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Evaluation of hygienic quality of ferment of local beer "dolo" used as condiment in Burkina Faso

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***Rabilé* is a ferment of local beer called "*dolo*" in Burkina Faso. It is used for local beer production and as condiment in sauces. Its process of production could be a source of contamination. So the quality of ferment needs to be guaranteed for consumers' health. The aim of the study was to control the hygienic quality of local ferment consumed in Burkina Faso. A total of 70 samples were purchased in seven localities of Burkina Faso. The standard plate count method was used for microbial analysis. The results show that aerobic mesophilic flora varied from 8.34 ± 0.72 to 10.07 ± 0.51 $\log_{10}$ UFC.g^{-1}; yeast and mold from 7.24 to 8.28 $\log_{10}$ UFC.g^{-1}; *Staphylococcus aureus* from 4.08 to 4.76 $\log_{10}$ UFC.g^{-1}; *Enterococci* from 3.47 to 4.61 $\log_{10}$ UFC.g^{-1} and total coliform from 2.12 to 2.32 $\log_{10}$ UFC.g^{-1}. *Salmonella* spp. and *Shiguella* spp. were not detected in any of the samples. So, 100% of the samples from Ouahigouya were contaminated with sulphite reducing bacteria (BRS). All the samples analyzed were contaminated by *S. aureus* and *Enterococci*. The hygienic control of the ferment showed that the contamination caused by food borne pathogens can lead to food poisoning. Pearson correlation matrix of microorganisms' distribution in the samples showed highest positive correlation between Enterococci and sulphite reducing bacteria at r = 0.888. Analysis of principal components exhibited the variability of microbial groups with 94.312% cumulative values of the variance and Eigen values ranging between 0.492 and 3.695. The data suggested that traditional beer ferments samples collected were not suitable in quality and there is need to ameliorate the process of production.**

Key words: Local ferment, dolo, hygienic quality, food, Burkina Faso.

INTRODUCTION

Traditional beer ferment of Burkina Faso is called "rabilé" in Mooré, "dambilé" in Dagara, "yantoro" in San, "li dabili" in Gourmatché, "seinbié" in Gouroussi, "kuinguié" in Bobo, "yiibou" in Bissa, also called Kpètè-Kpètè or Otchè

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in Benin, Umusemburo in Rwanda (Djêgui et al., 2015; Keita et al., 2016; Lyumugabe et al., 2014; N'tcha et al., 2016). It is used for cooking traditional beer and for preparation of certain sauces in some communities of Burkina Faso.

The local beer is cooked in Burkina Faso using *Sorghum bicolor* L, *Setaria italic* or *Zea mays*. The traditional process of local beer production involves three principal steps: malting, brewing and fermentation process. However, variations can occur according to ethnical group, the producers and the locality (Coulibaly et al., 2014; Songre-Ouattara et al., 2016). In African countries, sales of the local beer constitute an important source of money for the sellers. Moreover, local beer is used in some ceremonies like funeral, wedding and baptism (Maoura et al., 2005; Djegui et al., 2015; Keita et al., 2016).

Studies carried out by the different authors (Glover et al., 2009; Sawadogo-Lingani et al., 2010; Djegui et al., 2015; N'Guessan et al., 2015) have shown that *Lactobacillus* sp., *Leuconostoc* sp. and *Saccharomyces cerevisae* are principal microorganisms found in the fermentation of traditional beer. Sawadogo-Lingani et al. (2008), Kayode et al. (2012) and N'tcha et al. (2016) have revealed probiotic property of lactic acid bacteria isolated from fermented local sorghum beer. The ferment constitutes interesting nutriments source. Wijeyaratne and Jayathilak (2000) demonstrated that *Candida* sp. contains vitamin B (riboflavin (0.231 mg/g), and thiamin (0.178 mg/g). The percentage of its protein ranges from 50 to 60.31% (Dimova et al., 2010; Mondal et al., 2012; Ouédraogo et al., 2012) in the ferment source. Ouedraogo et al. (2017) revealed that *Candida utilis* NOY1 contains 54.8 ± 0.12% of protein. Thus, people in Burkina Faso used local ferment as condiment in sauces and to dress some dishes of chicken or pork meat.

