Hindawi
Journal of Food Quality
Volume 2022, Article ID 4236921, 12 pages
https://doi.org/10.1155/2022/4236921

Research Article

Microbiological Quality of Egusi Pudding, A Traditional Cake of Cucurbitaceae Sold in the City of Yaoundé, Cameroon

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Received 25 June 2021; Revised 24 November 2021; Accepted 17 December 2021; Published 4 January 2022

Academic Editor: Efstathios Giaouris

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Egusi pudding is one of the most popular traditional dishes of the Cameroonian population. Besides its nutritional values, it is also endowed with a sociocultural character. Nowadays, consumer demand for Egusi pudding has increased and the dish is sold as street food in several cities of Cameroon and mostly under uncontrolled hygienic conditions. The objective of this study was to assess the microbiological quality of Egusi pudding sold in the city of Yaoundé taking into consideration the protein sources and the sampling sites. Five types of Egusi pudding samples based on protein sources were randomly collected from 25 sellers distributed in 7 districts in the city of Yaoundé and their microbiological quality were assessed. The results showed that the total aerobic count of the different samples (2.97 ± 0.03 to 4.43 ± 0.05 Log CFU/g) was under the threshold value (5.47 Log CFU/g) recommended for food intended for human consumption. Loads of fecal coliforms (1.47 ± 0.00 to 5.47 ± 0.00 Log CFU/g) in 40% of samples, *Escherichia coli* (2.39 ± 0.12 to 5.43 ± 0.05 Log CFU/g) in 60% of samples, fecal streptococci (2.90 ± 0.15 to 4.74 ± 0.05 Log CFU/g) in 88% of samples, *Pseudomonas* spp. (3.39 ± 0.15 to 5.43 ± 0.06 Log CFU/g) in 100% of samples, and the presence of *Salmonella* spp. in 56% of samples revealed a poor level of hygiene of the vendors. Pathogens associated with unsafe food handling such as *Staphylococcus* spp. were found in 100% of samples at loads (3.84 ± 0.18 to 5.43 ± 0.05 Log cfu/g) higher than the norms of the European Commission. Potential toxigenic pathogens such as *Clostridium perfringens*, *Yersinia enterocolitica*, *Bacillus cereus*, and moulds were also found, respectively, in 100, 96, 96, and 100% of samples. Overall, the most contaminated samples were those made with sardine as protein source, 92.85% ($n = 23/25$), followed with beef (88.57%), mackerel (84.28%), cod (82.85%), and control (77.1%). The results of this study suggest that important measures should be taken by the Public Health Service in order to sensitize the producers and vendors of Egusi pudding on the respect of good hygiene, manufacturing practices, and the continuous monitor of the quality of traditional products sold in markets.

1. Introduction

Street foods can be defined as food and beverages prepared or cooked and sold by street vendors at similar public places for immediate consumption [1]. The practice of street food which is constantly evolving is rapidly spreading in the world's metropolises and especially in developing countries. The reasons are the high unemployment rate, low incomes, urbanization, migration to cities, demographic growth, lack of time for cooking, and poor culinary knowledge about the preparation process of some meals [2–4]. Street food allows 2.5 million of the population of various age groups to eat nutritious meals easily, conveniently, and cheaply outside their living home [1]. It also plays an important role in the cultural and social heritage of societies as several traditional foods of various flavors are marketed in the streets [5–8].

Despite these multiple advantages, street foods are a culture medium, susceptible to contamination by pathogens, and are vectors of microorganisms that might cause foodborne diseases [9]. In fact, they are often prepared, stored, marketed, and consumed in conditions which favor microbial growth and thus represent a potential risk of foodborne diseases. In 2005, more than 250 cases of foodborne illness were reported [10]. In 2015, the global burden of foodborne disease indicates that each year up to 600 million people or one in ten people becomes ill after consuming contaminated food, of whom 410,000 die, including 125,000 children under five (30% mortality) [10]. In addition to the health damage, these result in global financial losses of about $95.2 million per year in low- and middle-income countries, with more than $15 million spent on foodborne illness treatment [1].

In developing countries, particularly Cameroon, the street food sector is highly developed. In almost all cities, a wide variety of ready-to-eat food can be found on the streets, neighborhoods, schools, hospitals, businesses, and other public places. They include unprocessed, semiprocessed, and processed foods [11]. Among these street foods, Egusi pudding is very popular not only for its nutritional value but also for its sociocultural value. With regard to its sociocultural value, Egusi pudding remains the main dish during popular festivities such as births, weddings, and funerals but also in unfortunate events such as mourning [12]. Its preparation includes a mixture of the paste of decorticated seeds of Cucurbitaceae, water, and other ingredients such as salt and refined oil. Depending on the food habits which vary according to the culinary practice and the income, protein sources are incorporated in the paste. These protein sources are fresh eggs, meat, and fish. Then, the paste is wrapped in leaves and steam-cooked. The cooking duration depends on its volume and its composition [13, 14]. Nutritionally, Egusi pudding prepared with *Cucumeropsis manii* seeds is composed of protein (33.59 g/100 g DW), fat (40.80 g/100 g DW), fiber (8.18 g/100 g DW), carbohydrate (11.39 g/100 g DW), and minerals such as magnesium (348.0 mg/100g DW), zinc (6.42 mg/100 g DW), copper (0.8 mg/100 g DW), and iron (14.2 mg/100 g DW) [13–15]. Despite the great interest in Egusi pudding, it was remarked that most people decided to avoid its consumption particularly those sold in markets and street corners as well as those served during festivities. Moreover, it is always recommended to avoid its consumption during travelling. The main reasons are outbreaks of diarrhea, flatulence, and bloating incriminating its consumption [16]. In fact, the approximate level of hygiene applied by the manufacturers and vendors might favor its contamination with pathogens including *Bacillus cereus*, *Staphylococcus aureus*, and enterodiarrheic strains of *E. coli*. The microflora of Cucurbitaceae seeds is dominated by *Bacillus* spp., *Paenibacillus* spp., *Enterobacteriaceae*, *Pseudomonas* spp., and lactic acid bacteria [17]. According to Adekunle and Uma [18], the microbial profile of *C. mannii* seeds mainly used for Egusi pudding preparation is composed of bacteria and moulds such as *Aspergillus flavus, Fusarium solani, Rhizopus oryzae, Absidia blakelseeana,* and *Penicillium chrysogenum*. Previous studies conducted on lab-made Egusi pudding in the city of Ngaoundéré (Cameroon) revealed its contamination with coliforms, yeast, and moulds at levels below the threshold recommended values [16]. The authors concluded that the reduced shelf life of Egusi pudding could be associated with its residual microflora which can resist heat treatment [16]. Giving that almost all symptoms associated with the consumption of Egusi pudding as well as its reduced shelf life could be attributed to the presence of microorganisms, it therefore appears interesting to assess the microbiological quality of the food matrix as found on markets.

However, knowing that several sources of proteins are used for its preparation as well as environmental conditions including water source, we can hypothesize the variation of microbial quality according to the protein source and sampling site. The objective of this work was to assess the microbiological quality of Egusi pudding sold in the city of Yaoundé taking into consideration the sampling sites and the protein sources.

