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Microbiological quality of *Egusi pudding*, a traditional cake of Cucurbitaceae sold in the city of Yaoundé, Cameroon

Qualité microbiologique du pudding Egusi, un gâteau traditionnel de Cucurbitacées vendu dans la ville de Yaoundé, Cameroun

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ABSTRACT:

Egusi pudding is one of the most popular traditional dishes of the Cameroonian population. Besides its nutritional values, it also endowed with a socio-cultural character. Nowadays, consumers demand of *Egusi pudding* has increased and the dish is sold as street food in several Cameroonian city and most time under inadequate 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 sampling site and the source of proteins. Five samples per type of *Egusi pudding* were randomly collected from 25 participants distributed in 7 districts in the city of Yaoundé their microbiological quality was 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. The presence of fecal coliforms (1.47 ± 0.00 to 5.47 ± 0.00 Log CFU/g) in 20 % of samples, pathogenic strains of *Escherichia coli* (2.39 ± 0.12 to 5.43 ± 0.05 Log CFU/g) in 40 % of samples, and *Salmonella* spp. in 16 % of samples showed the poor level of hygiene of the vendors. Pathogens associated to unsafe food handling such as *Staphylococcus* spp. were found in all samples at loads (3.84 ± 0.18 to 5.43 ± 0.05 Log CFU/g) higher than the norms of the European Commission. Globally, the most contaminated samples were those made with sardine as protein source. Potential toxinogenic pathogens such as *Clostridium perfringens*, *Yersinia enterolitica*, *Bacillus cereus* and moulds were also found in all samples at different loads. The results of this study suggest that important measures should be taken by the Public Health Service in order to sensitize e the producers and vendors of *Egusi pudding* on the respect of good hygiene and manufacturing practices and to continuously monitor the quality of traditional products sold in markets.

Keywords: Street food, Cucurbitaceae, *Egusi pudding*, Microbiological quality, Pathogens, Yaoundé.

RÉSUMÉ :

Le mets de pistache est l'un des plats traditionnels les plus populaires de la population camerounaise. Outre ses valeurs nutritionnelles, il est également doté d'un caractère socioculturel. De nos jours, la demande des consommateurs pour le mets de pistache a augmenté et le plat est vendu comme nourriture de rue dans plusieurs villes camerounaises et la plupart du temps dans des conditions d'hygiène inadéquates. L'objectif de cette étude était d'évaluer la qualité microbiologique du mets de pistache vendu dans la ville de Yaoundé en prenant en considération le site d'échantillonnage et la source des protéines. Cinq échantillons par type de mets de pistache ont été prélevés au hasard chez 25 participants répartis dans 7 quartiers de la ville de Yaoundé ; leur qualité microbiologique a été évaluée. Les résultats ont montré que la flore mésophile aérobie totale des différents échantillons (2,97 ± 0,03 à 4,43 ± 0,05 Log CFU/g) était inférieure à la valeur seuil (5,47 Log CFU/g) recommandée pour les aliments destinés à la consommation humaine. La présence de coliformes fécaux (1,47 ± 0,00 à 5,47 ± 0,00 Log CFU/g) dans 20 % des échantillons, de souches pathogènes d'*Escherichia coli* (2,39 ± 0,12 à5,43 ± 0,05 Log CFU/g) dans 40 % des échantillons, et de *Salmonella* spp. dans 16 % des échantillons a montré le faible niveau d'hygiène des vendeurs. Les agents pathogènes associés à la manipulation dangereuse des aliments, tels que *Staphylococcus* spp., ont été trouvés dans tous les échantillons à des charges (3,84 ± 0,18 à 5,43 ± 0,05 Log CFU/g) supérieures aux normes de la Commission européenne. Globalement, les échantillons les plus contaminés étaient ceux fabriqués avec de la sardine comme source de protéines. Les résultats de cette étude suggèrent que des mesures importantes devraient être prises par le Service de Santé Publique afin de sensibiliser les producteurs et les vendeurs de mets de pistache sur le respect des bonnes pratiques d'hygiène et de fabrication et de surveiller continuellement la qualité des produits traditionnels vendus sur les marchés.

Mots clés : Aliments de rue, Cucurbitacées, Mets de pistache, Qualité microbiologique, Germes pathogènes, Yaoundé.

