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Impact of Traditional Process on Hygienic Quality of *Soumbala* a Fermented Cooked Condiment in Burkina Faso

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Abstract *Soumbala* is an African fermented locust beans used as a food condiment in Burkina that it quality could be affected due to hygienic practices. The present study aimed to show the implication of traditional process on hygienic quality of *soumbala*. *Soumbala* a fermented condiment was collected in six ethnical groups of Burkina Faso. A total of 120 samples were analysed using standards methods of microbiology. The microbial analysis showed a significant difference ($p < 0.05$) among microorganism groups following to the synthetized diagrams (A and B). Significant difference ($p < 0.05$) was established among microorganisms targeted in diagram A versus diagram B comparing separately the amount of *Bacillus sp.* (18.77 ± 0.29% VS 37.22 ± 0.076%), *Micrococcus sp.* (17.52 ± 0.65% VS 10.68 ± 0.38%) and *Staphylococcus sp.* (19.63 ± 0.35% VS 14.49 ± 0.2%), Yeasts and Molds (34.74 ± 0.098% VS 37.6 ± 0.26%), Total coliform (9.1 ± 0.16% VS 0). The results indicated that samples of *soumbala* contain various microorganisms due the impact of processing.

Keywords: *seeds, processing, fermentation, food, safety*

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1. Introduction

The need for additional protein supplies to promote sustainable livestock in most tropical area has increased the search for indigenous wild legumes or condiments basis seeds for least cost formulation and production. Two such legumes or condiments are *soumbala* and *bikalga,* commonly known as African locust bean, of tropical trees (*Parkia biglobosa* and *Hibiscus sabdariffa*) which are native to Africa and widely distributed in the savanna region (Adewusi et al. 1992). *Bikalga* and Soumbala are condiments of traditional uncontrolled alkaline fermentation of *Hibiscus sabdariffa and Parkii biglobosa seeds respectivelly. These* food condiments are used and produced as major condiment in many African countries including Burkina Faso, Mali, Niger, Nigeria, Cameroon and Sudan among others. *Bikalga* is also call as *Dawadawa botso* in Niger, *Datou* in Mali, *Furundu* in Soudan, *Mbuja* in Cameroon (Odunfa 1985; Yagoub et al. 2004). *Bikalga* and *Soumbala* are produced by women and create economical source (Ouoba et al. 2003). The seeds serve as source of useful ingredients for consumption as *Iru* in Yoruba, have some anti-nutritional factors (ANFs) such tannins, oxalate and hydrogen cyanide. ANFs were eliminated or reduced by application of heat and sprouting (Alabi et al. 2005). So fermentation has been reported to destroy some natural toxins which may occur in beans, improve the nutritive value, increase digestibility and enhance growth (Alabi et al. 2005; Bridget et al. 2004). The strains of *Bacillus* and *Staphylococcus* are implied fluently in the *soumbala* fermentation. *Bacillus genus* is involved to the initial fermentation of seeds characterized by the excretion of exopolysaccharides and lipopeptides (Savadogo et al. 2011). The *Staphylococcus* strains are responsible to the genesis of specific aromas. The excretion of lipopeptides can explain the use of these condiments as disinfectant in traditional medicine.

The *Bacillus subilis* and *Bacillus pumilus* are implied in the deterioration of the indigestibles polysaccharides and oligosaccharides (arabinogalactan, stachyose) content in *Parkia biglobosa* seeds (Ouoba et al. 2003; Ouoba et al. 2007). Kim and Park (2009) showed that *Bacillus cereus* isolated in a fermented cooked that showed have a high anticancer effects against AGS Human Gastric Adenocarcinoma cells. The final product from the fermentation of seeds, can present itself in several aspects according to the preferences: flour, curds or wads. This

product is used in the human food as condiment in sauces and can replace industrial soups.

The *soumbala* is a condiment very consumed by some ethnic groups of Burkina Faso. An investigation permitted to target ethnic groups where its consumption is current. It is about the *Bissa, Dagara, Dafing, Mossi and Samos*. They are also consumed by *Peulh, Bwaba, bobo, Sambla, Sénoufo* (Ouoba et al. 2003).

