

Microbial quality assessment of biltong produced and marketed in Lubumbashi, DR Congo

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World Journal of Advanced Research and Reviews, 2023, 20(02), 593–598

Publication history: Received on 10 September 2023; revised on 07 November 2023; accepted on 10 November 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.20.2.2134>

Abstract

Objective: The aim of this study was to evaluate the hygienic quality of biltong produced locally and sold in Lubumbashi.

Methods: Seventy-five biltong samples were taken randomly from five supermarkets in the city of Lubumbashi, at the rate of 15 samples per supermarket. Thus, the mesophilic aerobic flora (MAF), yeasts and molds, *Staphylococcus aureus* and *Clostridium perfringens*, were enumerated on these samples after a series of successive decimal dilutions and inoculations in selective media.

Results: The results of microbiological analyzes revealed that levels of microbial contamination varied depending on the points of sale. MAF (2.34 ± 0.12 to $3.79 \pm 0.17 \times 10^6$ CFU/g) as well as yeasts and molds (3.50 ± 0.17 to $4.25 \pm 0.50 \times 10^3$ CFU/g) constituted the predominant flora of the biltong studied in five large supermarkets in the city of Lubumbashi with 100% of samples presenting levels of contamination above acceptable standards. *S. aureus* and *C. perfringens* were identified at low levels compared to acceptable standards, i.e. from 1.80 ± 0.07 to $3.79 \pm 0.41 \times 10^2$ CFU/g and 15.33 ± 0.15 to $32, 33 \pm 0.34$ CFU/g, respectively.

Conclusion: This study highlighted the exposure of the population of Lubumbashi to food poisoning through the consumption of locally produced biltong. Improving its hygienic quality involves reducing the total flora present on the meat, pathogenic germs and spoilage germs based on the initial microbiological quality of the meat used, improving personal hygiene agents assigned to production and improving the manufacturing process.

Keywords: Biltong; Food preservation; Hygienic condition; Microbiological characteristics; Lubumbashi

1. Introduction

Biltong is commonly known as a South African salted dried ready-to eat meat product originating from South Africa [1, 2]. Traditionally, it is prepared from raw meat fillets from cattle, Austria and antelope [3]. The meat is cut into strips and then salted for several hours. Nitrites/nitrates may also be used as well as sugar, vinegar, pepper and other spices [4, 5, 6]. It is then dried, hanging in a well-ventilated area for 1 to 2 weeks. For larger-scale productions, drying chambers are used, allowing the drying time to be reduced to a few days [7]. Without refrigeration, it can be stored for months and consumed without any modifications [8].

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In South Africa, the production of Biltong from wild meat is highly valued [9] as a picnic and snack product because it can be stored at room temperature and it is poor in lipids and rich in proteins [10, 11].

In the Democratic Republic of Congo, biltong is produced by a few butchers and delicatessens run by expatriates and tends to gain ground in various markets in the city.

Some individuals manufacture it in large quantities to resell it in supermarkets in the city, its marketing is therefore expanding.

Biltong has been the subject of a certain number of research studies, particularly on the characterization of its physicochemical characteristics with a water content varying from 8 to 44%, a salt content from 3.5 to 7.7 % and a water activity (a_w) of 0.60 to 0.84 [5, 12]. Furthermore, these characteristics can correlate with the presence of microorganisms [5]. Thus, in less hygienic manufacturing and storage conditions for this product, pathogenic microorganisms can develop and cause health problems (food poisoning) in consumers. In Lubumbashi no study has been carried out to verify the hygienic quality of biltong sold on the local market. Thus, with the aim of ensuring the food safety of consumers, we undertook this study to assess the microbiological quality of biltong produced and marketed in Lubumbashi.

2. Material and methods

2.1. Origin of commercial biltong samples

This study was carried out in Lubumbashi, capital of the Haut-Katanga province (DR Congo). This province is located between 27°30' and 29°30' eastern longitude and between 7°15' and 13°30' southern latitude with a total area of 134,431 km². The analyzes were carried out in the Laboratory of Expertise, Hygiene and Food Technology of the Faculty of Veterinary Medicine of the University of Lubumbashi located within the University Veterinary Clinics, in the Golf District.

Five supermarkets were identified as Biltong sellers in Lubumbashi. In total, five batches of the product have been identified there. Sampling included a total of 75 biltong taken from these supermarkets at a rate of 15 samples per point of sale. These samples were packaged in waterproof 100 g polyethylene bags and were collected randomly then transported to the laboratory in a cooler containing melting ice to avoid any temperature variation likely to modify the microflora. When in the Laboratory, samples were kept in the refrigerator at 4 °C before analyses.

