International Journal of Nutrition and Food Sciences
2024, Vol. 13, No. 3, pp. 56-65
https://doi.org/10.11648/j.ijnfs.20241303.11

Research Article

Evaluation of the Microbiological Quality of Some Marketed Spices in Lomé (Togo)

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Abstract

Spices are a group of condiments usually added to foods in small quantities and used to flavor different dishes. However, although the conditions of production, conservation and sale can be a source of infection, little data is available to guide stakeholders and users. The objective of this study is to evaluate the microbiological quality of spices marketed in the city of Lomé. To do this, sampling was carried out and standardized microbiology methods from the French standardization association (AFNOR) were used to search for germs in the samples of spices considered. The results of the microbiological analysis revealed contamination to varying degrees. The presence of significant total mesophilic aerobic flora (FAMT) for approximately 65% of the samples, germs indicative of fecal contamination (Coliforms), yeasts and molds and anaerobic sulphite-reducing bacteria (ASR) for 30% of the samples were were revealed by the analyzes carried out. However, the presence of germs such as Staphylococcus aureus and Escherichia coli ($< 10^1$) is below the norm with a total absence of salmonella. The level of contamination of spices, the conditions of processing and sale constitute potential risks of toxic infections linked to their consumption in Lomé. Education and awareness of those involved in the marketing of these packaged spices and health monitoring become necessary to guarantee food use without major risk of poisoning.

Keywords

Spices, Hygiene Practice, Contaminant, Microbiological Quality, Lomé (Togo)

1. Introduction

In traditional cuisine, spices represent one of the essential ingredients, both traditional and modern dishes, in Togo as elsewhere. Numerous studies have reported that spices can be contaminated by microorganisms, particularly pathogenic germs, causing serious foodborne illnesses [1]. The question of the hygienic quality of spices remains relevant since marketing in its pure form or in powder form remains the main outlet for these products today [2]. Since, the production practices of these products present insufficiencies in relation to hygiene [3]. They are in fact subject to

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Received: 20 March 2024; **Accepted:** 22 April 2024; **Published:** 17 May 2024

contamination and can be a source of potential dangers for consumers when they are contaminated by pathogens.

Unsafe foods, including bacteria, cause more than 200 illnesses, ranging from diarrhea to cancer. It is estimated that 600 million people (1/10) are infected each year after consuming contaminated food, 420,000 die and 33 millions lose years of healthy life. The frequency of these food poisonings is mainly linked to street food [4]. These foods essentially consist of spices used, most of the time, as food additives. However, the incorporation of spices into foods as food additives may be the cause of their poor hygienic quality [5]. Indeed, spices, like many other agri-food products, are also exposed to a wide range of microbial and non-microbial contaminants, during collection, processing and in retail repackaging markets through dust, sewage, animal excreta and even human excreta [6]. Hence the need to carry out regular analyzes and checks in order to preserve the health of the consumer [7]. The general objective of this study, which is part of this context, is to evaluate the microbiological quality of spices sold in large markets and spice processing factories in the city of Lomé in order to contribute to the health safety of its foodstuffs.

2. Material and Method

2.1. Study Framework

2.1.1. Geographical Framework

The study was carried out in the Autonomous District of Greater Lomé (DAGL) and covered around twenty market.