2. Materials and Methods

2.1. Study Area. The study was conducted in 7 districts of the city of Yaoundé from September to November 2020. Yaoundé is located in the Centre Region of Cameroon (3° 52′ 12″ N and 11° 31′ 12″E). It has an area of 180.00 km^2 with a population of 2,440,462 inhabitants and a density of 13,558.1 inhabitants/km^2. The city has an altitude of 750 m, with a savannah climate and a dry winter [19]. The city of Yaoundé was chosen because it is an economic metropolis with multivarieties of cultural potential. The sector of street food is also well developed and increased as time passes.

2.2. Sampling Design and Samples Collection. A complete random sampling design was adopted in this study. A preliminary survey carried out with the producers of Egusi pudding in the city of Yaoundé revealed that *C. mannii* seeds (N°12355/HNC) were the Cucurbitaceae species mainly used for its preparation and there were 5 types of Egusi pudding according to the protein source commonly used for its preparation [20]. The 5 types included Egusi pudding made with beef, sardines, mackerel, and smoked cod as protein sources and another type that is free of protein source. 25 women who prepared daily and sold Egusi pudding were randomly chosen. From each of the selected women, 5 samples per type of Egusi pudding of approximately 250 g each were randomly collected. The different collection sites include Briqueterie, Nkolbissong, Mendong, Etoug-Ebe, Simbock, Essos, Grand Messa, Cité Verte, Mvog-Ada, Melen, Ngoa Ekélé, Nsimeyong, Mvog-Beti, Obili, Madagascar, Quartier Centre, and Mvan. These sites were chosen because they represent quarters of the city of Yaoundé where Egusi pudding is mostly sold every day and during the whole year [20]. Samples collected were introduced in an icebox and transported to the laboratory where analyses were performed.

2.3. Sample Processing. The normalized method ISO 7218 [21] was used for samples processing. Briefly, the 5 samples of each type of Egusi pudding collected from each vendor

were pulled and ground in aseptic conditions. Then, 25 g of the mixture was taken and introduced into a sterile conical flask of 1 L containing 225 mL of sterile peptone water (Hameau, Germany). After homogenization with a vortex (Heidolph top mix, USA), the mixture was left at room temperature for 30 min and serially diluted (10^{-1} to 10^{-6}).

2.4. Microbiological Analysis. The total mesophilic aerobic flora (TMAF) was determined using the Pour plate method NF ISO 4833 [22]. Briefly, 1 mL of each dilution was introduced into a sterile Petri dish followed by the addition of 20 mL of sterile Plate Count Agar (PCA, LiofilChem, Italy). The plates were homogenized and incubated at 30°C for 48 h under aerobic conditions. Spread plate method NF V 08-054 [23] was used for the enumeration of total and fecal coliforms. 100 μL of the different dilutions were surface-inoculated onto MacConkey agar (LiofilChem, Italy) in Petri dish followed with incubation for 24 h at 44°C for fecal coliforms and at 37°C for total coliforms. Milky white colonies on MacConkey were considered coliforms.

E. coli count was assessed following the NF ISO 4832 [24] method. 100 μL of the different dilutions were inoculated in Eosin methylene blue agar (EMB, Himedia, India) followed with incubation for 24 h at 37°C. Metal green colonies on EMB agar were considered as *E. coli*. Gram staining, catalase, methyl red, indole, Voges- Proskauer, culture on triple sugars iron agar, dextrose, maltose, lactose, sucrose, and mannitol were performed as confirmative tests for *E. coli*.

The normalized NF ISO 6888-2 [25], NF ISO 7932 [26], NF ISO 21527-1 [27], NF ISO 13720 [28], EN ISO 10273 [29], and NF T 90-416 [30] methods were used for enumeration of *Staphylococcus* spp., *Bacillus cereus*, *Pseudomonas* spp., yeasts and moulds, *Yersinia enterocolitica*, and fecal streptococci, respectively. In the protocol, 100 μL of the different dilutions was inoculated on the surface of sterile Mannitol Salt Agar (MSA, LiofilChem, Italy), *Bacillus cereus* agar (LiofilChem, Italy), Cetrimide agar (Himedia, India), Sabouraud agar supplemented with chloramphenicol (LiofilChem, Italy), Cin Agar Base (CIB, Himedia, India), and Slanetz Bartley agar (LiofilChem, Italy), respectively. Petri dishes were incubated at 37°C for 24 h. For yeasts and moulds, they were incubated at 25°C for 5 days. White colonies on MSA were considered as *Staphylococcus* spp. and those of *Bacillus cereus* showed blue coloration on *Bacillus cereus* agar. Red colonies corresponded to *Yersinia enterocolitica* on Cin Agar Base medium while those colored brown or pink on Slanetz Bartley agar medium corresponded to fecal streptococci colonies. Further confirmation tests for *B. cereus* (Gram, lecithinase, nitrate, Voges-Proskauer, and tyrosine) and *Y. enterocolitica* (xylose, trehalose, and salicin acidification, aesculin hydrolysis, tween-esterase, and pyrazinamidase activity) were also performed.

Anaerobic sulfite-reducing bacteria were enumerated according to the NF ISO 7937 [31] method. 100 μL of the different dilutions was inoculated into a Petri dish containing 20 mL of sterile and solidified Tryptone Sulfite Neomycin agar (TSN, LiofilChem, Italy). The plates were left at room temperature for 30 min and 5 mL of soft sterile TSN agar was added to create anaerobic conditions. The Petri dishes were incubated at 37°C for 24 h in anaerobic conditions. Uncolored colonies with black centres on TSN were considered as anaerobic sulfite-reducing bacteria.

The ISO 6579-1 (2017) [32] method was used to assess the presence of *Salmonella* spp. in Egusi pudding samples. A homogenized solution made of the sample (25 g) and sterile peptone water (225 mL) was incubated for 16 h at 37°C. Then, 1 mL of the suspension was transferred into a sterile tube containing 10 mL of Rappaport Vassiliadis broth (Humeau, Germany) and incubated for 24 h at 37°C. Thereafter, one loopful from the broth was streaked onto Salmonella and Shigella agar (SS, Himedia, India) and incubated at 37°C for 24 h. Uncolored colonies with black centres on SS agar were considered as *Salmonella* spp. Some microscopic (Gram staining) and biochemical tests (catalase, sugar fermentation, methyl red, indole, and Voges-Proskauer) were performed on presumptive colonies for confirmation.

2.5. Plates Reading. Well individualized colony-forming units (CFU) appearing on the Petri dishes after the incubation period were counted. All experiments were performed in triplicate and the results were expressed as Log colony-forming units per gram (Log CFU/g) of sample.

2.6. Statistical Analysis. Microbial loads of the different samples were expressed as means ± standard deviations. Duncan's Multiple Range test was performed to compare microbial loads of samples using IBM SPSS 22 software (SPSS Inc., IBM Corporation, Chicago, USA). Significant difference was set at $p < 0.05$. Principal component analysis was performed to visualize the association between the composition of Egusi pudding, the sampling site, and the bacterial load found in the samples.