2.2. Microbiological assays and strain identification

Microbiological analyzes were carried out at the food microbiology laboratory of Nouveaux Horizons University (Lubumbashi, DR Congo). Considering aseptic measures, 10 g of each sample was collected and transferred to a sterile Stomacher bag. Samples were homogenized using an Ultra Turrax (T25 basic IKA WERKE, Germany) at 9500 rpm. The sample was then submitted to a series of successive decimal dilutions (10^{-1} to 10^{-6}). In order to determine the hygienic quality of the samples, the following germs were sought:

2.2.1. Mesophilic aerobic flora (MAF)

MAF was sought and counted on Plate Count Agar (PCA) culture medium. Aseptically, the different dilutions formed were inoculated into Petri dishes using the so-called “double layer” method. Briefly, using a sterile pipette, 1 mL of sample was placed at the bottom of the Petri dish, then 12 mL of culture medium was added. After the culture medium had solidified, a second layer of 3 mL of medium was added. Subsequently, the Petri dish was placed in the incubator at 37 °C for 3 days. MAF colonies were counted according to Iso 4833-1 standards [13].

2.2.2. Yeasts and molds

These germs were counted on the Oxytetracyclin Glucose Yeast Extract (OGYE) culture medium. Oxytetracycline is used as a selective agent in this environment. One milliliter of each decimal dilution constituted was mixed with 15 mL of culture medium (Oxytetracyclin Glucose Yeast Extract) and allowed to solidify in a Petri dish. The dish was then incubated at 25 °C for 3 days. The yeast and mold colonies were counted according to the NF Iso 21527-2 standard [14].

2.2.3. *Staphylococcus aureus*

Seeding was carried out with 0.1 mL of each dilution, mixed in 15 mL of culture medium (Baird-Parker). After solidification, the Petri dishes were incubated at 37 °C for 48 h. The germ count was carried out according to the ISO 6888-1 standard [15].

2.2.4. *Clostridium perfringens*

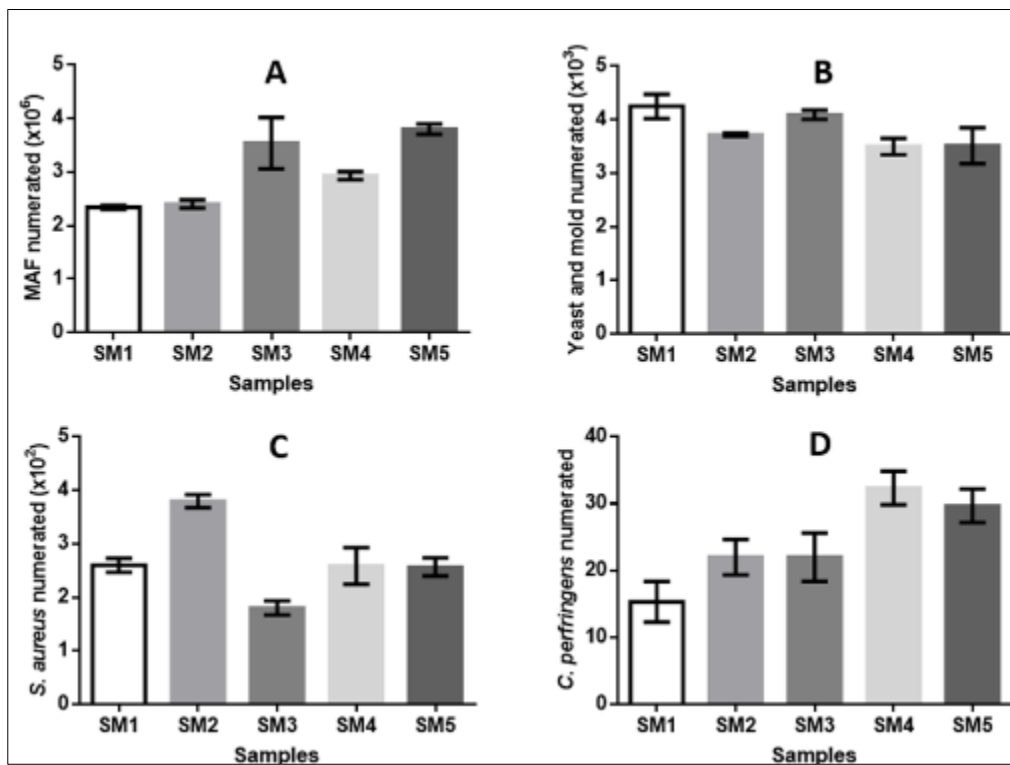
A deep inoculation was carried out with 1 mL of the bacterial suspension and 15 mL of TSN (Tryptone Sulfito Neomycin) medium. After solidification, the plates were incubated under anaerobic conditions at 37 °C for 18 to 24 h. The germ count was carried out according to the ISO 7937 standard [16].

