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MICROBIOLOGICAL PROFILE OF AGBELIMA, EBLIMA AND EPOMA, THREE TRADITIONAL FERMENTED PRODUCTS IN TOGO

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ABSTRACT: *Agbelima*, *eblima* and *epoma* are the principal cereal based fermented foods usually used by people of Togo especially from south. They are produced by traditional uncontrolled fermentation of *Manihot esculenta Crantz*, *Zea mays* and *Sorghum bicolor* respectively. It is significant to study the microbiological biodiversity of these Togolese fermented products in order to select microorganisms for a possible use as starter or probiotics. Fifteen samples of *Agbelima*, *eblima* and *epoma* respectively were collected from Adidogomè market, Agoè-assiyéyé market, Akodésséwa market, Gbossimè market and Hanoukopé market. All the five markets are located in Lomé city. The microbiological profile of *agbelima*, *eblima* and *epoma* showed that the total aerobic mesophilic flora varies from 9.32 to 10.97 $\log_{10}$ CFU/g. The lactic acid bacteria population ranges from 3.16 to 8.99 $\log_{10}$ CFU/g and 0 to 5.93 $\log_{10}$ CFU/g for yeasts. Molds represent the least counted germs (0 to 0.77 $\log_{10}$ CFU/g). The microbiological analysis of the samples revealed the existence of a great variability between the samples according to their source. The counted ferment microorganisms must be identified for a future use as starter.

Key words: Traditional Fermented Products, Total Aerobic Mesophilic Flora, Lactic Acid Bacteria, Yeasts and molds

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INTRODUCTION

Cereals are the most important source of the world's food and have significant impact in human diet throughout the world (Adebayo *et al.*, 2010). Cereal grains constitute an important group of substrate for fermented foods because they are widely applied in the preparation of a wide variety of dishes in developing countries especially in Africa countries (Obiri-Danso, 1994; Soro-Yao *et al.*, 2014). A majority of traditional fermented products consumed in Africa are processed by natural fermentation of cereals, tuber, fruits or fish, and are particularly important as weaning foods for infants and dietary staples for adults (Odunfa, 1985; Viéra-Dalodé *et al.*, 2007; Tou *et al.*, 2006). A wide range of cereal based fermented foods exist such as *ogi* and *mawè* in Benin, *kenkey* in Ghana, *injera* in Ethiopia, *poto-poto* in Congo, *ogi* and *kunu-zaaki* in Nigeria, *uji* and *togwa* in Tanzania, *kisra* in Sudan (Oyewole, 1997; Blandino *et al.*,2003; Soro Yao *et al.*, 2013). *Agbelima*, *eblima* and *epoma*, are fermented foods which are widely consumed in Togo (Ola, 2010) but can be found in Ghana, Bénin and Nigeria. They are produced by spontaneous traditional fermentation of *Manihot esculenta Crantz*, *Zea mays* and *Sorghum bicolor* respectively. West African countries are experiencing rapid changes in their social and economic environments, which are associated with changes in food consumption patterns. In response to increasing rates of urbanization, efforts are now geared towards developing small-scale facilities for the processing of fermented cereal foods (Trèche *et al.*, 2002).