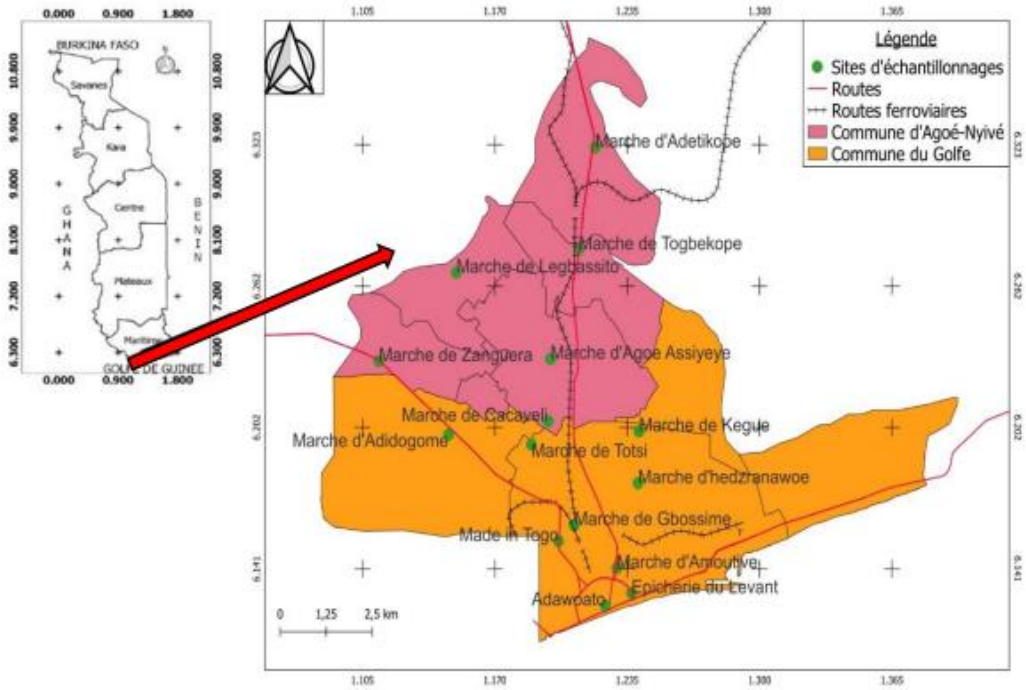


Figure 1. *Geographic Location Map of Prospected Markets in the Autonomous District of Greater Lomé (DAGL).*

Figure 1 shows that the District of Greater Lomé is located in the extreme southwest of Togo between 6°7'20" and 6°18'00" of North Latitude and 1°5'40" and 1°21' 60" East Longitude. The Autonomous District of Greater Lomé, according to the administrative reforms of 2017, brings together two prefectures (the Golf prefecture and the Agoè-Nyivé prefecture) and is subdivided into 13 municipalities over an area of 39,000 hectares [8].

2.1.2. Scientific Framework

The microbiological studies were carried out at the microbiology and food quality control laboratory (LAMICODA) of the University of Lomé

2.2. Material

2.2.1. Different Flora Sought in Spices and Corresponding Culture Media

The culture media used with the germs counted there and the culture parameters are summarized in Table 1.

Table 1. Germs Sought in Spices and Corresponding Culture Media.

Flora counted	Culture media	Temperature and incubation time	Observation	Standards used
TAMF	PCA Agar	30 ℃ for 72 hours	Lenticular colonies	NF V08-051
Coliforms	VRBL Agar	37°C for 24 hours	Pink-red colonies	NF V08-050
Anaerobic-sulfito-reducing	TSN	37°C for 24 to 48 hours	Black color	XP V08-06
Fungal flora (yeasts & molds)	Sabouraud Chloramphenicol	25°C for 72 hours	Cottony appearance	NF V08-059
Staphylococcus aureus	Baird Parker Chapman	37°C for 24 hours	Black colonies with light haloGolden yellow colonies	NF V08-057-1
Escherichia coli	TBX	44°C for 24 hours	Blue colonies	NF ISO 16649-2 (2001)
Salmonella	Broth report SS Selective Medium	37°C for 24 hours	Trouble Transparent colonies with or without black center	NF V08-052

2.2.2. Plant Material

The plant material used in this study consists of six different spices most used in traditional Togolese dishes (red peppers, black peppers, cloves, ginger, green anise and spice mixtures). Microbiological analyzes were carried out on these spices in packaged powder form.