1. INTRODUCTION

Street foods can be defined as food and beverages ready to be consumed, prepared or sold by street vendors especially on the streets and in similar public places for immediate consumption without processing or preparation (FAO, 2007). The practice of street which constantly evolves, is very widespread 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, the lack of time for cooking and the poor culinary knowledge about the preparation process of some meals (Nonga et al., 2015; Yannick et al., 2013; Kumar et al., 2006). Street food allow a large part of several million of the population of various age groups to eat nutritious meal easily, conveniently and cheaply outside their living home. It is also playing an important role in the cultural and social heritage of societies as several traditional foods of various flavors are marketed in streets (Vedant et al., 2017; Akusu et al., 2016; Rahman et al., 2014; Barro et al., 2006). Despite these multiple advantages, street foods are susceptible to contamination by pathogens and are vectors of microorganisms that might cause public health problems (Madueke et al., 2014). In fact, they are often prepared, stored, marketed and consumed in conditions which might favor microbial contamination and thus represent a potential risk of foodborne diseases.

In developing countries and particularly in Cameroon, the sector 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, semi-processed and processed foods (Nguendo, 2018). Among these street foods, *Egusi pudding* is very popular not only for its nutritional value but also for its socio-cultural value. Nutritionally, it is composed of protein (33.59 %), fat (40.80 %), fiber (8.18 %), carbohydrate (11.39 %) and minerals such as magnesium (348.0 mg/100 DW), zinc (6.42 mg/100 DW), copper (0.8 mg/100 DW) and iron (14.2 mg/100 DW) (Djiogue et al., 2017; Ponka et al.; 2006; Ponka et al., 2005). With regards to its socio-cultural value, *Egusi pudding* remains a main dish during popular festivities such as births, weddings, funerals but also unfortunate events such as mourning (Kouebou et al., 2013). Its preparation included a mixture of the paste of decorticated seeds of Curcubitaceae, water and other ingredients such as salt and refined oil. Depending on the food habit which vary according to the culinary practice and the incomes, 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 (Djiogue et al., 2017; Ponka et al., 2005). Despite the great interest for *Egusi pudding*, it was remarked that, most of person declared 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 travel. The main reasons are outbreaks of diarrhea, flatulence and bloating incriminating its consumption. Giving that almost all these symptoms are associated with the presence of microorganisms, it therefore appears interesting to assess the microbiological quality of that food matrix. However, knowing that several sources of proteins are used for its preparation, we can hypothesize the variation of microbial quality according to the protein source.

The objective of this work is to assess the microbiological quality of *Egusi pudding* sold in the city of Yaoundé taking into consideration the sampling site and the source of proteins.

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 km² with a population of 2,440,462 inhabitants and a density of 13,558.1 inhabitants per /km². The city has an altitude of 750 m, with a savannah climate and a dry winter (NIS, 2018). The city of Yaoundé was chosen because it is an economic metropolis with a multivariate cultural potential. The sector of street is also well developed and increased as time pass. Cucurbitaceae seeds used for the preparation of *Egusi pudding* currently found among street food, is available and mainly cultivated in the Centre Region of Cameroon.