Small-scale food processing industry is important for stimulating sustainable development in the rural and peri-urban areas of developing countries and for making food available to the increasing populations in urban areas. It provides a source of income and a means of poverty alleviation and contributes to variety in the diet and the food security of millions people. Small-scale food industries also provide linkages to local suppliers of agricultural raw materials and to income-generating activities such as the manufacture of machinery, packaging and ingredients (Chelule *et al.*, 2010). In spite of its socio-economic importance, the processes of fermentation vary from a one country to another, and inside the same country, from one producer to another according to local knowledge and food practices. The fermentation processes is often carried out on small or household scales and are characterized by the use of simple, non-sterile equipment, random or natural inoculums, unregulated conditions, sensory fluctuations, low self-life and unattractive packaging of the processed products (Olanrewaju *et al.*, 2009). During fermentation, the presence of microorganisms such as lactic acid bacteria (LAB), acetic acid bacteria, *Bacillus*, *Enterobacteriacea*, yeasts and molds have been reported (Coulin *et al.*, 2006; Djè *et al.*, 2009; Ouattara *et al.*, 2011; Papalexandratou *et al.*, 2011). However, LAB, yeasts and *Bacillus* are the predominant species involved in most of fermentations studied. To stabilize the foods quality, several studies are consecrated to the research of microbial flora being characterized by physiological and metabolic properties particular for their use as cultures starters (Oguntoyinbo et Narbad, 2012; Owusu-Kwarteng *et al.*, 2012; Ekwem 2014; Obinna-Echem *et al.*, 2014). The development of this new process of fermentation by using a starter adapted to the substrate and making it possible to develop the desired organoleptic properties in order to initiate, to accelerate and control fermentation constitutes a good solution. The strategy "of cultures starters" comprises several stages whose first is the microbiological characterization of the fermentative microorganisms present in the products resulting from an artisanal fermentation.

In Togo, there are lack of informations about *agbelima, eblima* and *epoma*. There are a few data on the microbes involved in their production (Ameyapoh *et al.*, 2009). In order to provide new data about the potential microorganisms to use as starters to produce safety *agbelima, eblima* and *epoma* in Togo, this study was undertaken with an aim of having a microbiological profile of these foodstuffs.

MATERIAL AND METHODS

Material and sampling

The biological material used in this study was constituted by three different types of fermented products (*agbelima*, *eblima* and *epoma*) collected in five markets of Lomé (Adidogomè, Agoè-assiyéyé, Akodésséwa, Gbossimè and Hanoukopé). Fifteen samples of 200 g of each product and three samples were purchased per market. They were put in Stomacher bags and were kept on ice at 0°C and quickly transported to the laboratory of Microbiology and Quality Control of Foodstuffs (University of Lomé) for analysis.

Microbiological analyses

Microbiological analyses were performed according to the standard methods. 10 g of each sample were crushed and suspended in 90 ml of sterile Salt Tryptone Solution (Bio-Rad, Bio-rad laboratories SAS, France). The solution was then homogenated in a Stomacher lab-blender (Model 4001, Seward Medical, U.K.) for 3 min and keep on at room temperature for 45 minutes under aseptic conditions to stimulate revivification of germs. From this solution decimal dilutions have been performed for seeding on suitable media depending on the germs.

Determination of Total Aerobic Mesophilic Flora (TAMF)

Total Aerobic Mesophilic Flora was determined according to the standard NF V08-051, using Plate Count Agar (PCA, Oxoid CM 325, Oxoid Ltd., England). 1 mL of each decimal dilution chosen has been inoculated into Petri dish and 15 mL of the PCA medium have been added and mixed. After solidification of the medium, petri dish were incubated at 30°C for 72 hours.

Determination of Yeast and molds

Yeasts and molds were enumerated on Sabouraud Dextrose Agar (SDA, Oxoid CM 41, Oxoid Ltd., England) supplemented with 250mg/100ml chloramphenicol (selective supplement, Oxoid) following recommendations of the standard NF ISO 7954. 0.1 mL of each dilution was spread on the surface of the medium. Then, the petri dish were incubated at 25°C for five days. The molds were identified based on examination of the colonial heads, phialides, conidiophores and presence or absence of footcells or rhizoids.

Determination of Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria was determined using the method previously described by Hounhouigan *et al.*, 1993. The enumeration was done on De Man Rogosa and Sharpe agar (MRS, Oxoid CM 361, Oxoid Ltd., England). 0.1 mL of the dilution has been spread on the surface of the precooled agar medium. The petri dish were then incubated under anaerobic conditions in an Anaerobic Gas-Pack system at 30°C for 48-72 h.

Statistical analysis

For microbial flora determination triplicate agar plates with 15 and 300 colonies were counted. Data collected were reported as $\log_{10}$ CFU/g. Statistical analysis was carried out by using the MINITAB statistical software package (MINITAB Inc. Release 14 for windows, 2004). Data, expressed as mean value ± standard deviation (SD) of three separate determinations. The data were statistically analyzed using one way ANOVA. Tukey test was used to compare means and significance was accepted at 5% ($P< 0,05$).