2.3. Identification of Spoilage and/or Pathogenic Flora in Powdered Spices Packaged and Sold in the Markets of the City of Lomé

2.3.1. Sampling of Spices

The collection of samples was carried out in the markets of six municipalities (Adawlato; Légbassito; Adéticopé; Agoè-Assiyéyé; Gbossimé; Hanoukopé). A pre-selection of spices was made based on their high frequency of consumption. Six widely consumed spices (E1; E2; E3; E4; E5; E6) were therefore selected for each site. At most one (01) sample (E) per spice and per seller was collected. Forty (40) samples were thus collected in the different markets and 10 samples of spice mixtures produced by industrial units specializing in the processing of spices were collected in two showcase in the city of Lomé (made in Togo showcase and grocery store the Levant). A total of 50 samples were collected and analyzed.

The samples were collected in sterile, well-labeled white plastic bags, then quickly transported to the laboratory in the best possible conditions and within an hour after collection. The samples were all analyzed immediately after being transported to the laboratory and after assigning identification codes to them. The weight of the samples taken was between 100 and 150 g.

2.3.2. Sample Collection

The sampling process involved red pepper (10 samples), black pepper (05), green anise (05), ginger (05), cloves (05) and spice blend (10 in markets and industrial units, all in the condition of packaged powder. The type of spices and the sites of the samples from the markets are represented in Table 2.

Table 2. Distribution of Spices According to theirs Places of Collection.

SPICES	MARKETS						TOTAL
	Adawlato	Legbassito	Adeticope	Agoè – Assiyéyé	Gbossime	Hanukope	
Red pepper	2	-	2	2	2	2	10
Black pepper	2	1	-	1	1	-	5
Clove	1	1	-	1	1	1	5

SPICES	MARKETS						TOTAL
	Adawlato	Legbassito	Adeticope	Agoè – Assiyéyé	Gbossime	Hanukope	
Ginger	1	1	1	-	2	-	5
Green anise	2	-	-	1	1	1	5
Spice blend	2	2	2	2	-	2	10
Total	10	5	5	7	7	6	40

2.3.3. Sampling Periods and Sampling Frequency for Spices

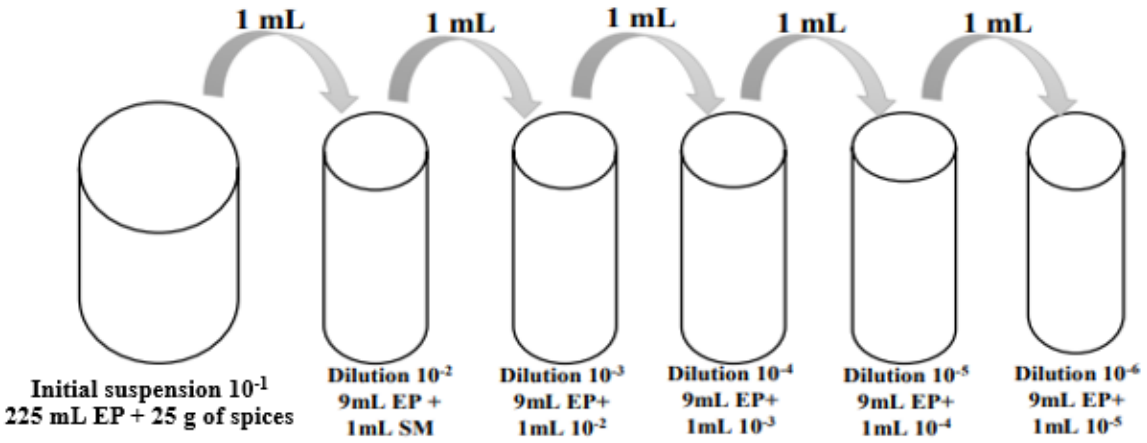
The samples were collected between October and November 2022, and samples were taken at a frequency of twice a week.

2.4. Microbiological Analyzes of the Spice Samples Considered

Preparation of the stock suspension and successive dilutions:

In accordance with the NF EN ISO 6887-1 standard, a test portion of 25 g of material to be analyzed (powdered spice sample) was taken. The test portion was therefore homogenized in Stomachers then diluted in 225 mL of Buffered Peptone Water, and thus constituted the stock solution for carrying out the different dilutions. The different decimal dilutions were made from the stock solution and in accordance with standard ISO 6887-2:2004.