Mainly studies have been done on the processing of local beer and fermentation, like technological property of starters from beer and fermentation of sorghum and beer qualities (N'Guessan et al., 2010; Abdoul-latif et al., 2012; Abdoul-latif et al., 2013; N'tcha et al., 2015; Djegui et al., 2016; Songre-Ouattara et al., 2016). It is observed that there is lack of information on hygienic and microbial qualities of local ferment used as starter for fermentation or condiment in sauces. The process used for local fermentation is sometime affected by hygienic conditions and it could be contaminated with food borne pathogens. The source of contamination could come from practice, tools, and producers themselves.

This study aimed to evaluate the hygienic quality of ferment extracted from local beer used in dishes in Burkina Faso. Quality hygiene can prevent certain contamination from pathogenic microorganism and thus enhance quality and safety.

MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Biotechnology, Biological Food and Nutrition Sciences at the Research Center in Biological Food and Nutrition Sciences (CRSBAN / University Ouaga I Pr Joseph KI-ZERBO). The seven ways of processing of local beer are followed in the sites of production like Ouahigouya, Fada N'Gourma, Réo, Bobo Dioulasso, Grarango and Tougan Gaoua in Burkina Faso.

Process of traditional beer and ferment production

An investigation was realized with 70 traditional beer producers and diagram production of traditional beer and ferment (Table 1).

Sample collection

The ferment of local beer was collected from 70 (seventy) sellers of local beer in 7 (seven) localities Ouahigouya, Fada N'Gourma, Réo, Bobo Dioulasso, Grarango, Tougan Gaoua (Table 1). From each producers, one hundred grams (100 g) of local ferment was sampled and maintained at 4°C in isothermal box for laboratory analysis.

Microbiological analysis

Samples microbiological analysis was carried out to determine the levels of aerobic mesophilic bacteria; yeast and molds, total coliform, thermo tolerant coliform including *Escherichia coli*, *Staphylococcus aureus*, *Enterococci*, sulphite reducing bacteria, *Salmonella* spp. and *Shiguella* spp. by standards microbiology system using the methods of De Souza et al. (2011) and Somda et al. (2014). The prevalence rates and levels of presumptive were determined in 70 samples.

Cell numeration

For each sample, 10 g was mixed with 90 mL trypton-salt water (0.85% w/v) and stored at ambient temperature for 30 min. Afterwards, tenfold serial dilutions were made for each suspension and 0.1 mL diluted suspension was used to inoculate specific culture media for microbial isolation and enumeration as recommended by the French Association of Normalization (AFNOR, 2009). The total number of microorganisms expressed as colony forming unit (cfu) per gram of sample was determined by standard plate count. The microorganisms were enumerated under the following conditions:

1. Total aerobic mesophilic bacteria with plate count agar (PCA) after 24 h incubation at 30°C.
2. Total coliforms and thermotolerant coliforms on Eosin Methylen Blue medium (EMB) after 24 h incubation at 37 and 44°C, respectively.
3. Yeasts and molds were counted in a Sabouraud after 3 to 5 days of incubation at 30°C, under aerobic condition.
4. Sulphite reducing bacteria (SRB) by most probably number (MPN) with tryptone-sulfite neomycin broth after 20 h incubation at 44°C.
5. *Salmonella* sp. and *Shigella* sp. were researched in a SS medium after 3 to 5 days of incubation at 37°C.
6. *S. aureus* by counting chapman coagulase positive colonies after 24 h incubation at 37°C.
7. *Enterococci* by counting Enterococcel agar after 24 h incubation at 37°C.

Standard identification methods including Gram stain morphology, colony morphology, production of catalase or oxidase, and lactose

Table 1. Different process steps of traditional beer and ferment production.