RESULTS

Microbiological characteristics of the fermented products

The population of total aerobic mesophilic flora, lactic acid bacteria, yeasts and molds present in samples collected from five markets of Lomé are shown in Table 1. Enumeration of the microorganisms carried out on all 45 samples indicated that they contained in various degree the wanted germs. Total aerobic mesophilic flora counted vary from 9.32 to 10.97 $\log_{10}$ CFU/g. Apart from this group, there exists a specific flora of which most representative was lactic acid bacteria whose population varied from 3.16 to 8.99 $\log_{10}$ CFU/g. The population of yeasts varied from 0 to 5.93 $\log_{10}$ CFU/g. Molds represented the least counted germs (0 to 0.77 $\log_{10}$ CFU/g) in the three analyzed products (Table 1).

Analysis of variance showed the existence of a significant difference between fermented food coming from the various markets ($p< 0,05$) in terms of lactic acid bacteria counts (*epoma*), yeasts and molds counts (*eblima*). However, no significant difference exists between the products analyzed as regards population of total aerobic mesophilic flora, lactic acid bacteria (*agbelima*, *eblima*), and population of yeasts and molds (*agbelima, epoma*).

Correlation coefficient (r) between the different studied variables

The correlation coefficient curve between total aerobic mesophilic flora and lactic acid bacteria population and between lactic acid bacteria and yeasts counted present in the analyzed products are respectively shown in Figure 1 and Figure 2 while their coefficient correlation is presented in Table 2. Analysis of Figure 1 show that the variation in the total aerobic mesophilic flora count is correlated positively with a variation in the lactic acid bacteria load of the three studied matrices whatever their source. The experimental results showed a weak correlation between the load of lactic acid bacteria and yeasts present in *agbelima* and *epoma* (Figure 2 and Table 2). Thus, an increase in the lactic acid bacteria count would slightly be correlated with a reduction in the yeast load on *epoma*, but it exists a good correlation between the lactic acid bacteria load and the yeasts population on *eblima* (Table 2). In general, LAB was the predominant microorganism and in many samples yeasts are presented in significant numbers.

Table 1. Distribution of the Microorganisms in the Fermented Products Samples ($\log_{10}$ CFU/g)

Products	Markets	TAMF	LAB	Yeasts	Molds
Agbelima	Adidogomè	10.44 ±0.19^{a}	8.60 ±0.47^{a}	1.86 ±1.73^{a}	0^{a}
	Agoè-assiyéyé	10.43 ±0.28^{a}	8.99 ±0.43^{a}	1.63 ±1.19^{a}	0.65 ±0.65^{a}
	Akodésséwa	10.60 ±0.17^{a}	8.01 ±0.61^{a}	2.07 ±3.15^{a}	0^{a}
	Gbossimè	10.97 ±0.09^{a}	8.18 ±0.26^{a}	2.08 ±2.03^{a}	0^{a}
	Hanoukopé	10.08 ±0.43^{a}	8.1 ±0.73^{a}	0^{a}	0^{a}
Eblima	Adidogomè	9.52 ±0.78^{a}	7.77 ±0.81^{a}	3.50±0.76^{ab}	0.36 ±1.36^{b}
	Agoè-assiyéyé	10.86 ±0.80^{a}	7.37 ±0.79^{a}	5.93 ±0.31^{a}	0.77 ±0.77^{b}
	Akodésséwa	9.51 ±0.84^{a}	7.11 ±0.33^{a}	5.88 ±0.47^{a}	0.47 ±0.54^{a}
	Gbossimè	9.32 ±0.42^{a}	7.51 ±0.39^{a}	4.06±0.58^{ab}	0^{b}
	Hanoukopé	9.67 ±0.78^{a}	7.03 ±0.89^{a}	2.31±1.66^{b}	0^{b}
Epoma	Adidogomè	10.95 ±0.05^{a}	7.07 ±0.11^{a}	3.03 ±0.40^{a}	0.16 ±1.08^{a}
	Agoè-assiyéyé	10.84 ±0.36^{a}	7.40 ±0.45^{a}	2.44 ±1.31^{a}	0.20 ±2.20^{a}
	Akodésséwa	10.03 ±0.22^{a}	7.07 ±0.40^{a}	3.05 ±0.90^{a}	0^{a}
	Gbossimè	10.30 ±0.48^{a}	7.06 ±0.39^{a}	3.06 ±1.01^{a}	0^{a}
	Hanoukopé	9.47 ±0.15^{a}	3.16 ±3.16^{b}	3.33 ±0.50^{a}	0^{a}