Thus, 9 mL of tryptone salt (TS) were introduced into a series of previously sterilized tubes. Using a sterile pipette, 1 mL of the stock solution was taken and introduced into a tube containing 9 mL of tryptone salt to make the 10^{-1} dilution. The 10^{-2} dilution was obtained by taking one (01) mL of the 10^{-1} dilution in 9 mL of tryptone salt, and so on until the 10^{-6} dilution (Figure 2).



EP: Peptone Water

Figure 2. *Method for Preparing the Different Solutions at the Desired Dilutions.*

The microbiological study consisted of searching for and counting hygiene indicator microorganisms in the samples following standard routine methods specific to each microorganism (Table 1).

Enumeration of microorganisms

Microbial enumeration was performed as follows: 1 mL of the initial suspensions or serial decimal dilutions was used to inoculate petri dishes incubated under appropriate conditions (Figure 2). The following figure 3 presents the method used for counting colonies on solid medium by seeding in the mass.

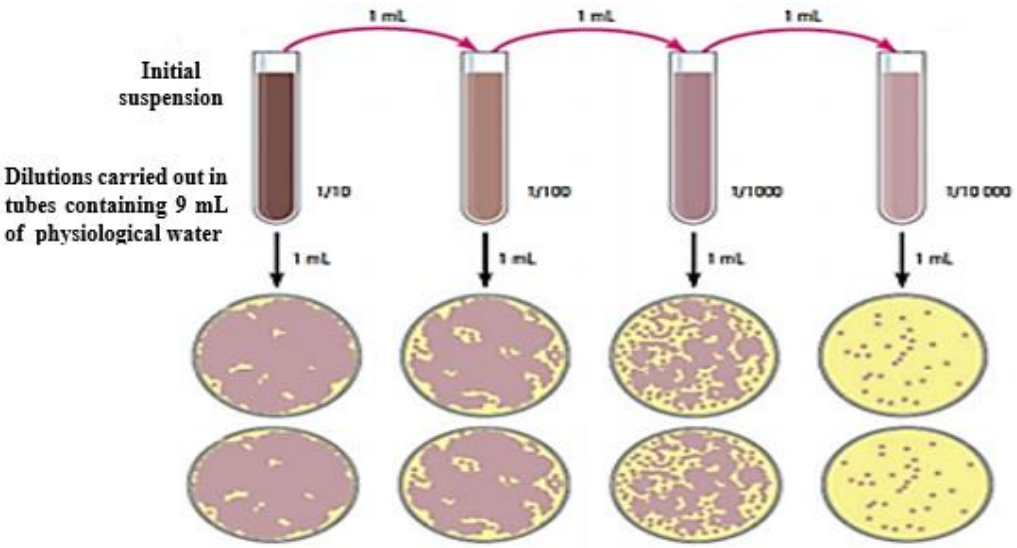


Figure 3. *Method Used for Counting Colonies on Solid Medium by Seeding into the Mass. Source: [1].*

2.5. Reading and Interpretation

A two-class sampling plan was used to determine the quality of the Samples analyzed in accordance with the microbiological criteria applicable to food additives in retail trade of the AFSSA standard 2007-SA-0174 (2008) according to regulation 2073/ 2005/EC relating to microbiological criteria applicable to foodstuffs. (Table 3)

Table 3. *Microbiological Criterion for Food Additives (Packaged Powdered Spices).*

	Sampling plan	FAMT	Total coliforms	*E. Coli*	L & M	ASR	*S. aureus*	Salmonella
Spices & spice blends (M)	n = 5; c = 2 n = 10; c = 4	5.10^5	10^2	10^2	10^5	10^4	10^2	Absence/25g

n = Sample size; c = Maximum number of defective sample units allowed; M ($X10^Y$): limit of acceptable hygiene concentrations FMAT: Total Aerobic Mesophilic Flora; ASR: Anaerobic-Sulfito Reductants; L & M: Yeasts and Molds.