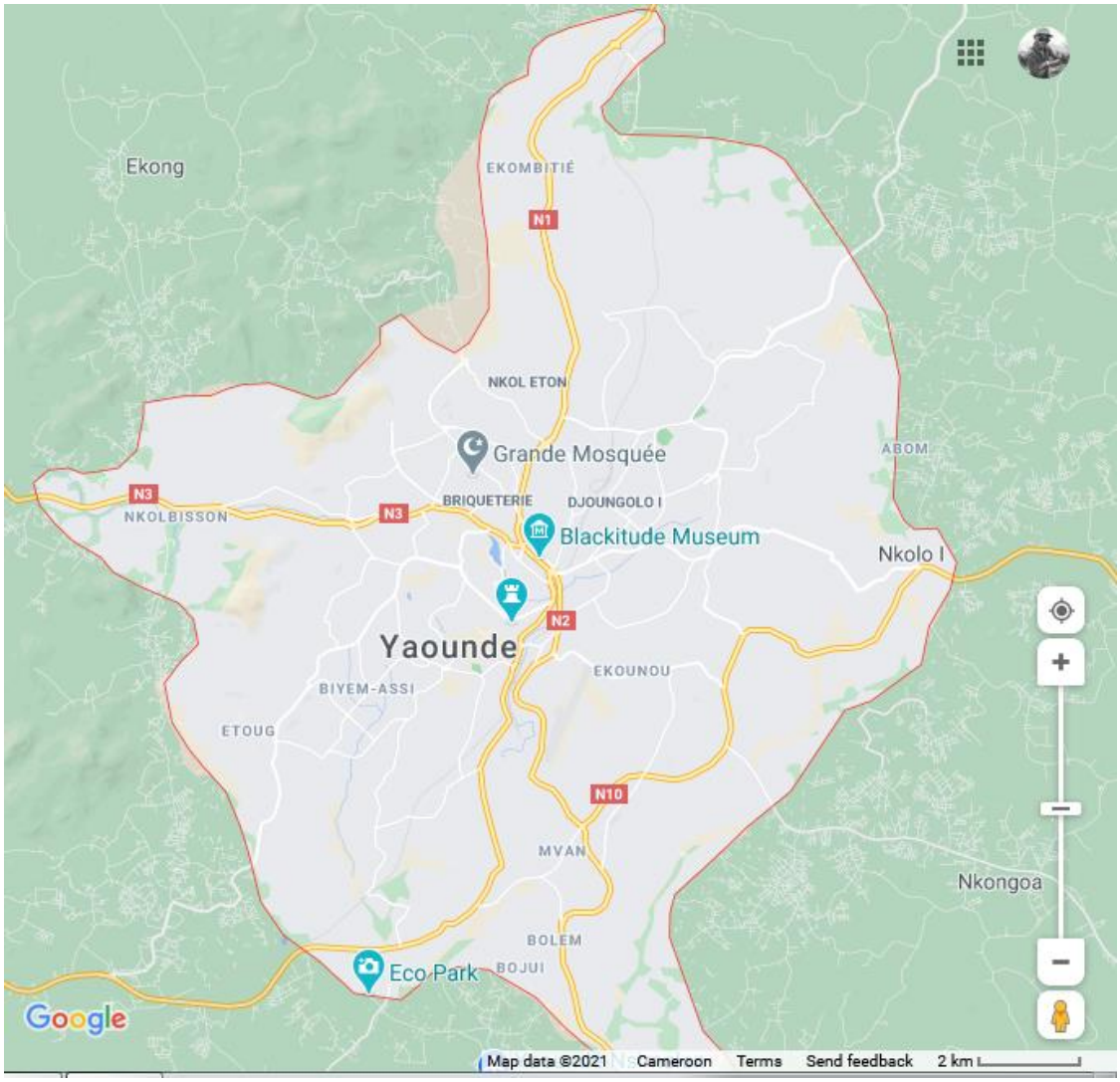

Figure 1: Map showing the location of the sampling sites in the city of Yaoundé (source: Google maps; 2021).

2.2. Sampling design and samples collection

A completely randomized sampling design was adopted in this study. Preliminary survey carried out with the producers of *Egusi pudding* in the city of Yaoundé revealed that there are 5 types according to the protein source commonly used for its preparation. The 5 types included: *Egusi pudding* made with beef, sardines, mackerel and smoked cod as protein sources and another type which is free of protein source. 25 women who's daily prepared and sold *Egusi pudding* were randomly chosen. From each of the selected women, 5 samples per type of *Egusi pudding* were collected. The 5 samples of approximately 250 g each, were randomly chosen among the whole production. They were labeled, introduced in an icebox and transported to the laboratory where analyses were performed.

2.3. Sample processing

The normalized method (ISO 7218, 2007) was used for samples processing. Briefly, the 5 samples of each type *Egusi pudding* collected from each vendor were pooled and ground in aseptic conditions. Then, 25 g of the mixture were taken and introduced into a sterile Erlenmeyer 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. Inoculation and culture conditions

The total mesophilic aerobic count was determined using the pour plate method (ISO 4833-1, 2013). Briefly, 1 mL of each dilution was introduced into a sterile Petri dish followed with the addition of 20 mL of sterile Plate Count Agar (PCA, LiofilChem, Italy). The plates were incubated at 37 °C for 48 h under aerobic conditions. Small and white colonies on PCA were considered as TMAF. Spread plate method (NFT-V-08-054, 2009) was used for the enumeration of total and fecal coliforms. 100 µL of the different dilutions was surface inoculated onto Mac Conkey agar (EMB, 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 Mac Conkey were considered as coliforms. *E. coli* count was assessed following the method (ISO 4832, 2006) 100 µL of the different dilutions was inoculated in Eosin methylene bleu agar (EMB, Himedia, India) followed with incubation for 24 h at 44 °C**.** Pink or red colonies with a possible area of bile precipitation around the colonies on EMB agar were considered as *E.coli*.

The normalized methods (ISO 10273, 2017; ISO 13720, 2010; ISO 21527-1, 2008; ISO 7932, 2004; ISO 6888-2, 1999; NFT-90-416, 1985) were used for enumeration of *Staphylococcus* spp., *Bacillus cereus*, *Pseudomonas* spp., yeasts and moulds, *Yersinia enterolitica* and fecal streptococci, respectively. In the protocol, 100 µL of the different dilution was inoculated at 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 Barthley agar (LiofilChem, Italy). Petri dishes were incubated at 37 °C for 24 h but for yeasts and moulds, they were incubated at 25 °C for 3 to 5 days. Colonies of yellowish colored aureole on Manitol

were considered as *Staphylococcus* spp. and those of *Bacillus cereus* showed blue coloration on *Bacillus cereus* agar. *Pseudomonas* spp. colonies stained blue-green on Cetrinide agar medium, while those colored red corresponded to *Yersinia enterolotica* on Cin agar base medium, those colored brown or pink on SLanetz Barthley agar medium corresponded to fecal streptococci colonies.