Total Aerobic Mesophilic Flora (TAMF), Lactic Acid Bacteria (LAB)

*Averages in column with different letters are significantly different at 5% level according to post hoc test of Tukey

Regrouping of analyzed products according to the similarities

Figure 3 presents the regrouping of the three fermented products taken in various markets according to their similarity based on the correlation coefficients and microbiological characteristics. Analysis of the figure shows that products were gathered in two great clusters E and F. The first cluster E was subdivided in three sub-groups (E1, E2 and E3). The sub-group E1 incorporated three products from Adidogomé market, *agbelima* collected from Agoè-Assiyéyé, Akodésséwa, Gbossimè markets, and *eblima* from Hanoukopé market. The second sub-group E2 was made up only of *agbelima* collected from Hanoukopé market. The sub-group E3 comprised *eblima* and *epoma* collected from Agoè-Assiyéyé, Akodésséwa, and Gbossimè markets. *Epoma* samples collected from Hanoukopé market are the only component of the second cluster named F. We observed heterogeneity on the level of the sub-groups E1 and E3 and homogeneity for the sub-group E2 and the second group.

Table 2. Matrix of Pearson's correlation between different variables

Variables	TAMF	LAB	Yeasts	Molds
Agbelima				
TAMF	1.00			
LAB	0.582	1.00		
Yeasts	0.562	0.384	1.00	
Molds	-0.153	0.408	0.049	1.00
Eblima				
TAMF	1.00			
LAB	0.644	1.00		
Yeasts	-0.203	0.545	1.00	
Molds	-0.824*	-0.293	0.521	1.00
Epoma				
TAMF	1.00			
LAB	0.828*	1.00		
Yeasts	-0.493	-0.145	1.00	
Molds	0.316	0.389	-0.658	1.00

Total Aerobic Mesophilic Flora (TAMF), Lactic Acid Bacteria (LAB)
*5% level of significance