3. Results

3.1. Microbial Quality of Egusi Pudding. Microbiological analyses of Egusi pudding samples revealed the presence of several groups of microorganisms at loads varying from a sample to another as a function of the protein source and the sampling site (Table 1). The TMAF counts of the different samples varied from 2.97 ± 0.03 to 4.43 ± 0.05 Log CFU/g. The least contaminated samples were those collected from the sites Etoug-Ebe (2.97 ± 0.03 Log CFU/g) and Cité Verte (3.81 ± 0.04 Log CFU/g) while the most contaminated ones were from sites Biyem-Assi (4.43 ± 0.05 Log CFU/g) and Obili (4.45 ± 0.02 Log CFU/g). An overall observation of Table 2 reveals that the type of protein source used in the preparation of Egusi pudding showed a significant effect on the TMAF counts. Although the differences were not significant ($p > 0.05$), the highest contamination was recorded with samples made with beef as the protein source (4.19 ± 0.22 Log CFU/g) while the least one was recorded with the sample free of protein source (3.89 ± 0.52 Log CFU/g).

Table 1: Mean microbial loads (Log CFU/g) of the different samples of Egusi pudding sold in the city of Yaoundé, Cameroon.

Samples	Sampling sites	TMAF	TC	FC	Enterobacteria	*E. coli*	FS
	Nkolbissong	4.16 ± 0.02^{fhijk}	4.65 ± 0.06^{f}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.35 ± 0.06^{c}
	Etoug-Ebe	2.97 ± 0.03^{a}	3.69 ± 0.12^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.74 ± 0.05^{g}
MCO ($n = 25$)	Simbock	4.06 ± 0.19^{cdefh}	5.00 ± 0.00^{i}	0.00 ± 0.00^{a}	3.97 ± 0.09^{b}	0.00 ± 0.00^{a}	3.00 ± 0.00^{b}
	Etoug-Ebe	4.21 ± 0.05^{hijkl}	5.09 ± 0.21^{jk}	4.09 ± 0.12^{de}	4.95 ± 0.06^{f}	2.90 ± 0.15^{e}	3.84 ± 0.08^{f}
	Briqueterie	4.09 ± 0.12^{defhi}	4.87 ± 0.05^{hi}	3.47 ± 0.00^{c}	3.43 ± 0.05^{c}	0.00 ± 0.00^{a}	4.90 ± 0.15^{j}
	Etoug-Ebe	3.90 ± 0.07^{cd}	3.74 ± 2.84^{cd}	0.00 ± 0.00^{a}	5.35 ± 0.06^{hij}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Essos	4.35 ± 0.06^{klm}	3.97 ± 0.03^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.09 ± 0.12^{b}
MMO ($n = 25$)	Grand Messa	4.30 ± 0.00^{jklm}	4.65 ± 0.06^{f}	0.00 ± 0.00^{a}	5.24 ± 0.08^{fgh}	2.39 ± 0.12^{b}	3.77 ± 0.00^{f}
	Cité Verte	3.81 ± 0.04^{b}	4.69 ± 0.00^{f}	4.17 ± 0.21^{e}	5.00 ± 0.00^{f}	2.74 ± 0.05^{d}	2.95 ± 0.06^{b}
	Mvog-Ada	4.35 ± 0.06^{klm}	5.39 ± 0.00^{lm}	5.30 ± 0.15^{gh}	5.47 ± 0.00^{j}	4.60 ± 0.15^{h}	4.74 ± 0.05^{j}
	Nsimeyong	3.94 ± 0.08^{bcde}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.43 ± 0.05^{ij}	4.54 ± 0.08^{h}	4.07 ± 0.10^{g}
	Cité Verte	4.39 ± 0.12^{lm}	5.00 ± 0.00^{ij}	0.00 ± 0.00^{a}	4.74 ± 0.05^{de}	3.74 ± 0.05^{g}	3.57 ± 0.04^{e}
MMA ($n = 25$)	Ngoa Ekélé	4.09 ± 0.12^{defhi}	4.77 ± 0.10^{h}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.32 ± 0.05^{h}
	Melen	3.97 ± 0.03^{bcdef}	0.00 ± 0.00^{a}	4.00 ± 0.00^{d}	4.69 ± 0.12^{de}	3.54 ± 0.08^{f}	3.39 ± 0.12^{cdf}
	Mvog - Betsi	3.87 ± 0.04^{c}	3.079 ± 0.00^{b}	0.00 ± 0.00^{a}	5.49 ± 0.06^{j}	0.00 ± 0.00^{a}	3.74 ± 0.55^{de}
	Melen	3.88 ± 0.01^{bc}	3.74 ± 0.05^{cd}	0.00 ± 0.00^{a}	5.25 ± 0.06^{gh}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Grand Messa	3.94 ± 0.08^{bcde}	3.87 ± 0.12^{cde}	0.00 ± 0.00^{a}	4.77 ± 0.15^{e}	4.87 ± 0.04^{i}	4.38 ± 0.11^{hi}
MSA ($n = 25$)	Obili	4.45 ± 0.02^{im}	5.43 ± 0.05^{mn}	0.00 ± 0.00^{a}	5.35 ± 0.06^{hij}	2.54 ± 0.08^{c}	4.38 ± 0.11^{hi}
	Madagascar	4.24 ± 0.08^{ijkl}	5.24 ± 0.08^{kl}	0.00 ± 0.00^{a}	5.43 ± 0.05^{ij}	3.43 ± 0.05^{f}	4.54 ± 0.08^{i}
	Biyem-Assi	4.43 ± 0.05^{im}	5.27 ± 0.04	5.35 ± 0.06^{hi}	5.11 ± 0.09^{f}	5.43 ± 0.05^{j}	3.75 ± 0.08^{f}
	Quartier Centre	4.13 ± 0.02^{efhij}	3.74 ± 0.05^{klm}	0.00 ± 0.00^{a}	3.39 ± 0.12^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Mvog-Betsi	4.24 ± 0.08^{ijkl}	3.90 ± 0.15^{dec}	1.47 ± 0.00^{b}	5.26 ± 0.04^{hi}	5.39 ± 0.12^{j}	4.29 ± 0.01^{h}
MVI ($n = 25$)	Mendong	4.35 ± 0.06^{gklm}	5.20 ± 0.15^{jk}	5.47 ± 0.00^{i}	5.11 ± 0.04^{f}	5.30 ± 0.00^{j}	3.44 ± 0.18^{cd}
	Mvan	3.84 ± 0.08^{b}	5.00 ± 0.00^{ij}	4.94 ± 0.08^{f}	4.60 ± 0.15^{d}	3.74 ± 0.05^{g}	2.90 ± 0.15^{b}
	Biyem-Assi	4.39 ± 0.12^{hilm}	5.47 ± 0.00^{n}	5.21 ± 0.04^{g}	5.47 ± 0.00^{j}	5.43 ± 0.05^{j}	3.54 ± 0.08^{cd}