This condiment is used preferentially in sauces and also in the preparation of the sauces of rice, *couscous* and other dishes basis of cereals. Several literature have been developed about fermented seed and related the extent of their physico-chemical properties and microbial aspect (Mohamadou et al. 2007; Ouoba et al. 2007; Parkouda et al. 2008; Oguntoyinbo et al. 2010; Parkouda et al. 2011).

The major problem of the set of the traditional process is located to the fluctuating quality of the different foods obtained. The processing and quality of *soumbala* is variable according *Mossi, Fulani, Bobo, Dagara, Samo, Bissa* groups. Indeed, the fermentation process which takes place spontaneously through the microflora development, can conduct to an organoleptic, microbiological or toxicological undesirable products (Rainbaul 1995).

The recipes and preparations of fermented condiments are unfit while the packaging and presentations are traditional; of course they still lack safety and quality controls. The physical conditions and infrastructure in the site of *soumbala* production are generally poor, with most seeds material displayed in the open. Under these conditions, the material is exposed to microbial and insect attack as well as the effects of moisture, dust, and temperature (De Souza et al. 2011).

Producers often collect seeds material on tree or market and prepare *soumbala* without any preliminary treatment to eliminate indigenous microbes. Sometimes, seeds processing is carried out in their homes in nonadequate hygienic conditions, and prepared condiments are stored in inappropriate conditions. Overall, the final product proposed to the consumers is sometimes of poor hygienic quality (Djikpo-Tchibozo et al. 2011).

This study was designed to value the impact of traditional process on hygienic quality of *Soumbala* a fermented cooked condiment in Burkina Faso. It will be able to prevent pathogens contamination and improve the hygienic and safety quality.

2. Material and Methods

The experiment was conducted in the Laboratory of Microbiology and Biotechnology at CRSBAN (University of Ouagadougou/Burkina Faso). The six processing kind of *soumbala* has been followed in the sites of production among ethnical groups as *Mossi, Fulani, Bobo, Dagara, Samo, Bissa* in Burkina Faso.

2.1. Diagrams of Production of *soumbala*

Table 1. Different process steps and their role in the production of *soumbala*

Process according to ethnic	Process Steps	Role of steps	Locality
Process of *mossi*	a, b, c, d, f, g, i,j	To obtain solid fermented product with pungent smell	Ouahigouya about 110 km of Ouagadougou
Process of *fulani*	a, b, c, d, f, g, i,j	To obtain solid fermented product with pungent smell	Dori about 400 km of Ouagadougou
Process of *samo*	a, b, c, d, e, f, g, h, i,j	To obtain soft to fell condiment with least pungent smell	Tougan about 300 km of Ouagadougou
Process of *bobo*	a, b, c, d, e, f, g, h, i,j	To obtain soft to fell condiment with least pungent smell	Bobo-Dioulasso about 365 km of Ouagadougou
Process of *dagara*	a, b, c, d, e, f, g, h, i,j	To obtain soft to fell condiment with least pungent smell	Dagara about 400 km of Ouagadougou
Process of *bissa*	a, b, c, d, f, g, i,j	To obtain solid fermented product with pungent smell	Garango about 200 km of Ouagadougou

a = *Parkia biglobosa* seed cleaning**, b** = seed cooking**, c** = cooling**, d**= addition of alkaline ash**, e** = first fermentation of seed**, f**= pounding**, g**= moulding**, h**=second fermentation, **i**= sundring**, j**= fermented condiment.

2.2. Sample Collection

Different samples of fermented condiments (*soumbala*) were taken to check the process effect on the microbial quality of these foods. Six (06) process of *soumbala* production were studied. A total 120 samples of *soumbala* were collected on six (06) sites of production with seller women according to the ethnical groups mentioned in table I. In each site, 20 samples of *soumbala* were collected and maintained at + 4°C in isothermal box then sent to the laboratory for analysis.

2.3. Microbiological Analysis

The levels of total mesophilic flora, total coliform, *Escherichia coli, Staphylococcus sp, Micrococcus, Salmonella*, Yeast and Molds were determined by standards microbiology system using the methods of Zinnah et al (2007) and De Souza et al (2011). The prevalence rates and levels of presumptive were determined in 120 samples.