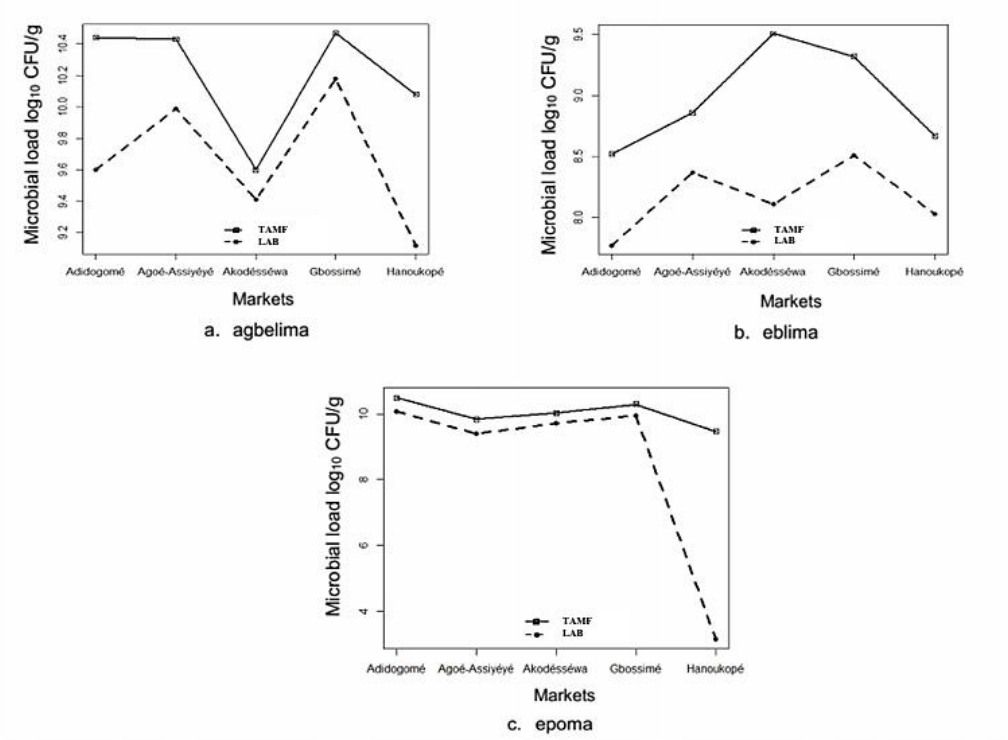

Figure 1. Correlation coefficient (r) curve of Total Aerobic Mesophilic Flora (TAMF) and Lactic Acid Bacteria (LAB)

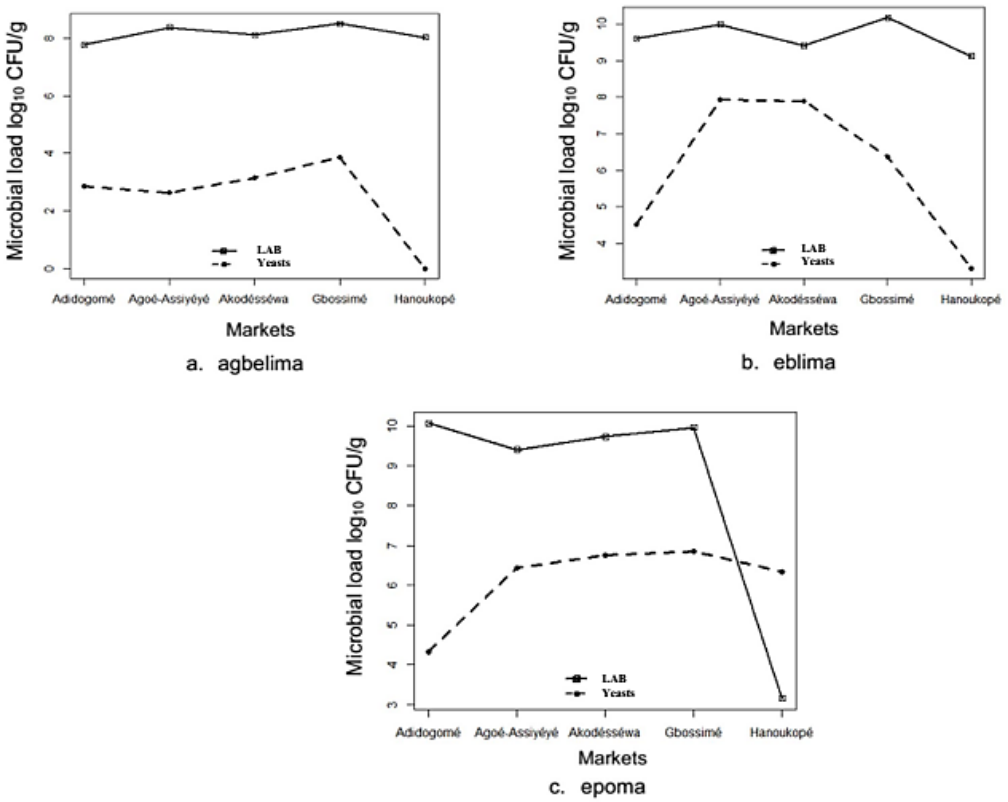

Figure 2. Correlation coefficient (r) curve of Lactic Acid Bacteria (LAB) and Yeasts