Samples	Sampling sites	*Staphylococcus* spp.	*Bacillus cereus*	*Yersinia enterocolitica*	*Pseudomonas* spp.
	Nkolbissong	4.82 ± 0.02^{def}	4.81 ± 0.04^{hij}	3.84 ± 0.08^{a}	4.84 ± 0.00^{a}
	Etoug-Ebe	4.92 ± 0.10^{fgh}	5.35 ± 0.06^{n}	0.00 ± 0.00^{a}	4.94 ± 0.04^{fgh}
MCO ($n = 25$)	Simbock	4.95 ± 0.06^{fgh}	4.90 ± 0.07^{ijk}	3.90 ± 0.03^{cd}	4.65 ± 0.08^{gh}
	Etoug-Ebe	5.31 ± 0.01^{hij}	3.24 ± 0.08^{b}	5.00 ± 0.00^{fgh}	4.20 ± 0.06^{ef}
	Briqueterie	4.62 ± 3.54^{c}	5.35 ± 0.06^{n}	3.84 ± 0.08^{cd}	3.39 ± 0.15^{d}
	Etoug-Ebe	5.24 ± 4.54^{i}	4.69 ± 0.12^{gh}	5.19 ± 0.01^{i}	4.57 ± 0.12^{b}
	Essos	4.97 ± 0.03^{gh}	0.00 ± 0.00^{a}	4.81 ± 0.01^{e}	4.95 ± 0.04^{e}
MMO ($n = 25$)	Grand Messa	4.74 ± 0.05^{cd}	5.09 ± 0.12^{lm}	4.74 ± 0.17^{e}	4.74 ± 0.06^{gh}
	Cité Verte	4.90 ± 0.00^{efg}	5.00 ± 0.00^{klm}	4.65 ± 0.06^{e}	4.84 ± 0.05^{ef}
	Mvog-Ada	5.24 ± 0.08^{ij}	4.41 ± 0.09^{ef}	5.00 ± 0.00^{fg}	3.79 ± 0.08^{fgh}
	Nsimeyong	4.87 ± 0.04^{defg}	4.54 ± 0.084^{fg}	4.04 ± 0.05^{d}	5.01 ± 0.02^{c}
	Cité Verte	4.77 ± 0.00^{de}	4.77 ± 0.10^{hij}	3.64 ± 0.07^{bc}	5.05 ± 0.16^{hij}
MMA ($n = 25$)	Ngoa Ekélé	4.00 ± 0.00^{b}	5.33 ± 0.10^{n}	4.75 ± 0.02^{ef}	4.77 ± 0.06^{ij}
	Melen	3.84 ± 0.18^{a}	3.62 ± 0.10^{c}	4.79 ± 0.07^{efg}	3.43 ± 0.10^{fg}
	Mvog - Betsi	4.87 ± 0.04^{defg}	4.35 ± 0.06^{e}	3.60 ± 0.15^{b}	4.74 ± 0.06^{b}
	Melen	4.97 ± 0.03^{gh}	3.62 ± 0.10^{c}	3.81 ± 0.18^{b}	3.95 ± 0.05^{ef}
	Grand Messa	4.95 ± 0.00^{fgh}	4.95 ± 0.06^{jkl}	3.84 ± 0.08^{cd}	5.20 ± 0.06^{c}
MSA ($n = 25$)	Obili	4.75 ± 0.02^{d}	4.77 ± 0.10^{hij}	4.86 ± 0.04^{efgh}	5.32 ± 0.15^{lm}
	Madagascar	5.43 ± 0.05^{j}	4.88 ± 0.05^{ijk}	4.81 ± 0.04^{efg}	5.24 ± 0.02^{mno}
	Biyem-Assi	5.06 ± 0.02^{h}	5.14 ± 0.04^{m}	5.02 ± 3.08^{ghi}	3.79 ± 0.08^{lmn}
	Quartier Centre	5.19 ± 0.01^{i}	5.39 ± 0.00^{n}	3.62 ± 0.03^{bc}	5.13 ± 0.02^{c}
	Mvog-Betsi	5.30 ± 0.00^{ij}	5.43 ± 0.05^{n}	4.65 ± 0.064^{e}	5.43 ± 0.06^{jk}
MVI ($n = 25$)	Mendong	4.84 ± 0.88^{defg}	4.39 ± 0.12^{ef}	5.09 ± 0.02^{h}	4.77 ± 0.05^{o}
	Mvan	5.30 ± 0.00^{ij}	3.95 ± 0.06^{d}	3.77 ± 0.10^{bc}	5.39 ± 0.10^{fg}
	Biyem-Assi	5.43 ± 0.05^{j}	14.74 ± 0.05^{hi}	4.97 ± 0.03^{fghi}	5.39 ± 0.00^{no}

Samples	Sampling sites	ASR bacteria	*Clostridium* spp.	Yeast and moulds	*Salmonella* spp.
	Nkolbissong	4.81 ± 0.14^{abcd}	3.17 ± 0.00^{a}	$5.24 \pm 0.08^{g\ hi}$	−
	Etoug-Ebe	4.87 ± 0.23^{bcde}	4.95 ± 0.06^{f}	4.74 ± 0.05^{b}	+
MCO ($n = 25$)	Simbock	5.17 ± 0.00^{ghi}	5.24 ± 0.08^{ijk}	3.87 ± 0.04^{a}	+
	Etoug-Ebe	5.13 ± 0.05^{fghi}	5.31 ± 0.01^{j}	4.74 ± 0.05^{b}	+
	Briqueterie	4.95 ± 0.17^{def}	5.43 ± 0.05^{k}	5.02 ± 0.02^{def}	−

Table 1: Continued.

	Etoug-Ebe	5.16 ± 0.33^{ghi}	4.74 ± 0.05^{cd}	5.23 ± 0.07^{ghi}	−
	Essos	4.74 ± 0.05^{ab}	4.74 ± 0.05^{cd}	4.87 ± 0.04^{bcd}	−
MMO ($n = 25$)	Grand Messa	4.65 ± 0.06^{ab}	5.09 ± 0.12^{fhi}	5.43 ± 0.06^{j}	−
	Cité Verte	4.69 ± 0.00^{ab}	4.74 ± 0.05^{cd}	4.74 ± 0.05^{b}	+
	Mvog-Ada	5.09 ± 0.02^{fg}	4.87 ± 0.12^{de}	3.74 ± 0.05^{a}	+
	Nsimeyong	4.95 ± 0.06^{def}	4.69 ± 0.01^{cd}	4.74 ± 0.05^{b}	−
	Cité Verte	4.92 ± 0.03^{def}	4.39 ± 0.00^{b}	4.90 ± 0.07^{cd}	+
MMA ($n = 25$)	Ngoa Ekélé	4.84 ± 0.18^{abcd}	4.65 ± 0.06^{c}	4.92 ± 0.10^{d}	+
	Melen	5.30 ± 0.00^{ij}	5.15 ± 0.03^{hij}	5.35 ± 0.06^{ij}	−
	Mvog - Betsi	5.30 ± 0.15^{ij}	5.35 ± 0.06^{jk}	5.35 ± 0.06^{ij}	+
	Melen	5.37 ± 0.03^{j}	4.35 ± 0.06^{b}	5.09 ± 0.12^{efg}	−
	Grand Messa	4.97 ± 0.03^{def}	5.30 ± 0.15^{jk}	5.36 ± 0.08^{ij}	−
MSA ($n = 25$)	Obili	5.02 ± 0.08^{efg}	4.87 ± 0.12^{k}	5.26 ± 0.11^{hi}	+
	Madagascar	5.19 ± 0.01^{ghij}	5.43 ± 0.05^{j}	5.35 ± 0.06^{ij}	+
	Biyem-Assi	5.27 ± 0.03^{hij}	4.81 ± 0.04^{de}	5.30 ± 0.00^{gij}	+
	Quartier Centre	5.11 ± 0.14^{fgh}	5.09 ± 0.12^{fhi}	5.00 ± 0.00^{cdef}	−
	Mvog-Betsi	4.95 ± 0.06^{def}	5.24 ± 0.08^{ijk}	4.84 ± 0.08^{bc}	+
MVI ($n = 25$)	Mendong	5.37 ± 0.03^{j}	5.40 ± 0.10^{k}	4.95 ± 0.06^{cde}	+
	Mvan	4.90 ± 0.07^{cde}	5.24 ± 0.08^{ijk}	5.13 ± 0.06^{fgh}	−
	Biyem-Assi	5.37 ± 0.03^{j}	5.00 ± 0.12^{efh}	4.87 ± 0.04^{bcd}	+