2.3.1. Preparation of Sample

Each sample of *soumbala* was properly mixed to ensure homogenisation of the microorganisms present in the fermented product. 10 g of each of *soumbala* was introduced aseptically into 90 ml (1:10 dilution) of buffered Peptone water (0.85% (wv^{-1}) Biomerieux, France) into stomacher blister and stored at ambient temperature for 30 min. The mixture introduce in a McCartney bottle was mixed with a vortex (Gemmy Industrial Corporation, Italy) for 5 min. A serial dilution of 10^{-1} to 10^{-6} was made and 1 ml of each of dilution was pipetted in duplicate into appropriately marked Petri dishes.

2.3.2. Microbial Numeration

All media used in this study were purchased from Biomerieux (France). The total number of microorganisms expressed as Colony Forming Unit (cfu) per gram of sample was determined by standard plate count. The important approach in numeration was to detect the phenotypic aspect of microorganisms targeted.

-The total aerobic mesophilic flora (FAMT) was counted in Plate Count Agar (PCA), after 24 hours of incubation at 30°C under aerobic conditions.

-Eosin Methylen Blue medium (EMB) was carried out, to enumerate total coliforms and *E. coli*, after 48 hours of incubation, at 37°C and 44°C ±0.5°C respectively, under aerobic conditions.

-Yeasts and molds were counted in a Sabouraud after 3 - 5 days of incubation at 30°C, under aerobic conditions.

-*Salmonella* and *Shigella sp* was researched in a SS medium after 3 - 5 days of incubation at 37°C, under aerobic conditions.

-Suspected pathogenic *Staphylococcus sp*, were counted in the Chapman positive coagulase after 24 hours of incubation at 37°C, under aerobic conditions.

-Micrococ*cus sp*, were counted in the Chapman medium after 24 hours of incubation at 37°C, under aerobic conditions.

-Bacill*us sp*, were counted in the Man Rogosa and Sharpe medium after 24 hours of incubation at 37°C, under aerobic conditions.

-Sulphite Reducing Bacteria (SRB) were screened with tryptone-sulfite neomycin broth after 20 hours incubation at 44°C ± 0.5°C.

Standard identification methods included Gram stain morphology, colony morphology, production of catalase or oxidase, lactose fermentation were used for microorganisms phenotypical identification. Coagulase production, pigment production, anaerobic production of acid from glucose of have been used confirm *Staphylococcus sp.* Mannitol salt agar (MSA) selective both was used to differentiate Staphylococcus sp. (Salt tolerance) and Micrococcus sp. (Salt intolerance). *Bacillus sp.* was identified by Voges-Proskauer (VP) test which reveal acetoin produced from glucose.

2.4. Statistical Analysis

Data obtained were expressed as means ± standard deviation (SD). Statistical analysis was done using Stata/IC 10.0 from StataCrop LP. All enumeration values analysed were transformed to log10 values due to the abnormal nature of the data. Frequency of microbial groups was calculated using Microsoft excel. A one way analysis of variance (ANOVA) was performed on enumeration values (duplicate means) obtained for each sample per medium. Comparison between mean values for the parameters considered was performed using the least significant difference test at 0.05 significance level.

3. Results

3.1. Diagrams of Production of *soumbala*

Production of *soumbala* is a traditional process and involves many steps, including cleaning, boiling in alkaline conditions, pounding, fermentation steaming, drying and roasting. The different steps of process used for *soumbala* production have been regrouped in two diagrams.