Each box selected must contain a maximum of 300 colonies and at least 30 colonies. Counting was performed after the recommended incubation period (Table 1). The number of microorganisms per gram of product was calculated from the boxes selected and according to the following formula:

$$N = (\sum \text{colonies}) / (V \text{ in ml} \times 1.1\ d1)$$

N: concentration in number of CFU per gram of initial product;

∑: sum of colonies counted on the two boxes selected.

V: volume of inoculum applied to each box in milliliters.

d1: dilution factor of the first box retained.

2.6. Statistical Analysis

The data were analyzed using analysis of variance (ANOVA) and Excel 2013 software to determine the significant difference between the analyzed sample data.

3. Results and Discussions

3.1. Microbiological Analysis Results

Microbiological characteristics of the analyzed samples

Three types of microorganisms are conventionally sought during the microbiological analysis of foodstuffs. These are spoilage flora, indicators of fecal contamination and pathogenic microorganisms responsible for food poisoning [9].

According to Table 4, of the 40 samples taken from the markets, 45% were of unsatisfactory quality compared to the required TAMF load. Compared to total coliforms and ASR, the samples were of "Unsatisfactory" quality at respective rates of 45% and 17%. The overall dissatisfaction rate was

therefore 15%. *E. coli*, *S. aureus*, and *Salmonella spp.* were not detected in the samples analyzed (Table 4).

Table 4. *Summary of the Contamination Rate of Spices Analyzed by Germs.*

	Red pepper	Black pepper	Clove	Green anise	Ginger	Spice blend	Contamination rate
TAMF	80%	30%	20%	30%	60%	50%	45%
TC	70%	80%	20%	20%	0%	80%	45%
E. Coli	0%	0%	0%	0%	0%	0%	0%
Y&M	0%	0%	0%	0%	0%	0%	0%
ASR	30%	0%	0%	0%	0%	70%	17%
S. aureus	0%	0%	0%	0%	0%	0%	0%
Salmonella	0%	0%	0%	0%	0%	0%	0%
Rate by type of spice	26%	16%	6%	7%	9%	29%	15%

TAMF: Total Aerobic Mesophilic Flora; TC: Total Coliforms; ASR: Anaerobic-Sulfito Reductants; S. aureus: Staphylococcus aureus; Y & M: Yeasts and Molds.
CFU/g: colony forming unit per gram. Abs: Absent.

The following figure 4 shows the overall conformity rate of the different spices analyzed in relation to each germ.