2.3. Statistical analysis

The mean value and standard deviation were calculated from the data obtained from three individual packets of the same product. One-way ANOVA was used to test the significance of the differences between the means at $p < 0.05$ of five supermarkets (SMI, SMII, SMIII, SMIV and SMV). All statistics were performed using Graphpad Prism 6.

3. Results

The results of this study including the levels of contamination of biltong samples by mesophilic aerobic flora (MAF), yeasts and molds, *Staphylococcus aureus* and *Clostridium perfringens* are presented in Figure 1 and Table 1 below.



A: contamination by mesophilic aerobic flora (MAF), B: contamination by yeasts and molds, C: contamination by *S. aureus*; D: contamination by *C. perfringens*. SM: supermarket.

Figure 1 Contamination of biltong samples marketed in the city of Lubumbashi

In general, the contamination levels of the samples are different depending on the supermarkets selling biltong. MAF was more predominant in supermarkets V (3.79 ± 0.17), III (3.54 ± 0.21) and IV (2.93 ± 0.19) respectively. The analyzes carried out revealed that the differences were significant for these three points of sale, however the difference between supermarkets I and II was not statistically significant (Figure 1, A).

Regarding yeasts and molds (Figure 1, B), contamination was highest in supermarket I (4.25 ± 0.50), followed by supermarket III (4.09 ± 0.92). Statistical differences were only significant when comparing supermarkets I and III versus supermarkets IV and V.

S. aureus was enumerated in high quantities in the samples from supermarket II (Figure 1, C). The differences were statistically significant when comparing these supermarkets, except for the comparison between supermarkets IV and V.

C. perfringens contamination were higher in samples from supermarkets IV and V, statistically different with those from other outlets (Figure 1, D).

Table 1 Average microbiological contamination of biltong sold in supermarkets in relation to reference thresholds

Microorganisms	Level of contamination found in samples (C/g)					
	SM I	SM II	SM III	SM IV	SM V	Standards
MAF ($\times 10^6$)	2.34 \pm 0.12 ^a	2.40 \pm 0.09 ^a	3.54 \pm 0.21 ^b	2.93 \pm 0.30 ^a	3.79 \pm 0.17 ^b	3 $\times 10^5$
Yeast and molds ($\times 10^3$)	4.25 \pm 0.50 ^a	3.72 \pm 0.17 ^b	4.09 \pm 0.92 ^a	3.50 \pm 0.17 ^b	3.51 \pm 0.29 ^b	10 ² -10 ³
<i>S. aureus</i> ($\times 10^2$)	2.59 \pm 0.35 ^a	3.79 \pm 0.41 ^b	1.80 \pm 0.07 ^c	2.59 \pm 0.17 ^a	2.57 \pm 0.13 ^a	5 $\times 10^2$
<i>C. perfringens</i>	15.33 \pm 0.15 ^a	22.00 \pm 0.28 ^b	22.00 \pm 0.19 ^b	32.33 \pm 0.34 ^c	29.67 \pm 0.44 ^b	50

SM: supermarket, MAF: mesophilic aerobic flora, CFU: colony forming units. Different letters represent a significant difference at the 0.5%.

The results of this Table indicate that, in general, in all supermarkets, the average contaminations by MAF and by yeasts and molds are higher than the limit value set by the standards, however the contaminations by *S. aureus* and *C. perfringens* were below the acceptable standard. For all the supermarkets consulted, contamination by MAF were higher compared to the other microorganisms studied.

4. Discussion

The analyzes carried out on 75 biltong samples revealed that mesophilic aerobic flora (MAF) as well as yeasts and molds constituted the predominant flora of the biltong studied in five large supermarkets in the city of Lubumbashi. Indeed, in each supermarket 100% samples contained MAF with an average value of 3.07×10^6 CFU/g. With regard to the standards laid down by the International Commission on Microbiological Specifications for Food (ICMSF) [17], the average levels of MAF are higher than the maximum values allowed by the microbiological criteria (3×10^5 CFU/g) and which must be met by the raw and dried charcuterie products ready to eat, to be officially recognized as suitable for consumption.