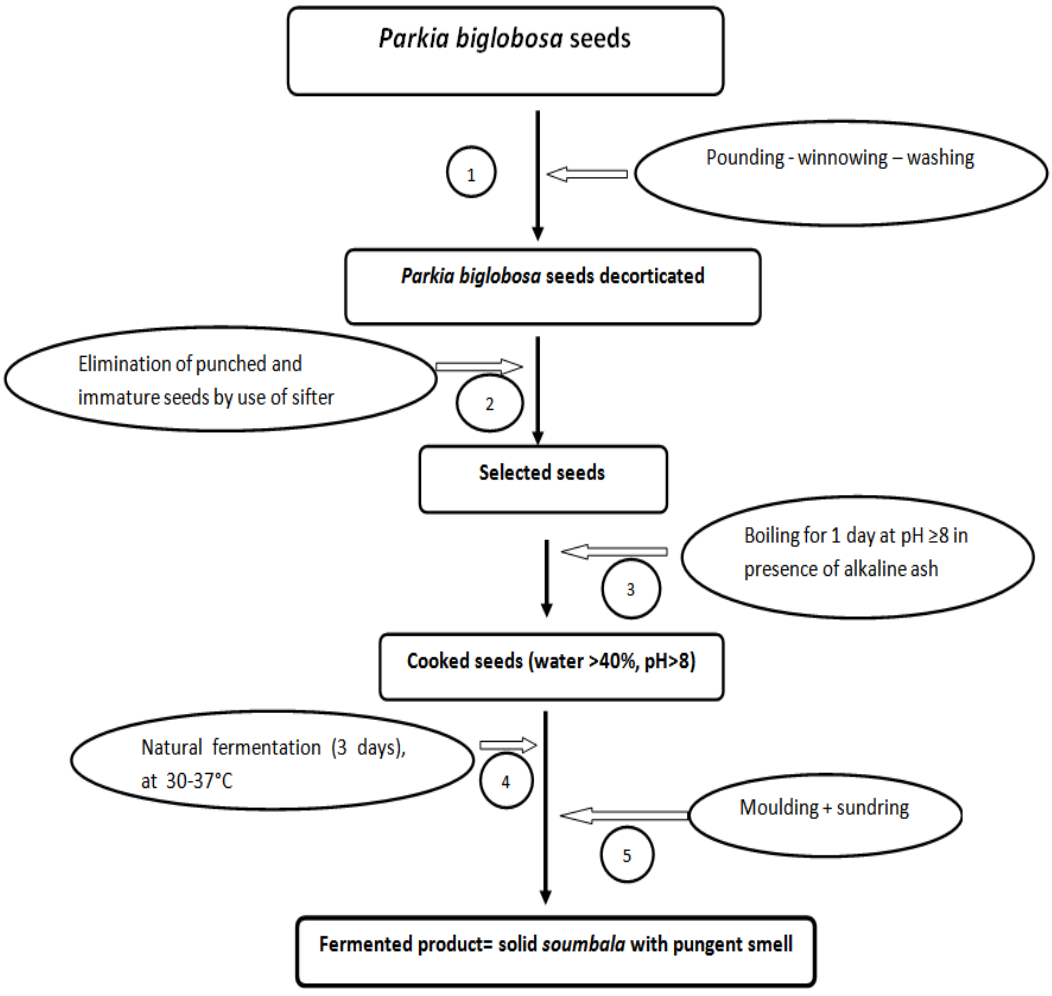

Figure 1. Diagram **A** relating to different steps of *soumbala* production in *Mossi, Bissa, Fulani*