$N = 125$ samples; TMAF = total mesophilic aerobic flora; TC = total coliforms; FC = fecal coliforms; FS=fecal streptococci; MCO = Egusi pudding free of protein source; MMO = Egusi pudding with cod as protein source; MMA = Egusi pudding with mackerel as protein source; MSA = Egusi pudding with sardine as protein source; MVI = Egusi pudding with beef as protein source, values with different letters within a column are significantly different ($p < 0.05$) according to Duncan' multiple range test. $N = 125$ samples; MCO = Egusi pudding free of protein source; MMO = Egusi pudding with cod as protein source; MMA = Egusi pudding with mackerel as protein source; MSA = Egusi pudding with sardine as protein source; MVI = Egusi pudding with beef as protein source, values with different letters within a column are significantly different ($p < 0.05$) according to Duncan' multiple range test. $N = 125$ samples; ASR = anaerobic sulfite-reducing; − = absence; + = presence; MCO = Egusi pudding free of protein source; MMO = Egusi pudding with cod as protein source; MMA = Egusi pudding with mackerel as protein source; MSA = Egusi pudding with sardine as protein source; MVI = Egusi pudding with beef as protein source, values with different letters within a column are significantly different ($p < 0.05$) according to Duncan' multiple range test. $N = 125$ samples; TMAF = total mesophilic aerobic flora; ASR = anaerobic sulfite-reducing; − = absence; + = presence; MCO = Egusi pudding free of protein source; MMO = Egusi pudding with cod as protein source; MMA = Egusi pudding with mackerel as protein source; MSA = Egusi pudding with sardine as protein source; MVI = Egusi pudding with beef as protein source, values with different letters within a column are significantly different ($p < 0.05$) according to Duncan' multiple range test.

Table 2: Distribution of the means microbial loads (Log CFU/g) of the samples of Egusi pudding according to the protein sources.

Microflora	Source of proteins				
	Beef	Mackerel	Cod	Sardine	Control
TMAF	4.19 ± 0.22^{a}	4.05 ± 0.20^{a}	4.14 ± 0.26^{a}	4.18 ± 0.26^{a}	3.89 ± 0.52^{a}
Total coliforms	4.66 ± 0.78^{a}	2.56 ± 2.46^{b}	4.48 ± 0.65^{a}	4.71 ± 0.83^{a}	4.66 ± 0.56^{a}
Fecal coliforms	3.41 ± 2.5^{a}	0.8 ± 1.78^{a}	1.89 ± 2.62^{a}	1.07 ± 2.39^{b}	1.51 ± 2.08^{a}
Enterobacteria	4.76 ± 0.83^{a}	4.07 ± 2.30^{a}	4.21 ± 2.36^{a}	5.18 ± 0.25^{a}	2.47 ± 2.31^{a}
E. coli	3.97 ± 2.33^{a}	2.36 ± 2.19^{a}	1.94 ± 1.96^{a}	3.25 ± 2.14^{a}	0.58 ± 1.29^{a}
Fecal streptococci	2.83 ± 1.65^{a}	3.81 ± 0.37^{a}	2.91 ± 1.77^{a}	3.41 ± 1.93^{a}	3.96 ± 0.83^{a}
Staphylococcus spp.	5.21 ± 0.22^{a}	4.47 ± 0.50^{a}	5.01 ± 0.21^{a}	5.03 ± 0.24^{a}	4.92 ± 0.25^{a}
Bacillus cereus	6.78 ± 4.49^{a}	4.52 ± 0.62^{a}	3.83 ± 2.16^{a}	4.67 ± 0.60^{a}	4.73 ± 0.86^{a}
Yersinia enterocolitica	4.42 ± 0.68^{a}	4.16 ± 0.57^{a}	4.87 ± 0.21^{a}	4.46 ± 0.59^{a}	3.31 ± 1.91^{a}
Anaerobic sulfite-reducing bacteria	5.14 ± 0.22^{a}	5.06 ± 0.22^{a}	4.86 ± 0.23^{a}	5.14 ± 0.16^{a}	4.98 ± 0.15^{a}
Clostridium perfringens	5.19 ± 0.15^{a}	4.84 ± 0.39^{a}	4.83 ± 0.15^{a}	4.95 ± 0.42^{a}	4.82 ± 0.93^{a}
Pseudomonas spp.	5.22 ± 0.27^{a}	4.6 ± 0.66^{a}	4.57 ± 0.46^{a}	4.7 ± 0.76^{a}	4.40 ± 0.63^{a}
Yeasts and moulds	4.95 ± 0.11^{a}	5.05 ± 0.28^{a}	4.80 ± 0.65^{a}	5.27 ± 0.10^{a}	4.72 ± 0.52^{a}

TMAF = total mesophilic aerobic flora, values with same letters within a column are not significantly different ($p < 0.05$) according to Duncan' multiple range test.

With respect to the total coliforms, 92% of samples analyzed were contaminated and the loads recorded varied from 0 to 5.47 ± 0.00 Log CFU/g. Total coliforms were absent in the samples of Egusi pudding made with mackerel as the protein source and sold in the sites Melen and Nsimeyong while they were found at highest loads in those from the sites Mvan with cod as the protein source (5.39 ± 0.00 Log CFU/g), Obili with sardine as protein source (5.43 ± 0.05 Log CFU/g), and Biyem-Assi with beef as protein source (5.47 ± 0.00Log CFU/g). Based on the protein source, the most contaminated samples (4.71 ± 0.83 Log CFU/g) were made with sardine while those made with mackerel were the least contaminated (2.56 ± 2.46 Log CFU/g) ones (Table 2). Only 20% of samples made with mackerel as protein source were contaminated compared to 100% for the other protein sources.

Opposite to total coliforms, fecal coliforms were absent in several sampling sites independent of the source of protein used in the preparation of Egusi pudding samples. The loads recorded in this study for the 40% of samples contaminated ranged from 0 to 5.47 Log CFU/g. The most important proportion of contaminated samples was observed with samples prepared with beef as protein source (80%). The highest mean load of 3.41 ± 2.51 Log CFU/g was recorded with these samples (Table 2). As for fecal coliforms, *E. coli* were also absent in several samples of Egusi pudding independently of the protein source. In contaminated samples (60%), loads ranging from 2.54 ± 0.08 to 5.43 ± 0.05 Log CFU/g were observed (Table 1). Although the proportions of contamination were 80% with samples prepared with sardine and beef as protein sources, the highest *E. coli* load (3.97 ± 0.33 Log CFU/g) was recorded with beef as the protein source (Table 2).