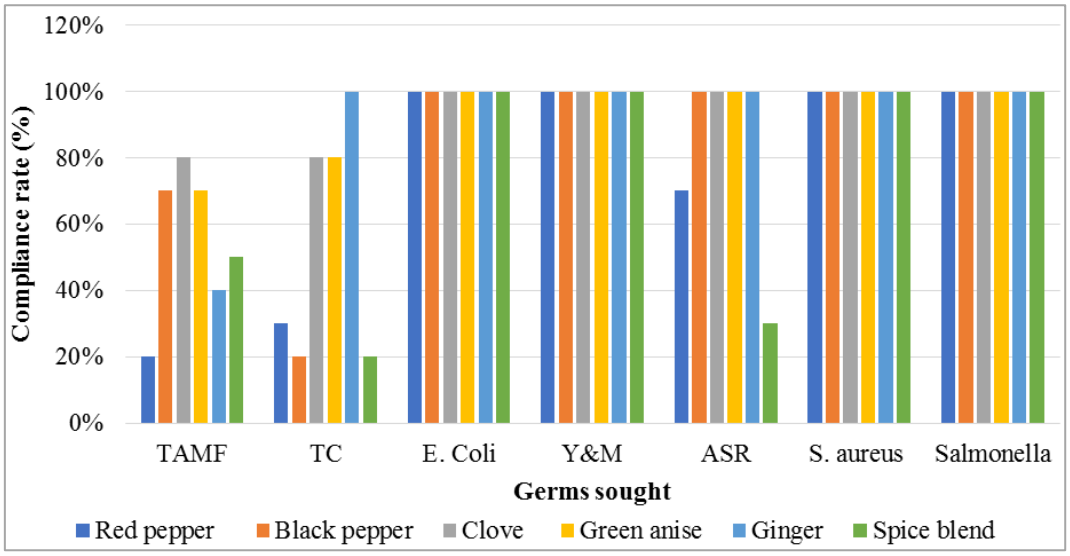


Figure 4. *Overall Rate of Germs in Spice Samples.*

TAMF: Total Aerobic Mesophilic Flora; TC: Total Coliforms; ASR: Anaerobic-Sulfito Reductants; S. aureus: Staphylococcus aureus; Y & M: Yeasts and Molds.
CFU/g: colony forming unit per gram. Abs: Absent.

Table 5 summarizes the average values of germs counted in the spices as well as the batch acceptability criterion.

Table 5. Summary of Average Values of Germs Counted in Spices.

	Red pepper	Black pepper	Clove	Green anise	Ginger	Spice blend	Criteria
TAMF	17.10^5	14.10^5	$63.2.10^5$	26.10^5	24.10^5	24.10^5	5.10^5
TC	13.10^2	2.10^2	$10.4.10^2$	74.10^2	9.10^2	5.10^3	10^2
E. Coli	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	10^2
Y & M	8.10^2	2.10^2	8.102	04.10^2	23.10^2	23.10^3	10^5
ASR	10^2	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	10^1	10^4
S. aureus	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	$< 10^1$	10^2
Salmonella	Abs	Abs/25g	Abs/25g	Abs/25g	Abs/25g	Abs	Abs/25g

TAMF: Total Aerobic Mesophilic Flora; TC: Total Coliforms; ASR: Anaerobic-Sulfito Reductants; Y & M: Yeasts and Molds. CFU/g: colony forming unit per gram; Abs: Absent.

The average contamination loads of the samples analyzed are recorded in Table 5. For the total flora, it varied from $14.10^5 \pm 63.2.10^5$ CFU/g. It varied from $2.10^2 \pm 74.10^2$ CFU/g for total coliforms and $2.10^2 \pm 23.10^3$ CFU/g for yeasts and molds.

It varies from 10^1 to 10^2 for ASR. Furthermore, the average microbial load of *E. coli*, *S. aureus* was less than 10^1.

3.2. Comparison of Spice Blend Results

From the results of microbiological analysis of the spice mixtures (Figure 5), it appears that the spice mixtures sold in the markets are 50% of satisfactory quality compared to the TAMF, while those produced in the industrial units are at 70%. They are respectively 20% and 30% satisfaction with respect to the total coliforms counted.

Furthermore, 30% of the spice mixtures sold in the markets are of satisfactory quality compared to the ASR, while we observe 100% satisfaction for the mixtures packaged in processing factories. On the other hand, these two types of spice mixture are of satisfactory quality compared to the other germs counted (*Escherichia coli*, *Staphylococcus aureus*, and Absence of *Salmonella*).