Anaerobic sulfite-reducing germs were enumerated according to the method (ISO 7937, 2004). 100 µL of the different dilution was inoculated into a Petri dish containing 15 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 .Uncolored colonies with black centers on TSN were considered as anaerobic sulfite-reducing bacteria.

The method (ISO 6579-1, 2017) was used to assess the presence of *Salmonella* spp. in *Egusi pudding* samples. A homogenized solution made of 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 of the broth was streaked onto Salmonella and Shigella agar (SS, Himedia, India) and incubated at 37 °C for 24 h. Uncolored colonies with black centers on SS agar were considered as *Salmonella* spp.

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 duplicate 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 association between the composition of *Egusi pudding*, the sampling site and the loads and group of pathogens found therein the samples.

3. RESULTS AND DISCUSSION

3.1. Microbial quality of Egusi pudding

Owing to its richness in nutrients, *Egusi pudding* contributes to the nutritional status of consumers but it also constitutes a favorable environment for the growth and proliferation of microorganisms including pathogens responsible for foodborne illness (Toe et al., 2017; Moutafs et al., 2007) and spoilage microorganisms. Hence, in this study, the microbiological quality of *Egusi pudding* samples sold in several quarters in the city of Yaoundé was assessed.

Microbiological analyses of *Egusi* pudding samples revealed the presence of several group of microorganisms at loads ranging significantly ($p<0.05$) from a sample to another independently of the protein source and the sampling site (Table 1). The total aerobic counts of the different samples varied significantly ($p<0.05$) 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 Citéverte (3.81 ± 0.04 Log CFU/g) while the most contaminated one were from sites Biyem-Assi (4.43 ± 0.05 Log CFU/g)and Obili (4.45 ± 0.02 Log CFU/g).In order to assess the effect of the protein source on the level of contamination of the *Egusi pudding* samples, mean loads of samples made with the different protein sources independently of the sampling sites were calculated and the results are presented in Table 2. Globally, the type of protein source used in the preparation of *Egusi pudding* showed a significant effect on the total aerobic counts. The highest contamination was recorded with samples made with beef as protein source (4.19 ± 0.22 Log CFU/g) while the least one was recorded with sample free of protein source (3.89 ± 0.52 Log CFU/g).

This observation could be ascribed to the preparing and selling conditions of these dishes which vary according to the culinary practices of each vendors (Toe et al., 2017; Baba-Moussa et al., 2006).

Egusi pudding free of protein source was less contaminated compared to the others. This information strengthened the hypothesis that the proteins sources which offer the favorable environment to the proliferation of microorganisms, might represents a source of contamination. Indeed, protein source such as beef, contained their own microflora of pathogenic and spoilage microorganisms (Mouafo et al., 2020). Addition of their microflora to those of the paste of Cucurbitaceae seeds will increase the final load of the dishes. Taking into consideration the protein source used in the preparation of *Egusi pudding,* the highest TMAF loads were recorded with samples containing sardine as proteins 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 source such as beef, mackerel and cod. Hence, microorganisms can easily penetrate into the cells and have access to nutrients which will favor their proliferation. A high susceptibility of sardine to get contaminated and spoiled by microorganisms was highlighted (Kaktcham et al., 2019). Globally, the TMAF loads of the different samples independently of the sampling sites and protein sources, were below the threshold value recommended the European Commission regulation regarding food intended for human consumption (5.47Log CFU/g). These findings suggest that *Egusi pudding* might be suitable for human consumption. However, the TMAF give only an indication of the general contamination of food (Mouafo et al., 2020; Adesetan et al., 2017). It does not provide information of 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 searched in the different *Egusi pudding* samples.

The microbiological criteria for ready-to-eat meals include microorganisms such as *Salmonella* spp., *E. coli*, *Staphylococcus* spp., *Clostridium perfringens, Bacillus cereus* for which the level of contamination