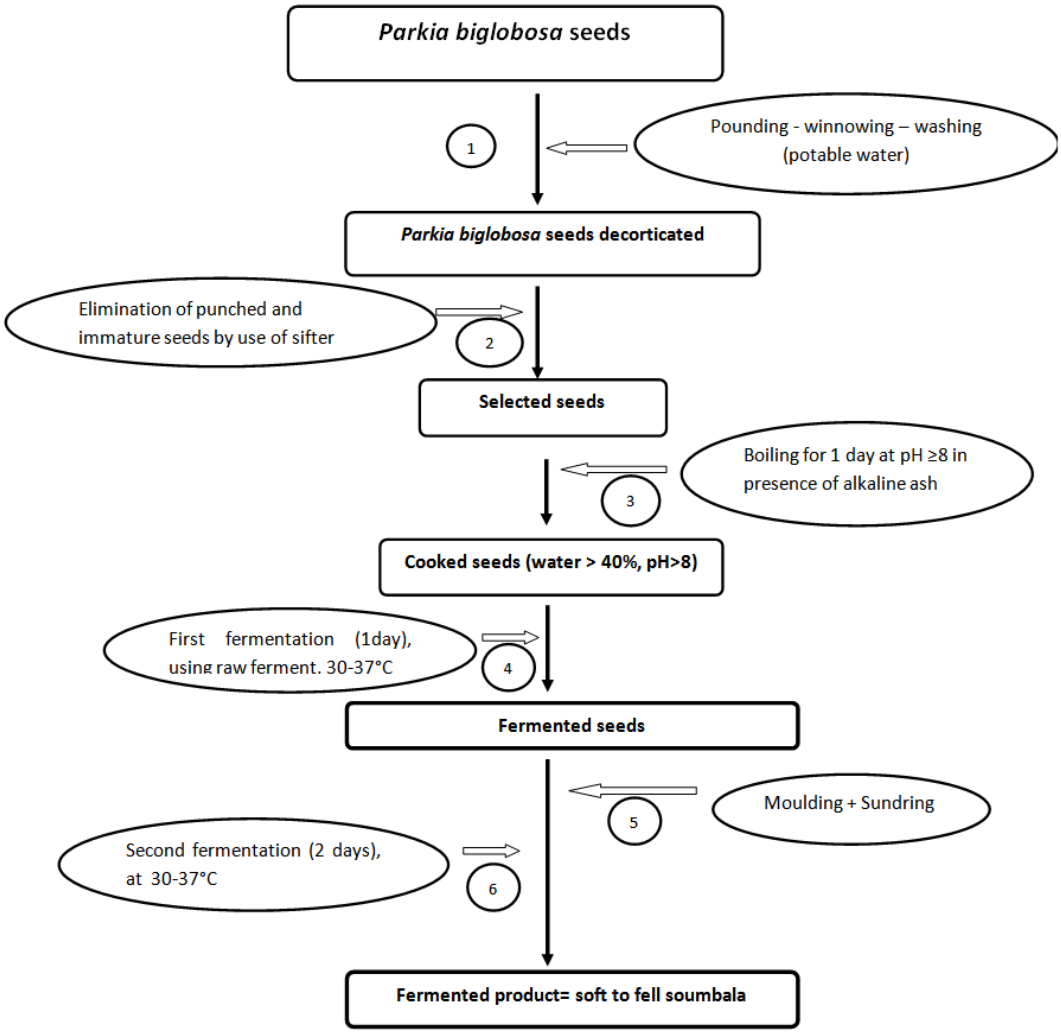

Figure 2. Diagram **B** relating to different steps of *soumbala* production in *Dagara, Bobo, Samo* ethnicals groups

The Figure 1 showed the similar steps process used by ethnicals groups as *Mossi, Bissa, Fulani* while the Figure 2 denoted same steps in *Dagara, Bobo, Samo* ethnical groups. The two diagrams A and B showed that the main difference of process between ethnic groups reside in the method fermentation. Even the ingredients used and major route is similar among the groups, the particular difference reside to the safety of finished product intended to the human consumption. The taste, flavour, smell, color, stiffness of soumbala from diagram B were clearly appreciated by the consumers than one of diagram A. These characteristics of fermented products are the major difference between the samples of soumbala obtained.

During the fermentation, proteolysis followed by the degradation of carbohydrates seems to be the main biochemical and smell of seeds. This hypothesis is supported by the found of Ouoba, Parkouda, Diawara, Scotti and Varnam (2007).

They showed that proteolytic, lipolytic and amylolytic activities during the fermentation involve the change of fermented product.

3.2. Numeration of Isolates Microorganisms

The microbial numeration on different samples of *soumbala* was studied and is presented in Figure 3.

The results in Figure 3 present the phenotypic microorganisms identified and show that neither *Salmonella* nor Sulphite Reducing Bacteria were found in the *soumbala*. Total coliform were detected in Diagram A and empty in Diagram B. The samples were much contaminated by total aerobic microorganisms. It was observed that the number of total mesophilic microorganisms from diagram A were most represented ($\log_{10}$ (UFC.g^{-1}): 9.42±0.03) was highest than diagram B ($\log_{10}$ (UFC.g^{-1}): 8.50±0.02) with a significant degree $p<0.05$. Yeast and molds were count were significantly different ($\log_{10}$ (UFC.g^{-1}): 7.86±0.02 in diagram A versus $\log_{10}$ (UFC.g^{-1}): 6.63±0.06) in diagram B) with $p<0.05$). A significant difference ($p<0.05$) is noted concerning the presence of *Bacillus sp*, the diagram A ($\log_{10}$ (UFC.g^{-1}): 4.38±0.12) contain a less count than diagram B ($\log_{10}$ (UFC.g^{-1}): 6.46±0.02). And yet the notable remark was the presence of total coliform ($\log_{10}$ (UFC.g^{-1}): 2.13±0.06) in the *soumbala* provide from diagram A. The count of *Staphylococcus sp* and *Micrococcus sp* is higher from diagram A ($\log_{10}$ (UFC.g^{-1}): 4.58±0.15 and 4.09±0.02) than diagram B ($\log_{10}$ (UFC.g^{-1}): 2.51±0.05 at 1.85±0.11) with $p<0.05$.