Except for samples of Egusi pudding from Ngoa Ekélé made with mackerel as the protein source, from Essos with cod as the protein source, and from Nkolbissong and Etoug-Ebe free of protein source, enterobacteria were found in the rest of samples (84%) at higher loads ranging from 3.43 ± 0.05 to 5.49 ± 0.06 Log CFU/g (Table 1). As shown in Table 2, samples made with sardine as the protein source were the most contaminated ones as they scored the highest mean load (5.18 ± 0.25 Log CFU/g).

Fecal streptococci were not found in samples of Egusi pudding from Etoug-Ebe made with cod and sardine as protein sources, as well as those from Quartier Centre made with beef as the protein source. However, they were found in the rest of the samples (88%) regardless of the collection site and the protein source at loads ranging from 2.90 ± 0.15 to 4.74 ± 0.05 Log CFU/g (Table 1). The samples free of protein source and those with mackerel as protein source scored the highest means loads of fecal streptococci of 3.96 ± 0.83 and 3.81 ± 0.37 Log CFU/g, respectively (Table 2).

Independent of the sampling sites and the protein sources, *Yersinia enterocolitica* was found in 96% of samples at loads ranging from 3.60 ± 0.15 to 5.19 ± 0.01 Log CFU/g, except the samples from Etoug-Ebe from which no contamination was noticed (Table 1). Taking into consideration the protein source (Table 2), samples made with cod as protein source were the most contaminated (4.87 ± 0.21 Log CFU/g) while those free of protein scored the least mean load (3.31 ± 1.91 Log CFU/g). 80% ($n = 20/25$) of samples free of protein source were contaminated while 100% ($n = 20/25$) of those made with the different protein sources were contaminated.

As for fecal coliforms and *E. coli*, *Salmonella* spp. were also absent in several samples of Egusi pudding independently of the protein source and the sampling site. Among the 56% of contaminated samples, 60% of those made with beef, sardine, and cod as the protein sources were contaminated while only 40% of ones made with mackerel and free of protein source were contaminated.

Staphylococcus spp. were present in 100% of Egusi pudding samples at loads ranging from 3.84 ± 0.18 to 5.43 ± 0.05 Log CFU/g (Table 1). The samples with the highest loads were collected from Madagascar and Biyem-Assi (5.43 ± 0.05 Log CFU/g) while those with the lowest loads were collected from Ngoa Ekélé (4.00 ± 0.00 Log CFU/g) and Melen (3.84 ± 0.18 Log CFU/g). The regrouping

according to the protein source revealed that samples made with beef appear as the most contaminated ones with a mean load of 5.21 ± 0.22 Log CFU/g (Table 2).

B. cereus was found in 96% of Egusi pudding samples at loads ranging from 3.24 ± 0.08 to 5.43 ± 0.05 Log CFU/g. Only samples from Essos with cod as the protein source were not contaminated. Samples made with beef as the protein source were the most contaminated ones as they scored the highest mean load of 6.78 ± 4.49 Log CFU/g (Table 2).

All samples of Egusi pudding independently of the sampling site and the protein source were contaminated with *Pseudomonas* spp. at loads varying from 3.39 ± 0.15 to 5.43 ± 0.06 Log CFU/g (Table 1). Samples free of protein source were the least contaminated (4.40 ± 0.63 Log CFU/g) while those made with beef scored the highest contamination with a mean load of 5.22 ± 0.27 Log CFU/g (Table 2).

The presence of anaerobic sulfite-reducing bacteria in Egusi pudding samples was assessed and the results obtained show that all samples were contaminated (Table 1). Loads ranging from 4.65 ± 0.06 to 5.37 ± 0.03 Log CFU/g were noticed. The least contaminated sample was from Grand Messa with cod as the protein source and the highest ones were from Melen with sardine as the protein source (5.37 ± 0.03 Log CFU/g) and from Biyem-Assi with beef as the protein source (5.37 ± 0.03 Log CFU/g). Considering the protein sources, samples made with beef and sardine scored the highest mean loads of 5.14 ± 0.16 and 5.14 ± 0.22 Log CFU/g, respectively.

Bacteria belonging to the group of ASR such *Clostridium* spp. were found in all samples with loads that vary significantly with the sampling site and the source. The lowest load was with samples collected at Nkolbissong (3.17 ± 0.00 Log CFU/g) while the highest load (5.40 ± 0.10 Log CFU/g) was recorded with samples from Briqueterie and Madagascar. Beef was the protein source for which the Egusi pudding samples were most contaminated (5.19 ± 0.15 Log CFU/g).

Yeasts and moulds were found in 100% of Egusi pudding samples at loads ranging from 3.74 ± 0.05 to 5.43 ± 0.06 Log CFU/g. The samples with the highest loads were collected at Mvog-Ada (5.43 ± 0.06 Log CFU/g) and Grand Messa (5.36 ± 0.08 Log CFU/g). Those with the lowest loads were collected at Mvog-Ada (3.74 ± 0.05 Log CFU/g) and Simbock (3.87 ± 0.04 Log CFU/g). The addition of protein sources in Egusi pudding increases their loads in yeasts and moulds as the least contaminated samples were those free of protein source (4.72 ± 0.52 Log CFU/g). However, among the protein sources, sardine seems to be the protein source for which the Egusi pudding was the most contaminated (5.27 ± 0.10 Log CFU/g).

Overall, the percentages of contaminated samples according to the protein sources were as follows: 92.85% ($n = 23/25$) of samples with sardine, 88.57% ($n = 22/25$) of samples with beef, 84.28% ($n = 21/25$) of samples with mackerel, 82.85% ($n = 20/25$) of samples with cod, and finally 77.14% ($n = 19/25$) of samples free of protein source.

3.2. Principal Component Analysis. In order to visualize the association between the microbial quality of the Egusi pudding, the protein source, and the sampling site, a principal component analysis was carried out. Figure 1(a) shows the distribution of the different variables on the axis systems F1 & F3. With regard to microorganisms, two groups were observed. The first is made with *E. coli, Staphylococcus* spp., total and fecal coliforms, fecal streptococci, *B. cereus, Y. enterocolitica,* enterobacteria, and anaerobic sulfite-reducing bacteria. The second group is formed with *Pseudomonas* spp., yeast, and moulds.

Taking into consideration the overall distribution on the axis systems F1 & F3 (Figure 1(b)), three groups can easily be distinguished. The first group includes pathogens, Egusi pudding made with sardine as the protein source, and the sampling sites Mvan, Obili, Mendong, Mvog-Betsi, Mvog-Ada, Madagascar and Biyem-Assi. The second group with the least pathogens is composed of the sampling sites Melen, Simbock, Ngoa Ekélé, and Briqueterie and the Egusi pudding made with mackerels and beef and one free of protein source. The third group includes spoilage microorganisms positively associated with Egusi pudding made with cod and the sampling sites Nsimeyong, Simbock, Cité Verte, Nkolbissong, Essos, Quartier Centre, and Grand Messa.