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

	Sampling sites	TMAF	Total coliforms	Fecal coliform	Enterobacteria	*E. coli*	Fecal streptococci	*Staphylococcus* spp.	*Bacillus cereus*	*Yersinia enterolitica*	*Pseudomonas* spp.	ASR bacteria	*Clostridium perfringens*	Yeast and moulds	*Salmonella* spp.
MCO (n=25)	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}	4.82 ± 0.02^{def}	4.81 ± 0.04^{hij}	3.84 ± 0.08^{a}	4.84 ± 0.00^{a}	4.81 ± 0.14^{abcd}	3.17 ± 0.00^{a}	5.24 ± 0.08$^{g\ hi}$	-
	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}	4.92 ± 0.10^{fgh}	5.35 ± 0.06^{n}	0.00 ± 0.00^{a}	4.94 ± 0.04^{fgh}	4.87 ± 0.23^{bcde}	4.95 ± 0.06^{f}	4.74 ± 0.05^{b}	+
	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}	4.95 ± 0.06^{fgh}	4.90 ± 0.07^{ijk}	3.90 ± 0.03^{cd}	4.65 ± 0.08^{gh}	5.17 ± 0.00^{ghi}	5.24 ± 0.08^{ijk}	3.87 ± 0.04^{a}	+
	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}	5.31 ± 0.01^{hij}	3.24 ± 0.08^{b}	5.00 ± 0.00^{fgh}	4.20 ± 0.06^{ef}	5.13 ± 0.05^{fghi}	5.31 ± 0.01^{j}	4.74 ± 0.05^{b}	+
	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}	4.62 ± 3.54^{c}	5.35 ± 0.06^{n}	3.84 ± 0.08 ^{cd}	3.39 ± 0.15^{d}	4.95 ± 0.17^{def}	5.43 ± 0.05^{k}	5.02 ± 0.02^{def}	-
MMO (n=25)	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}	5.24 ± 4.54^{i}	4.69 ± 0.12^{gh}	5.19 ± 0.01 ^{i}	4.57 ± 0.12^{b}	5.16 ± 0.33^{ghi}	4.74 ± 0.05^{cd}	5.23 ± 0.07^{ghi}	-
	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}	4.97 ± 0.03^{gh}	0.00 ± 0.00^{a}	4.81 ± 0.01^{e}	4.95 ± 0.04^{e}	4.74 ± 0.05^{ab}	4.74 ± 0.05^{cd}	4.87 ± 0.04^{bcd}	-
	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}	4.74 ± 0.05^{cd}	5.09 ± 0.12^{lm}	4.74 ± 0.17 ^{e}	4.74 ± 0.06^{gh}	4.65 ± 0.06^{ab}	5.09 ± 0.12^{fhi}	5.43 ± 0.06^{j}	-
	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}	4.90 ± 0.00^{efg}	5.00 ± 0.00^{klm}	4.65 ± 0.06 ^{e}	4.84 ± 0.05^{ef}	4.69 ± 0.00^{ab}	4.74 ± 0.05^{cd}	4.74 ± 0.05^{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}	5.24 ± 0.08^{ij}	4.41 ± 0.09^{ef}	5.00 ± 0.00^{fg}	3.79 ± 0.08^{fgh}	5.09 ± 0.02^{fg}	4.87 ± 0.12 ^{de}	3.74 ± 0.05^{a}	+
MMA (n=25)	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}	4.87 ± 0.04^{defg}	4.54 ± 0.084^{fg}	4.04 ± 0.05 ^{d}	5.01 ± 0.02^{c}	4.95 ± 0.06^{def}	4.69 ± 0.01^{cd}	4.74 ± 0.05^{b}	-
	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}	4.77 ± 0.00^{de}	4.77 ± 0.10^{hij}	3.64 ± 0.07^{bc}	5.05 ± 0.16^{hij}	4.92 ± 0.03^{def}	4.39 ± 0.00^{b}	4.90 ± 0.07^{cd}	+
	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}	4.00 ± 0.00^{b}	5.33 ± 0.10^{n}	4.75 ± 0.02^{ef}	4.77 ± 0.06^{ij}	4.84 ± 0.18^{abcd}	4.65 ± 0.06^{c}	4.92 ± 0.10^{d}	+
	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}	3.84 ± 0.18^{a}	3.62 ± 0.10^{c}	4.79 ± 0.07^{efg}	3.43 ± 0.10^{fg}	5.30 ± 0.00^{ij}	5.15 ± 0.03^{hij}	5.35 ± 0.06^{ij}	-
	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}	4.87 ± 0.04^{defg}	4.35 ± 0.06^{e}	3.60 ± 0.15 ^{b}	4.74 ± 0.06^{b}	5.30 ± 0.15^{ij}	5.35 ± 0.06^{jk}	5.35 ± 0.06^{ij}	+
MSA (n=25)	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}	4.97 ± 0.03^{gh}	3.62 ± 0.10^{c}	3.81 ± 0.18 ^{b}	3.95 ± 0.05^{ef}	5.37 ± 0.03^{j}	4.35 ± 0.06^{b}	5.09 ± 0.12^{efg}	-
	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}	4.95 ± 0.00^{fgh}	4.95 ± 0.06^{jkl}	3.84 ± 0.08 ^{cd}	5.20 ± 0.06^{c}	4.97 ± 0.03^{def}	5.30 ± 0.15^{jk}	5.36 ± 0.08^{ij}	-
	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}	4.75 ± 0.02^{d}	4.77 ± 0.10^{hij}	4.86 ± 0.04^{efgh}	5.32 ± 0.15^{lm}	5.02 ± 0.08^{efg}	4.87 ± 0.12^{k}	5.26 ± 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}	5.43 ± 0.05^{j}	4.88 ± 0.05^{ijk}	4.81 ± 0.04^{efg}	5.24 ± 0.02^{mno}	5.19 ± 0.01^{ghij}	5.43 ± 0.05^{j}	5.35 ± 0.06^{ij}	+
	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}	5.06 ± 0.02^{h}	5.14 ± 0.04^{m}	5.02 ± 3.08^{ghi}	3.79 ± 0.08^{lmn}	5.27 ± 0.03^{hij}	4.81 ± 0.04^{de}	5.30 ± 0.00^{gij}	+
MVI (n=25)	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}	5.19 ± 0.01^{i}	5.39 ± 0.00^{n}	3.62 ± 0.03^{bc}	5.13 ± 0.02^{c}	5.11 ± 0.14^{fgh}	5.09 ± 0.12^{fhi}	5.00 ± 0.00^{cdef}	-
	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}	5.30 ± 0.00^{ij}	5.43 ± 0.05^{n}	4.65 ± 0.064^{e}	5.43 ± 0.06^{jk}	4.95 ± 0.06^{def}	5.24 ± 0.08^{ijk}	4.84 ± 0.08^{bc}	+
	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}	4.84 ± 0.88^{defg}	4.39 ± 0.12^{ef}	5.09 ± 0.02^{h}	4.77 ± 0.05^{o}	5.37 ± 0.03^{j}	5.40 ± 0.10^{k}	4.95 ± 0.06^{cde}	+
	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}	5.30 ± 0.00^{ij}	3.95 ± 0.06^{d}	3.77 ± 0.10^{bc}	5.39 ± 0.10^{fg}	4.90 ± 0.07^{cde}	5.24 ± 0.08^{ijk}	5.13 ± 0.06^{fgh}	-
	Biyem - Asssi	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}	5.43 ± 0.05^{j}	14.74 ± 0.05^{hi}	4.97 ± 0.03^{fghi}	5.39 ± .00no	5.37 ± 0.03^{j}	5.00 ± 0.12^{efh}	4.87 ± 0.04^{bcd}	+