In accordance with the AFNOR limits (Association Française de Normalisation [AFNOR], 2009), all of samples investigate were acceptable in hygienic sight according to all enumerated microorganisms.

3.3. Microbial Assessment

The distribution microbial following the diagram production of *soumbala* is presented in the Table 2.

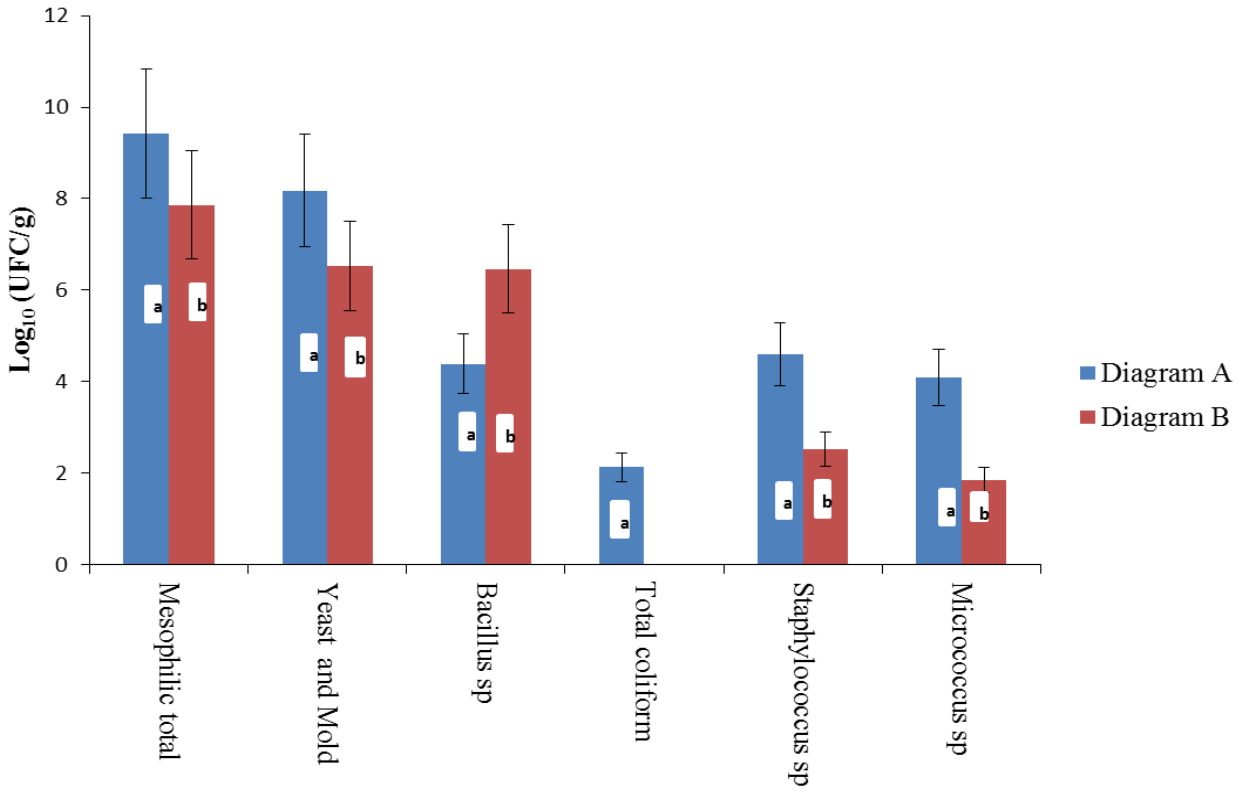

Figure 3. Number of isolates microorganisms following two diagrams of condiments production

Letters are used in alphabetical order to indicate variation value of microorganisms. The values are means ± standard deviation of three replicates. Means with different alphabets on the same column group indicate are significantly different at P<0.05 at 95% confidence level.