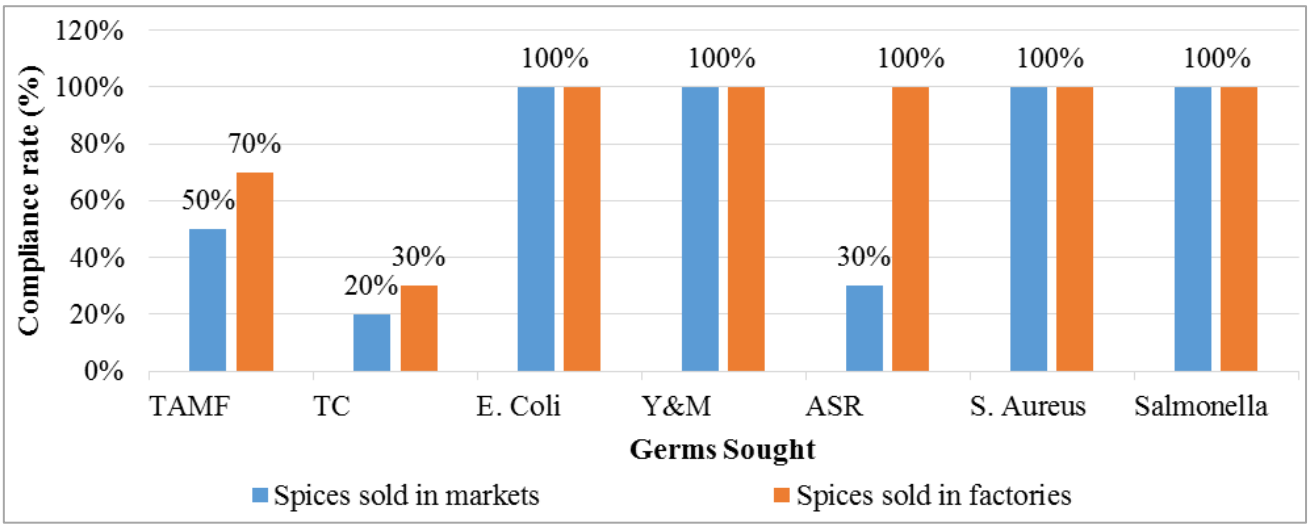


Figure 5. Comparison of Satisfaction Rates for Microbiological Quality of Spice Blends.

TAMF: Total Aerobic Mesophilic Flora; TC: Total Coliforms; ASR: Anaerobic-Sulfito Reductants; S. aureus: Staphylococcus aureus; Y & M: Yeasts and Molds.
CFU/g: colony forming unit per gram. Abs: Absent.

4. Discussion

The results of the various germ counts carried out during this study provide proof of a failure in hygiene practices and the implementation of good manufacturing practices (GMP). Indeed, the cleanliness of handling, the effectiveness of treatment processes, the state of freshness of the products are informed by the microbial load in the total flora of the spices. The results obtained easily show that TAMF contaminated the spices qualified as unsatisfactory (45% of the total samples analyzed) with regard to the Process Hygiene Criteria (CHP). This contamination rate is slightly below that reported by Ahoyo et al. (2010) [10], who reported a contamination rate of 74% by this group of microorganisms. This may be due to some improvements made in the prospected markets following health checks. The high rate of contamination of food samples analyzed by TAMF may be linked to poor quality of the raw material and practices related to their handling, including hygiene and/or temperature and time control [11, 12]. Sellers do not choose raw materials and ingredients based on relevant criteria. The data from this study show that spices are not spread or served in good conditions throughout the service. This can promote the multiplication of microorganisms in spices [13]. In addition, the results of the survey show that several sellers use spices that have been unsold for a long time to mix with those produced recently, with the aim of optimizing profits by minimizing losses. The contamination of red peppers and spice blends with total flora should raise concerns, as these spices have a high coliform load and are widely consumed in the city of Lomé Likewise, some of the seasonings added (salt, flour, onion, fish, etc.) can also contribute microorganisms to the initial flora of the base product. "Fecal" coliforms: *Citrobacter*, *Klebsiella* and more particularly *E. coli* are faithful indicators of fecal contamination of food [1].

Fortunately, the highest satisfaction rate (100%) was observed with these sprouts. Food contamination by *E. coli* is indeed an indicator of fecal contamination of ready-to-eat products [14]. The presence of fecal coliforms and *E. coli* in the samples would indicate poor hygiene in processing, which could result from the processor, the equipment in contact and/or the immediate environment of the product [15, 16]. In the case of this study, the absence of these germs can be explained by the fact that these spices have antimicrobial activities which can prevent the multiplication of germs.

The unsatisfactory microbiological quality of spices in relation to certain germs could also be directly linked to the quality of the water used by sellers during the processing of these spices. However, the high loads obtained for red peppers can be explained by the complexity of this product which contains other products (cooking salt) which do not undergo or which undergo little heat treatment depending on the production process adopted by the sellers. Indeed, cooking salts are often contaminated by total germs, notably thermotolerant coliforms [17]. The presence of *Salmonella*, Staphylococci and ASR in foods indicates their unsanitary nature.