N=125 samples; TMAF=Total mesophilic aerobic flora;ASR=Anaerobic sulfito-reducing; −=absence; +=presence; MCO=*Egusi pudding* free of protein source; MMO=*Egusi pudding* with cod as protein source; MMA=*Egusi puddin*g 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.

generally varies according to the physicochemical composition of the food as well as the preparation, storage and selling conditions (Anoman et al., 2018; Adesetan et al., 2017).

With respect to the total coliforms, the loads recorded varied significantly ($p<0.05$) from 0 to 5.47 ± 0.00 Log CFU/g. Total coliforms were absent in the samples of *Egusi pudding* made with mackerel as 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 protein source (5.39 ± 0.00 Log CFU/g), Obili with sardine as protein source (5.43 ± 0.05 Log CFU/g), Biyem-Assi with beef as protein source (5.47 ± 0.00 Log 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) one (Table 2).

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 sulfito-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 aeruginosa	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.

Opposite to total coliforms, fecal coliforms were absent in several sampling site independently of the source of protein used in the preparation of *Egusi pudding* samples. The loads recorded in this study ranged from 0.00 to 5.47 ± 0.00. 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, 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) were recorded beef as protein source (Table 2).

Excepted samples of *Egusi pudding* from Ngoa Ekélé made with mackerel as protein source, from Essos with cod as protein source and the one from Nkolbissong and Etoug-Ebe free of protein source, enterobacteria were found in the rest of samples at higher loads ranging from3.43 ± 0.05 to5.49 ± 0.06Log CFU/g (Table 1). As shown in Table 2, samples made with sardine as protein source were the most contaminated one as they scored the highest mean load (5.18 ± 0.25 Log CFU/g).

Fecal streptococci were not detected 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 protein source. However, they were found in the rest of samples 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).

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. The presence of these pathogens in *Egusi pudding* could be justified by the non-respect of good hygiene practices during the preparation of these dishes. In fact, the presence of these pathogens in food indicates a fecal pollution of these latter's and a poor food handling (Madueke et al., 2014). 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 inhibit some pathogens. This hypothesis suggests a possible post-contamination of *Egusi pudding* samples in the different sites. The presence of pathogens in processed food associated to a post-contamination was highlighted in the literature (Akusu et al., 2016; El-Marnissi et al., 2012; Clarence et al., 2009; Moutafs et al., 2007).