Table 2. Percentage distribution of various microbial genera in *soumbala*

Microorganisms	Percentage of incidence (%)	
	Diagram A	Diagram B
Yeast and Mold	34.74± 0.098[a]	37.6± 0.26[a]
Bacillus sp	18.77±0.29[bc]	37.22± 0.076[a]
Total coliform	9.1±0.16[e]	0
Staphylococcus sp	19.63±0.35[b]	14.49± 0.2[b]
Micrococcus sp	17.52±0.065[c]	10.68± 0.38[c]

Letters are used in alphabetical order to indicate value of microorganisms. The values are means ±standards deviation of three replicates. Means with different alphabets are significantly different at p < 0.05 at 95% confidence level.

Results in Table 2 showed a significant difference (p<0.05) between microbial communities presence in *soumbala* resulting of diagrams A and B of production. Significant difference (p<0.05) was established among microorganisms targeted in diagram A versus diagram B comparing separately the amount of *Bacillus sp.* (18.77±0.29% VS 37.22± 0.076%), *Micrococcus sp.* (17.52±0.65% VS 10.68± 0.38%) and *Staphylococcus sp.* (19.63±0.35% VS 14.49± 0.2%), Yeasts and Molds (34.74± 0.098% VS 37.6± 0.26%), Total coliform (9.1±0.16% VS 0).

4. Discussion

The *soumbala* collected on the sites of production in hot season are varied and undergo inopportune manipulations including the process of fermentation. These practices can reduce their quality. The *soumbala* is kept to an elevated ambient temperature. Temperature coupled an absence of cold chain causes a strong contamination (Gonfa et al. 2001). In the present work the microbiological quality of the *soumbala* examined is relatively acceptable. The rationale determining microbiological quality of the fermented products is to prevent deterioration due to the presence of foodborne pathogens and food intoxication risk.

Any sample was kept to limit to the quality of the international norms. It means unrespect of hygiene condition. The samples most contaminated are especially those of the diagram A due probably to the quality of water used during the production.

The detection of *S. aureus* and Sulphite Reducing Bacteria (SRB) is for their toxicological properties. So *S. aureus* is the leading species of the genus *Staphylococcus* implicated in food poisoning infection while SRB are a group anaerobic sporulating bacteria including *Clostridium*, their spores resist after heat treatment and may cause damages after contaminated product consumption (De Souza et al. 2011). The contamination of samples pathogen may occur in *soumbala* processing or during the handling water and implement used also during the storage.

Similar shapes of contamination have been described on fermented products in several countries of Africa by some authors as Godefay and Molla (2000); Gonfa, Foster and Holzapfel (2001).

A number superior to 4.0 log (ufc/g) of *Mesophilic total* indicates deficiency condition of hygiene in the production or storage about Yamani, Al Kurdi, Haddadin and Robinson (1999). The microbiological quality of the condiment remained important for its conservation

(Guinot-Thomas et al. 1995). The strong rate of yeasts and molds in *soumbala* samples could be explained as well as by the state of containers cleanliness used and the methods of transformation (Gadaga et al. 2000; Beukes et al. 2001).

The massive presence of yeasts and molds is also the expression of a strong outside contamination and a bad hygiene of the implement. The importance of *Staphylococcus aureus* and Total coliform in the fermented product could result to a lack of hygiene or a strong contamination of the raw material allowing germs to escape the bacteriocin produced by the *Bacillus* kind. *Staphylococcus aureus* and Total coliform are responsible to the food damage. The proliferation of the microorganisms on the *soumbala* could be explained by the necessary mineral and nitrogenous elements found in abundance on the raw material and used by these microorganisms (Somda et al. 2011).

5. Conclusion

The results showed that *soumbala* contain various microorganismes. The presence of the indicator germs of hygiene lack shows that the *soumbala* consumed by an important part of the population can especially contribute to reduce the quality and time conservation then create economical impact.

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