The samples analyzed were 100% satisfactory for these germs, contrary to the data reported by Ahoyo et al. [10] with 27% for *S. aureus*. The low loads obtained compared to other germs can be explained by the proven antimicrobial activity of spices and the processing processes of certain spices by sellers before their use. Added to this is the fact that some sellers only source from suppliers recognized for the good hygienic quality of their products.

As a comparison of the two types of spice blends analyzed, the results indicated that the samples of spice blends sold in the markets analyzed contain more microorganisms than those packaged in industrial units. According to previous studies [18-20], spices in themselves have significant antibacterial activities which ensure them good conservation in optimal conditions without immediate degradation. It should be noted that the lower average level of total aerobic mesophilic flora in spices packaged in industrial units compared to those sold in the markets is explained by the implementation of hygiene and manufacturing rules in the factories. [21] reported that, when spice mixtures are packaged under suitable hygienic conditions, the total flora does not exceed 10^4 CFU/g. International regulations therefore agree on the fact that a load greater than 10^5 CFU/g means significant contamination [22]. The low number of germs or even their absence is the result of the good health of the handlers, the intrinsic properties and the very good cleanliness of the environment of work conditioning and control of hygienic conditions for handling samples (in industrial units).

Furthermore, the total absence of salmonella in all samples (100%) should be taken with caution, because depending on the nature of the isolation medium and the possible presence of competing germs such as coliforms, this research may prove false negative [11]. Overall, considering the microbial load of TAMF and Total Coliforms, the clove, green anise, black pepper, and ginger samples were less corrupted than the red pepper and spice blend samples. Indeed, Samia et al. [23], showed that the variability of total coliform contamination from one seller to another could depend on the density of market attendance, which influences environmental hygiene and therefore product contamination.

However, observations made during the survey at certain sites show a lack of hygiene in relation to the 5M. In general, the contamination of spices is much more linked to the environment (circulation of two-wheeled vehicles, grouping of waste in the middle of the market by sellers, poor state of maintenance of the environment around the displays, etc.) and staff hygiene to which is added the low qualification of the workforce [1].

5. Conclusion

Spices have always occupied an important place, particularly in Togolese cuisine and elsewhere. However, when hygienic conditions are not respected, the result is that meals based on these spices present a considerable risk to the health of the consumer, due to the presence of potentially pathogenic microorganisms. This study showed that spices could be contaminated by different microorganisms, in particular pathogenic germs such as Staphylococcus aureus, responsible for food poisoning. In addition, the presence of germs indicative of fecal contamination such as coliforms and anaerobic-sulphite-reducing organisms (in powdered red peppers) demonstrate the lack of hygiene during the harvest of these spices and, above all, during their processing and processing packaging.

The preparation of spices of good microbiological quality therefore requires compliance with numerous hygiene rules at several levels: raw materials and preparation environment (materials, conservation, premises, personnel). It is therefore essential to improve the production conditions of these spices. This requires better training for those involved, who often ignore basic hygiene rules. In addition, it is important to ensure good hygiene practices from harvesting the spice to its packaging and distribution, avoiding possible re-contamination by various vectors. The implementation of an effective cleaning-disinfection program in spice production and sales areas also remains necessary. All the results obtained in the present study suggest that the contaminations certainly occurred during the grinding and packaging of these spices in the processing processes, the imported seeds being generally free of some of these germs. This could be due to poor practices observed in production sites, linked to the lack of knowledge of hygiene rules by most processors.

Abbreviations

DAGL: Autonomous District of Greater Lomé
LAMICODA: Microbiology and Food Quality Control Laboratory
TAMF: Total Aerobic Mesophilic Flora
TC: Total Coliforms
ASR: Anaerobic-Sulfito Reductants
CFU/g: Colony Forming Unit Per Gram
GMP: Good Manufacturing Practices
CHP: Process Hygiene Criteria

Conflicts of Interest

The authors declare no conflicts of interest.

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