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.60 % of samples made with beef, sardine and cod as protein sources were contaminatedwhile only 40 % of those made with mackerel and free of protein source were contaminated.

Staphylococcus spp. were present in all samples of *Egusi pudding* 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 sample made with beef appear as the most contaminated one with a mean load of 5.21 ± 0.22 Log CFU/g (Table 2).

The samples of *Egusi pudding* were all contaminated with *Staphylococcus* spp. at levels exceeding the standards established by the European Commission regulation (European Commission Regulation

2073/2005, 2005) which is 2 Log CFU/g. According to European Commission regulation (European Commission Regulation 2073/2005, 2005), *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 84 % 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 cook food might result in its contamination with pathogens such as fecal coliforms, *Staphylococcus* spp., fecal streptococci, enterobacteria and *Salmonella* spp. 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 conditions where street foods are sold was reported by Koffi-Nevry et al. (2011). Bezirtzoglou et al. (2000) also noticed that the improper food handling during its street foods selling led to contamination with pathogens such as *Staphylococcus* spp.

B. cereus were found in *Egusi pudding* samples at loads ranging from 3.24 ± 0.08 to 5.43 ± 0.05 Log CFU/g. Samples made with beef as protein source were the most contaminated one as they scored the highest mean load of 6.78 ± 4.49 Log CFU/g (Table 2). Excepted the samples from Essos with cod as protein source where *B. cereus* were not found, the rest of samples were contaminated with loads far high than the threshold value established by the microbiology criteria of the European Commission regulation (European Commission Regulation 2073/2005, 2005). The presence of *B. cereus* might result from reheating of *Egusi pudding*. Indeed, the rest of unsold products were stored at home and reheated the next day for selling purpose. Hence, in these conditions *B. cereus* due to its spore-forming ability might easily grow until reach high proportions as observed in this study.

The presence of anaerobic sulfite-reducing bacteria in *Egusi pudding* samples was assessed and the results obtained show that all samples were contaminated. 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 protein source and the highest one was from Melen with sardine as protein source (5.37 ± 0.03 Log CFU/g) and from Biyem-Assi with beef as 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 perfringens* were found in all samples with loads which vary significantly with the sampling site and the protein sources. The lowest load was collected at Nkolbissong (3.17 ± 0.00 Log CFU/g) while the highest load (5.40 ± 0.10 Log CFU/g) were 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).

It was observed in this study that the loads of *Clostridium perfringens* 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. These group of pathogens are known for their

spore-forming ability. Hence, their presence in *Egusi pudding* might be justified by the hypothesis on the positive effect of reheating on the proliferation of these microorganisms. The presence of thermoresistant microorganisms in street foods was reported (Maïwore et al., 2018; Mayoré et al., 2018). The authors used their spore-forming ability to justify their presence in street foods.

Independently of the sampling sites and the protein sources, *Yersinia enterolitica* was found in samples at loads ranging from 3.60 ± 0.15 to 5.19 ± 0.01 Log CFU/g, excepted the sample 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). *Yersinia enterolitica* were found in 98.67 % of samples with loads above the limits recommended by the European Commission regulation which is 2 Log CFU/g **(**. The poor level of hygiene of vendors might be the responsible of this contamination as highlighted by (Toe et al., 2017) in their studies.

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). Sample 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).

Yeasts and moulds were found in *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). Addition of protein source 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, amongst the protein sources,sardine seems to be the protein source for which the *Egusi pudding* was the most contaminated in yeasts and moulds (5.27 ± 0.10 Log CFU/g).

Spoilage microorganisms such as *Pseudomonas,* yeasts and molds, were found in the almost samples at levels higher than the threshold values of the European Commission (European Commission Regulation 2073/2005, 2005). Their spoilage ability is due to their ability to produce enzymes such as lipase and protease (Nychas et al., 2008). Hence, their presence in *Egusi pudding* suggests a reduced shelf life of the products

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 2A shows the distribution of

the different variables on the axis system F1 & F2. With regards to the microorganisms, two group of are observed. The first is made with*E. coli, Staphylococcus* spp., total and fecal coliforms, fecal streptococci, *B. cereus, Y. enterolitica,* enterobacteria and anaerobic sulfite-reducing bacteria. All these microorganisms are known for their pathogenic potential. Hence, this group represents pathogens found in *Egusi pudding* samples. The second group is formed with *Pseudomonas* spp., yeast and moulds which are mainly known for their spoilage ability.