Process according to ethnical group	Process steps	Role of steps	Locality
Mossi	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Ouahigouya about 110 of km Ouagadougou
Bissa	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Garango about 200 km of Ouagadougou
Dagara	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Dissine about 389.2 km of Ouagadougou
Samo	a, b , c, e, f, g, h, i, j, l	To obtain alcoholic drink and traditional ferment	Tougan about 214.3 km of Ouagadougou
Bobo	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Bobo-Dioulasso about 351 of Ouagadougou
Gourmatche	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Fada N'Gourma about 221 km of Ouagadougou
Gouroussi	a, b , c, e, f, g, h, i, j, k, l	To obtain alcoholic drink and traditional ferment	Reo about 129.6 km of Ouagadougou

a, Soaking; b, germination; c, drying in the sun; d, mashing and added vegetal mucilage; e, decantation/filtration and residues cooking; g, decantation/filtration; h, mixing with surviving collected; i, cooking; j, cooling; k, alcohol fermentation; l, decantation.

fermentation were used for microorganisms phenotypical identification.

Microbial quality of traditional beer ferment was assayed using the critical limits of French Association of Normalization (AFNOR).

Statistical analysis

Descriptive statistics were established to report the variability of the different parameters involved in the evaluation of the traditional fermentation. Microbial counts were transformed into decimals logarithmic. Log transformed counts of microbiological indicators data were analyzed using factorial analysis of variance between means of microorganism's number with respect to different sources. Correlation between different microbial indicators counted was determined. A p-value of < 0.05 or 0.0001 was considered statistically significant. Associations between microbial groups were performed through Pearson correlation at 5%. The Pearson's correlation coefficients between variables log number of different groups of tested microorganisms in traditional beer ferments samples were calculated. Principal component analysis was performed in order to identify the microbial groups or localities, which are best represented.

RESULTS AND DISCUSSION

Diagram of production of traditional beer and ferment

Traditional beer production is a complex, long process and varies according to the producers, geographic locality and ethnical group. It included mainly three steps such as: malting, brewing and fermentation. The different steps of process used for traditional beer and ferment production are synthetized in Figure 1.

The figure demonstrates the different steps in the local beer production. A similarity was found in the principal points of preparation: malting, brewing and fermentation. The main difference based on ethnical group essentially concerned the frequency of filtration and cooking during mixing and fermentation. Moreover, the mixture of some mucilages of *Abelmochus esculentus*, *Curculigo pilosa*, *Gladiolus klattianus* enzymes or leaves of *Adansonia digitata*, *Boscia senegalensis*, *Grewia bicolor* differed according to the producer. There is no significant difference between the producers of the different localities. The malting process involves soaking, germination and sun drying. Lyumugabe et al. (2012) and Abdoul-latif et al. (2013) showed that malting process allows the activation of synthesis of hydrolytic enzymes (α-amylases, β-amylases, proteases, etc.). During the brewing process, starch and proteins are converted into fermentable sugars and peptides. The alcohol fermentation lasts between 8 and 12 h, and it is produced by the old drink or the old leaven.

This diagram of production has concordance with some authors like Lyumugabe et al. (2012), Abdoul-latif et al. (2013) and Coulibaly et al. (2014) who found three principal steps during beer cooking. Some authors like Abdoul-latif et al. (2013), Atter et al. (2014) and Coulibaly et al. (2014) have added that the disadvantage of "dolo" preparation is lack of time.

Numeration of isolated microorganisms

The main microbial groups were counted and converted to $\log_{10}$ CFU.g^{-1} in analysis of local starter (Table 2). All the 70 samples of local ferment did not contain *Salmonella* sp. and *Shigella* sp. However, all samples contained aerobic mesophilic bacteria, yeast and mold, *S. aureus*, *Enterococci* and sulphite reducing bacteria. Aerobic mesophilic bacteria count ranged from 8.34 to 10.07 $\log_{10}$ (CFU.g^{-1}); yeast and mold from 7.24 to 8.28