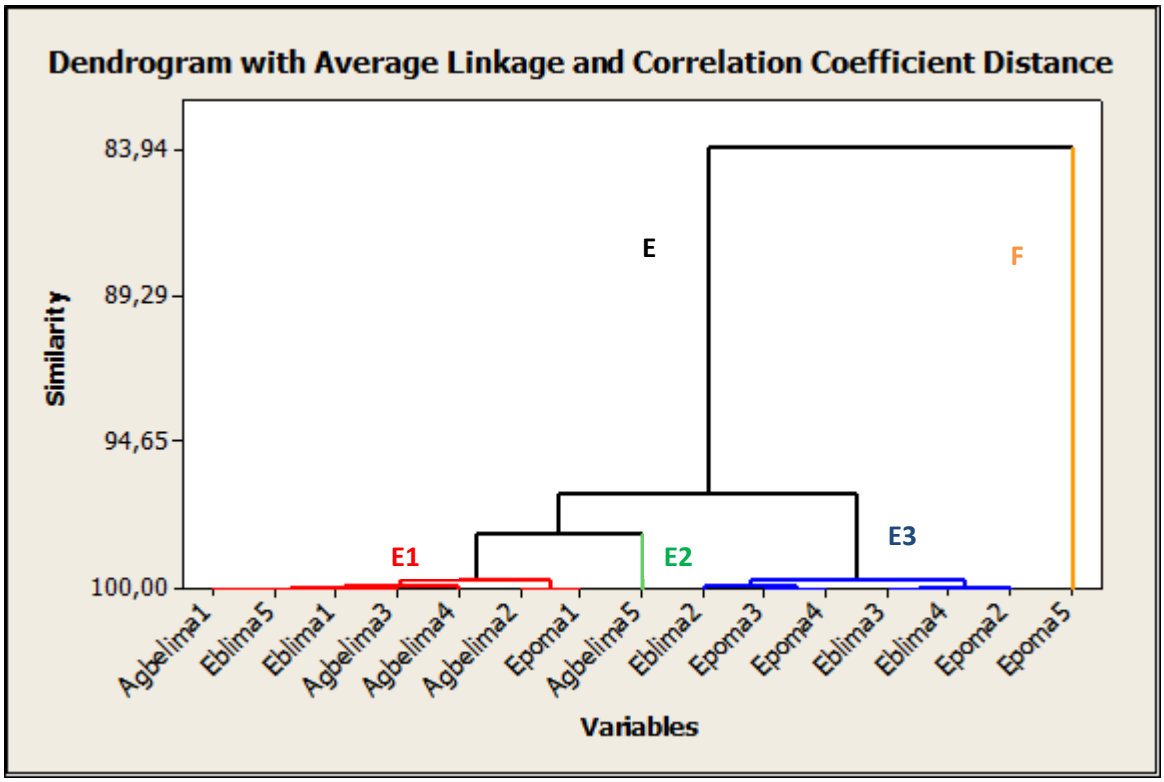

Figure 3. Classification of products taken in the various markets according to their similarity with 98% based at the distance enters the correlation coefficients (r)

Legend. 1: Adidogomè, 2: Agoè-Assiyéyé, 3: Akodésséwa, 4: Gbossimè and 5: Hanoukopé

DISCUSSION

The fermented products prepared from cereal (*eblima* and *epoma*) and cassava (*agbelima*) samples analyzed in this study were produced using traditional and ancient methods of natural fermentation. These fermented foods vary depending on their quality, taste, ingredients, and production methods, which are vary among regions, provinces, and even families (Owusu-Kwarteng *et al.*, 2012). Therefore, various beneficial microorganisms are present in different fermented starch products. We analyzed 45 samples to determine their microbiological biodiversity.