Taking into consideration the overall distribution on the axis system F1 & F2 (Figure 2B), three groups can easily be distinguished. The first group contained pathogens, *Egusi pudding*made with sardine as protein source and the sampling sites Mvan, Obili, Mendong, Mvog-Betsi, Mvog-Ada, Madagascar and Biyem-Assi. In these sites, the main protein source used in the preparation of *Egusi pudding* was sardine. However, samples from these sites contained pathogens and their consumption might represents a risk of foodborne disease. The second group is composed with the sampling sites Melen, Simbock, Ngoa Ekélé and Briqueterie and the *Egusi pudding* made with mackerels, beef and the one free of protein source. This second group is opposed to the first one. This observation means that samples from these sites contained less pathogens. The third group included spoilage microorganisms positively associated with *Egusi pudding* made with cod and the sampling sites Nsimeyong, Simbock, Cite verte, Nkolbissong, Essos, Quartier Centre and Grand Messa. This observation suggests that samples made in these sites contained more spoilage microorganisms.

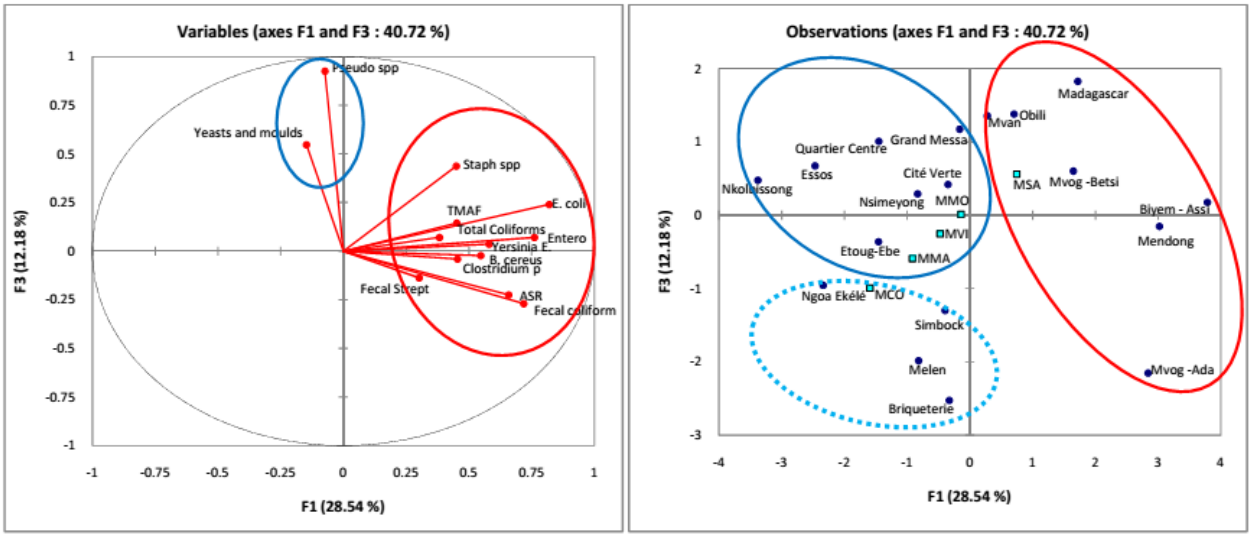

Figure 2: Distribution of the studied parameters on the axis systems F1 & F2.

● = sampling areas; ■ = protein source; TMAF = Total mesophilic aerobic flora, Entero = Enterobacteria, Fecal strept = *Fecal streptococci;* Staph spp.= *Staphylococcus* spp*; Yersinia E = Yersinia enterolitica*, ASR = Anaerobic Sulfito-reducing bacteria; MCO = *Egusi pudding* free of protein source; MMO = *Egusi pudding* with cod as protein source; MMA = *Egusi puddin*g with mackerel as protein source; MSA = *Egusi pudding* with sardine as protein source; MVI = *Egusi pudding* with beef as protein source.

An important observation made in this study was the fact that, independently of the sampling sites, *Egusi pudding* free of protein sources were less contaminated samples. This showed that, protein source might be the leading cause of high contamination of *Egusi pudding* samples. In fact, the protein sources have their own microflora and their addition to the Cucurbitaceae paste during the preparation of *Egusi pudding* will increase the final contamination.

4. CONCLUSION

This study demonstrated that a great proportion of *Egusi pudding* samples marketed in the city of Yaoundé was of poor microbiological quality. The presence of pathogens such as coliforms, *Staphylococci*, *Salmonella*, enterobacteria, *Yersinia* at level higher than the values recommended by the norms suggests a potential health risk for consumers. Potential toxinogenic microorganisms such as moulds, *Clostridium perfringens* and *B.cereus* were present in almost 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.

5. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

6. ACKNOWLEDGMENT

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