0.067	-0.012	0.253	1	-0.044
	P	0.861	0.727	0.950	0.177		0.818
L & M	R	-0.820 **	-0.542 **	-0.390 *	-0.411 *	-0.044	1
	P	0.000	0.002	0.033	0.024	0.818	

** . The correlation is significant at the 0.01 level (two-tailed); *. The correlation is significant at the 0.05 level (two-tailed).
Legend: r: pearson correlation; **p**: p valued; **TE**: Water Content; **Staph**: *Staphylococcus* sp; **L & M**: Yeasts and Molds.

With respect to the principal component analysis, Figure 2 shows on the one hand that the higher the water content of samples *charmout* analyzed, the higher these samples contain high number of microorganisms mainly the total counts (FAMT), total coliforms (CT) and thermotolerant coliforms (CTT) and also Staphylococci. And on the other hand, the less the moisture content of these samples, the more they contain a high amount of yeasts and molds and a high amount of fat.

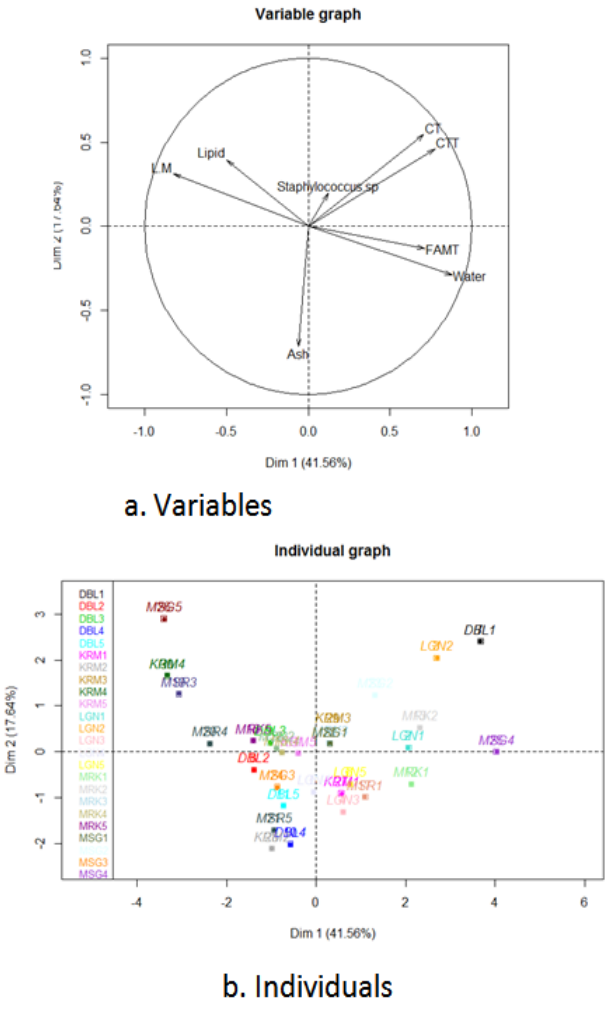

Figure 2. Diagram of PCA analysis

All these links call into question the water content of certain samples of *charmout* analyzed in this study. This calls for a decrease in the humidity level for a much more stable product.

4. Conclusion

The study on hygienic practices in the production chain of "*charmout*" dried meats in the six localities of Chad, allowed us to know the mode of production, conservation, and marketing of *charmout* as well as the appreciation of *charmout*. hygiene practices in the production line of this dried meat product.

Analysis of all the results from this study shows that throughout the process, from production to marketing, basic hygiene rules are not respected??? by producers. This failure could be a source of chemical and microbiological contamination of the finished product, which could lead to the occurrence of food poisoning. Enumeration of microorganisms showed that the charmout samples analyzed have a high load of FAMT, CT, CTT, *Staphylococcus* sp and yeasts, and molds. Microbial loads that are beyond the limit of acceptability as regards the standards are, therefore not satisfactory for human consumption. Thus, it is imperative to take into account the application of good hygiene and manufacturing practices as well as the formal ban on the use of dangerous chemicals during the production of charmout dried meats in Chad.

Conflict of Interest

The authors have not declared any conflict of interest.

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