4. Discussion

In this study, the microbiological quality of Egusi pudding samples sold in several quarters in the city of Yaoundé was assessed. TMAF was found in all Egusi pudding samples with varying loads independently of the sampling site and the protein source. With respect to the sampling sites, the most contaminated samples were those collected from Etoug-Ebe (2.97 ± 0.03 Log CFU/g) and Cité Verte (3.81 ± 0.04 Log CFU/g). This observation could be ascribed to the preparation and selling conditions of these dishes which vary according to the culinary practices of each vendor as previously highlighted by several authors in the literature [33, 34].

Taking into consideration the protein source used in the preparation of Egusi pudding, the highest TMAF loads were recorded with samples containing sardine as the protein source. This result could be explained by the fact that the tissue of the muscle of sardine is soft compared to the rest of protein sources such as beef, mackerel, and cod [35]. Hence, microorganisms can easily penetrate into the cells and have access to nutrients that will favor their proliferation. Egusi pudding free of protein source was less contaminated compared to the others. This information strengthened the hypothesis that the protein sources offer a favorable environment for the proliferation of microorganisms and thus might represent a source of contamination [20]. Indeed, protein sources such as beef contained their own microflora of pathogenic and spoilage microorganisms [36]. In addition to their microflora, the paste of Cucurbitaceae seeds will increase the final load of the dishes. Several microorganisms including bacteria and fungi were reported as inherent microflora of Cucurbitaceae seeds [17, 18, 37]. The mean TMAF count obtained in this study with Egusi pudding free of protein sources (3.89 ± 0.52 Log CFU/g) is comparable to the 4.16 Log CFU/g of TMAF reported by Manejo [16] in their works conducted in the city of Ngaoundéré (Cameroon) on Egusi pudding free of protein source.

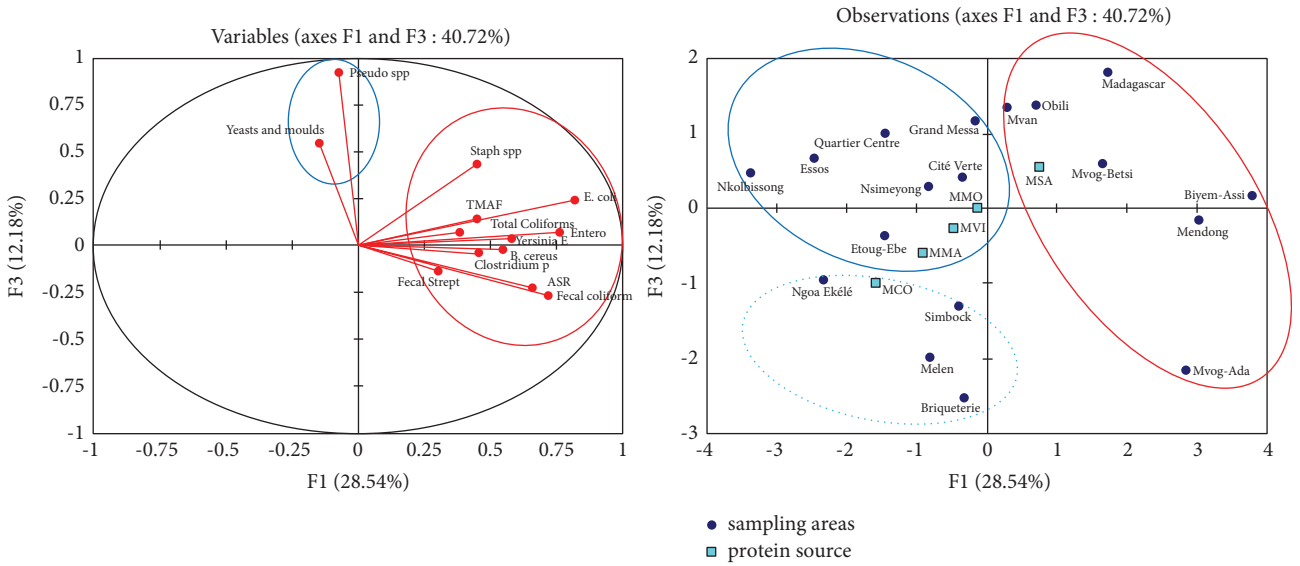

Figure 1: Distribution of the studied parameters on the axis systems F1 & F3. ● = sampling areas; ■ = protein source; TMAF = total mesophilic aerobic flora, Entero = enterobacteria, Fecal strept = Fecal streptococci; Staph spp. = *Staphylococcus* spp.; *Yersinia E* = *Yersinia enterocolitica,* ASR = anaerobic sulfite-reducing bacteria; MCO = Egusi pudding free of protein source; MMO = Egusi pudding with cod as protein source; MMA = Egusi pudding with mackerel as protein source; MSA = Egusi pudding with sardine as protein source; MVI = Egusi pudding with beef as protein source.

Globally, the TMAF loads of the different samples independently of the sampling sites and protein sources were below the threshold value recommended by the European Commission regulation regarding street food intended for human consumption without further treatment (5.47 Log CFU/g) [38]. These findings suggest that Egusi pudding might be suitable for human consumption. A similar conclusion was stated in the literature as the authors found a TMAF of Egusi pudding under the threshold recommended value [16]. However, the TMAF gives only an indication of the general contamination of food [36]. It does not provide information on the group of microorganisms present in these foods and which might constitute a risk for consumers' health. In this light, several groups of microorganisms were screened.

Total and fecal coliforms, fecal streptococci, and enterobacteria were found in the great majority of samples independently of the sampling sites and the source of proteins. Although *Enterobacteriaceae* are part of the inherent microflora of Cucurbitaceae seeds [17, 18, 37], the presence of these pathogens in Egusi pudding could be justified by the nonrespect of good hygiene practices during the preparation of these dishes. In fact, the presence of these pathogens in food indicates fecal pollution of the latter and poor food handling [9]. The absence of these pathogens in some sites might arise from the fact that the preparation process of Egusi pudding includes a long heat treatment (100°C for 2 to 3 h) which might destroy some pathogens. This hypothesis suggests that packaging, storage, and transportation after processing affect the microbial quality. The presence of pathogens in processed food associated with a postcontamination was also highlighted in [7, 39–41].

The samples of Egusi pudding were all contaminated with *Staphylococcus* spp. at levels exceeding the standards established by the European Commission [38] which is 2 Log CFU/g. The presence of these pathogens commonly found on human skins, noses, and saliva also demonstrated the nonrespect of good hygiene practices during the preparation of Egusi pudding and its selling. Bezirtzoglou et al. [42] noticed that improper food handling during street foods selling led to contamination with pathogens such as *Staphylococcus* spp. The observation made in this study is worrisome as it is well known that *S. aureus* might produce its enterotoxin in food and the consumption of that food could lead to foodborne intoxication [43].