The natural fermentation of these foodstuffs is unpredictable. Spontaneous fermentation typically results from the competitive activities of different microorganisms where by strains best adapted and with the highest growth rate during the process (Ouattara *et al.*, 2011). The microbiology of many West African fermented cereal products has shown that the fermentation process is natural and involves mixed cultures of lactic acid bacteria, yeasts and molds (Ouattara *et al.*, 2011; Owusu-Kwarteng *et al.*, 2012; Ekwem 2014). Among the bacteria associated with food fermentation, LAB are of predominant importance. The LAB are known to be associated with many natural and man-made environments. It was reported elsewhere by Assanvo *et al.* 2006 that samples from plant material showed the greatest diversity of LAB with *Lactobacillus* strains being predominant in food-related ecosystems. Among the counted microorganisms, lactic acid flora has as a primary role in the hydrolysis of starch leading to production of sugars, of lactic and acetic acids and this activity is supplemented by the cellulosic activity of molds (Assanvo *et al.*, 2006). All this leads to release in the environment sugars which represent the raw material for other microorganisms. The medium of fermentation remains always beneficial for the presence of lactic acid bacteria due to the pH which becomes acid due to their action. Indeed, lactic acid flora, mesophilic germs supports acid pH, thus making it possible to easily understand their preponderance in the various analyzed fermented products (Amoa-Awua *et al.*, 1996).

Coefficient of Pearson's correlation between total mesophilic flora and lactic acid bacteria of the various analyzed products are higher than 0.5, thus it exists a good correlation between the two variables. Lactic acid bacteria and yeasts are often associated in indigenous African fermented products. Evolution of LAB microbial load seems to be accompanied by the development of yeasts. This would be the result of a symbiotic association between yeasts and lactic acid bacteria. Yeasts could provide vitamins, amino-acids and growth factors for others microorganisms like lactic acid bacteria while the bacterial end-products could be used by the yeasts as an energy source (Nout, 1991; Leroi et Pidoux, 1993).Thus the alcohol produced by yeast, the acids produced by the bacteria and the anaerobic conditions induced by fermentation, will allow to remove filamentous fungi and the bacteria associated with food deterioration (Mensah *et al.*, 1991). Oyewole (2001) had announced that the growth of *Lactobacillus plantarum* strain had been improved considerably by the presence of *Candida krusei* during fermentation of the cassava for the production of *fufu*. The coexistence and symbiotic association between LAB and yeasts in African traditional fermented products have been reported by several authors (Hounhouigan *et al.*, 1993; Oyewole, 2001; Kayodé *et al.*, 2007). Besides their role in the build-up of the sour taste, typical flavor and unique aroma of fermented products, some yeast has been reported to show amylolytic, protease and phytase activities. This enzymatic ability according to Amoa-Awua *et al.* (1996) may contribute to breaking down maize starch and also allow better access to nutritionally essential minerals. These foodstuffs microflora is composed of a stable association of LAB and yeasts in particular due to metabolic interactions therefore this microbial ecology may influence the product characteristics and quality (Narvhus, 2003). The action of yeasts and the bacteria during the fermentation of the sorghum allowed to obtain *tchoukoutou* with a stable pH and a characteristic taste (Kayodé *et al.*, 2007).

Numerical classification allows us to classify fermented products and to gather them according to their similarity. This analysis enabled us to classify in two great groups the alimentary matrix. The processes of fermentation vary from one producer to another according to local knowledge and food practices. Assanvo *et al.* (2006) noted a various practices at nine producing leaven of cassava for the production of *attiéké* in three villages of Ivory Coast. These results also confirm studies of several authors (Hounhouigan *et al.*, 1993; Greppi *et al.*, 2013; N'tcha *et al.*, 2015) through their research carried out on the characteristics of traditional fermented products containing cereals and cassava which reported that the distribution frequencies of counted microorganisms vary according to localities and ingredients used for their manufacture. These authors showed that there was variability between productions within the same workshop and workshop to another. The resultant is fluctuation of quality of various food obtained. It is thus essential to standardize the manufacturing processes by defining parameters which make it possible to manufacture products of constant quality in time and space.

CONCLUSION

This study has complemented the knowledge on microbiological profile of three fermented products prepared and consumed in Lomé (Togo). Microbiological analysis allowed to compare the three fermented products according to their source. Lactic acid bacteria are dominant compared to the other bacteria present in analyzed alimentary matrix. Lactic acid bacteria existing in fermented products are correlated positively with total mesophilic flora load. Lactic acid bacteria and yeasts are microorganisms responsible of the traditional fermentation in these products. These microorganisms isolate from the uncontrolled fermentation process must be identified and characterized for a potential use as a starter or probiotic culture.

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