On a technological level, a numerous mesophilic flora indicates that the process of microbial alteration is strongly underway. Furthermore, food with too many flora is considered unfit for consumption [18]. High numbers of MAF can be explained by unhygienic handling of meat from the slaughterhouse to the butchery via the cutting plant. The hygiene of personnel and utensils assigned to meat processing can also be incriminated in the sense that it also contributes largely to the hygienic quality of the processed product and also impacts the finished product [19]. Naidoo and Lindsay [1], working on the microbial ecology of biltong during production and at the point of sale in South Africa, found values between 10^6 and 10^7 CFU/g of mesophilic aerobic germs despite the sudden drying treatment by the product. Similarly, Matsheka et al. [3] numbered MAF between 10^2 to 10^7 CFU/g in biltong samples produced in butcheries in Gaborone (Botswana). We can clearly notice that MAF found in the biltong samples marketed in Lubumbashi are within the limits of the values obtained by Naidoo and Lindsay [1] and Matsheka et al. [3] but are on the other hand lower than those of 10^7 CFU/g obtained by Mbawala et al. [20] in Cameroon.

Regarding yeasts and molds, when their proliferation in foods reaches an excessive level, they can cause the products to deteriorate in terms of taste, texture, appearance and cause significant economic losses [21]. Our samples revealed their presence in high numbers in all samples. According to the ISO 6888-1:2021 [13], the accepted norm is between 10^2 - 10^3 CFU/g. From a technological point of view, biltong is a ready-to-eat raw product obtained by marinating in vinegar, adding cooking salt and spices before undergoing dehydration by air drying. These various treatments aim to acidify, lower the activity of the water available in the product in order to make it edible and extend its shelf life. However, yeasts and molds can grow in certain foodstuffs despite precautions such as acidity and low water activity. In addition, some yeast and mold spores resist heat, freezing, antibiotics and irradiation. Under certain conditions, mold species can synthesize mycotoxins which are toxic metabolites, making them potentially pathogenic for humans.

Staphylococcus aureus produces an enterotoxin responsible for food poisoning. It is often found in dehydrated products including biltong. By listing this germ in the samples we investigated, we were able to highlight an average value that met the international standards published in the Canadian Food Inspection Agency (CFIA) [22], range from 10^2 and 10^4 UFC/g. In general, the contamination values by *S. aureus* recorded in biltong produced in Lubumbashi are lower than those obtained by Mbawala et al. [20] in Cameroon as well as Naidoo and Lindsay [1] in South Africa, respectively: 2.85

$\times 10^4$ and 10^3 CFU/g. *Clostridium perfringens* is known to be responsible for necrotic enteritis and plays a role in foodborne illnesses. As such, they are of interest to us in evaluating the quality of biltong given their frequent presence in meat products [23]. The search for *C. perfringens* in biltong marketed in Lubumbashi revealed average values below the standard (Table 1). Although identified in small quantities, this germ is responsible for significant food infections. As these spores are very resistant to heat, it is advisable to cook the meat well above 100 °C. During cooling after cooking, these spores can germinate and produce toxins [24].

5. Conclusion

This study aimed to assess the hygienic quality of biltong produced and marketed in Lubumbashi. We explored the hygiene indicators during handling such as the MAF, responsible for spoilage of food such as yeasts and molds and finally the pathogenic germs responsible for toxic infections such as *Staphylococcus aureus* as well as *Clostridium perfringens*. This study report that the biltong manufactured and marketed in Lubumbashi is of unsatisfactory hygienic quality. Indeed, the loads of MAF, yeasts and molds are higher than the microbiological standards which raw and dried charcuterie products must meet to be recognized as suitable for consumption. Considering the results obtained during the microbiological examinations, it is up to us to note that our concern about the microbiological quality of biltong sold in supermarkets is justified because these products present risks to the health of consumers.

Reducing mesophilic aerobic flora as well as yeasts and mold would be possible by improving hygienic conditions during the meat processing process, including the hygiene of staff and utensils used for the preparation of biltong. Thus, improving the hygienic quality of biltong produced and marketed in Lubumbashi involves reducing the total flora present on the meat, pathogenic germs and spoilage germs. With the aim of improving the initial microbiological quality of the meat used for the manufacture of biltong, improving the personal hygiene of agents assigned to production and improving the biltong manufacturing process are effective conditions.

Further studies on the physicochemical characteristics of biltong samples produced and marketed locally would help to understand the possible correlation between these characteristics and the level of microbial contamination of this commodity.

Compliance with ethical standards

Acknowledgments

The authors would like to thank Nouveaux Horizons University (UNH) through its food microbiology laboratory where the sample analyzes took place.

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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