According to the microbiological criteria of the European Commission [37], *Salmonella* spp. must be absent in food intended for human consumption. Regardless of the protein source and the sampling sites, *Salmonella* spp. was present in 56% of the samples. This could arise from the packaging used by vendors. For Egusi pudding preparation, the mixture of Cucurbitaceae seeds paste with other ingredients is packaged with leaves before being cooked. During the cooking process, the structure of leaves is disintegrated and improper handling of the cooked food might result in its contamination. Moreover, the air-exposure during its selling could also result in its contamination with microorganisms which might be present in dust. The presence of pathogens in relation to the selling conditions of street foods was reported by Koffi-Nevry et al. [44]. The consumption of Egusi pudding containing *Salmonella* spp. as observed in this could lead to foodborne diseases. In fact, the consumption of food contaminated with *Salmonella* spp. was associated with symptoms such as febrile diarrhea, vomiting, and abdominal pain and in severe cases typhoid fever and septicemia [45]. Given that, there are harmful strains of *E. coli* such as EHEC, ETEC, and *E. coli* O157 which are implicated in several foodborne diseases [46, 47].

It therefore appears necessary to perform a molecular identification (16S rRNA gene analysis) of the *E. coli* strains isolated in this study. Moreover, the presence of streptococci which produced beta-hemolytic toxins (group A and B) suggests potential health risks associated with the consumption of Egusi pudding [48].

It was observed in this study that loads of *Clostridium* spp. of all samples were higher than the norms (2 Log CFU/g) independently of the sampling sites and the source of proteins. Similar observations were also noticed with anaerobic sulfite-reducing bacteria. Except for the samples from Essos with cod as protein source where *B. cereus* was not found, the rest of the samples were contaminated (96%) with loads higher than the threshold value established by the microbiology criteria of the European Commission regulation [37]. The air exposition of the raw materials used for the preparation of Egusi pudding as observed on markets associated with the spores' forming ability of this group of pathogens could explain their origin in the samples analyzed in this study. *Bacillus* spp. were highlighted as the dominant microflora of Cucurbitaceae seeds [17]. Hence, their heat resistance ability could also justify the high contamination origin of Egusi pudding samples. In these conditions, the reheating of leftovers of Egusi pudding might lead to the proliferation of these spores forming bacteria. The presence of thermoresistant microorganisms in street foods was also reported by Maïwore et al. [49] and Mayoré et al. [50]. However, the results of this study are worrisome taking into consideration the fact that bacteria belonging to *Clostridium* genera such as *Clostridium perfringens* and *Clostridium botulinum* are incriminated in outbreaks of foodborne intoxication with such symptoms: necrotizing pneumonia and enterocolitis, nausea, vomiting, diplopia, dysphagia, and, in severe cases, motor paralysis [51, 52]. At the concentrations found in Egusi pudding samples, *B. cereus* can produce the heat-stable emetic toxin which might lead to diarrhea, vomiting, and abdominal cramps following consumption of Egusi pudding as reported in the literature by Six et al. [53]. Abdominal pain, diarrhea, distending, and flatulence were also noticed as symptoms associated with the consumption of Egusi pudding [16]. Moreover, *B. cereus* also produces enterotoxins such as hemolysin BL, hemolysin NHE, and cytotoxin K which are the leading cause of diarrheal syndrome associated with the consumption of food contaminated with *B. cereus* [54, 55].

Yersinia enterocolitica was found in 96% of samples with loads above the limit recommended by the European Commission which is 2 Log CFU/g [37]. The poor level of hygiene of vendors might be responsible for this contamination as highlighted by Toe et al. [32]. Knowing that *Y. enterocolitica* is incriminated in several outbreaks of diseases such as febrile diarrhea in young children, erythema nodosum in adults, and arthritis or bone foci [52], the results of this study highlight that its continuous monitoring in Egusi pudding should be performed.

Spoilage microorganisms such as *Pseudomonas* spp., yeasts, and moulds were found in all samples at levels higher than the threshold values of the European Commission [37]. They could originate either from dust contamination or from the seeds of *C. manii* used to prepare Egusi pudding. In fact, bacteria such as *Pseudomonas* spp. were found in the seeds of Cucurbitaceae [17]. Besides, several moulds like *A. flavus*, *F. solani*, *R. oryzae*, *Penicillium* spp., *Mucor* spp., *A. blakelseeana*, and *P. chrysogenum* constituted the natural microflora of Cucurbitaceae seeds [18, 36]. The spoilage capacity of these microorganisms is due to their ability to produce enzymes such as lipase and protease [56]. Hence, their presence in Egusi pudding suggests a reduced shelf life of the products and could confirm the reports of Manejo [16] who noticed that the maximum shelf lives of Egusi pudding stored at room and refrigeration temperatures were 24 and 48 h, respectively. Besides, the ability of moulds to resist heat treatment through spores' formation and to produce mycotoxins which are not only thermostable but also the leading causes of several diseases including cancers as reported by the International Agency for Research on Cancer [57] suggests potential health risks associated with the consumption of Egusi pudding.

The pathogens were mainly found in the sites Mvan, Obili, Mendong, Mvog-Betsi, Mvog-Ada, Madagascar and Biyem-Assi, Melen, Simbock, Ngoa Ekélé, and Briqueterie. A regrouping according to protein sources revealed that samples from these sites were either made with sardine, mackerel, and beef or were free of protein sources. This observation questioned the respect of good hygiene practices during the preparation of these dishes and also the handling, selling, and storage conditions of leftovers of Egusi pudding. The lack of application of hygiene rules by the vendors, especially during the preparation, storage, and selling, was highlighted by Mayoré et al. [50] as the potential causes of the presence of pathogens in street vended food. Besides, Nguendo [11] reported the quality of water used in the cooking practices as well as for hands and utensils washing as one of the other important leading causes of the presence of pathogens in street food.

Spoilage microorganisms were found in samples collected in the sites Nsimeyong, Simbock, Cité Verte, Nkolbissong, Essos, Quartier Centre, and Grand Messa. Principal component analysis revealed that samples from these sites were made with cod as the protein source. This observation suggests that Egusi pudding from these sites might have a reduced shelf life and cod might represent a source of spoilage microorganisms.

5. Conclusion

This study demonstrated that a great proportion of Egusi pudding samples marketed in the city of Yaoundé were of poor microbiological quality. The contamination levels were significantly affected by the protein sources and the sampling sites. The presence of pathogens such as coliforms, *E. coli, Staphylococci, Salmonella* spp., enterobacteria, and *Yersinia enterocolitica* at levels higher than the values recommended by the norms revealed the weak level of hygiene of the vendors and suggests a potential health risk for consumers. Potential toxigenic microorganisms such as moulds, *Clostridium* spp., and *B. cereus* were present in almost all samples thus questioning their suitability for human consumption knowing the harmful effect of these toxins on human health.

Hence, important measures should be taken by the government in order to sensitize the producers and vendors of Egusi pudding and to regularly control the quality of these highly appreciated traditional foods.

Data Availability

The data used in this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to thank the head of the Laboratory of Valorization and Quality Control (LAVACOQ) of the Institute of Fishery Sciences, the structure in which the research work was carried out. They also gratefully acknowledge sellers of Egusi pudding of the different collective eating places of Yaoundé for their kind cooperation during the sampling process. The authors acknowledge the Local Resource Exploitation (LOREXP) of the University of Ngaoundéré, where the paper was presented at the 1st International Conference "Value Chains and Integral Transformation of Local Resources," April 20 to 23, 2021, Ngaoundéré, Cameroon.

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