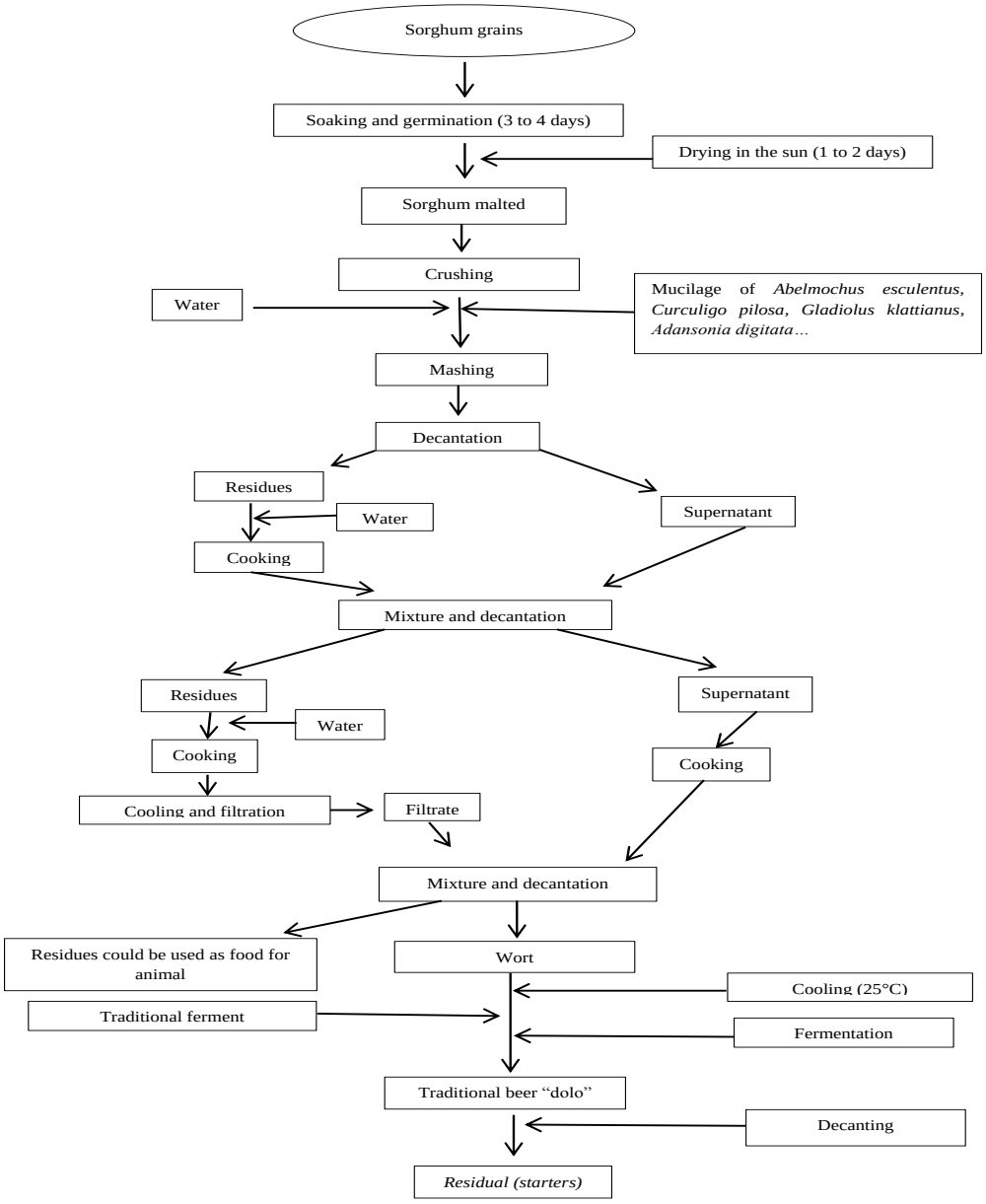

Figure 1. Diagram of production of local beer and starters.

Table 2. Microbial profile in traditional ferment.

Locality	Fada N'Gourma	Ouahigouya	Tougan	Réo	Garango	Gaoua	Bobo Dioulasso	p-value
	Range count of microorganisms per g for all sample, in log_{10} (CFU.g^{-1})							
Mesophilic aerobic bacteria	9.21	9.38	10.07	8.87	8.42	8.34	9.32	<0.0001
Yeasts and molds	8.23	7.82	8.28	7.76	7.24	7.27	7.9	<0.0001
Staphylococcus aureus	4.11	4.52	4.76	4.4	4.41	4.08	4.71	<0.0001
Total Coliform	2.14	2.32	nd	nd	2.22	nd	nd	
Thermo tolerant coliform	nd	nd	nd	nd	nd	nd	nd	<0.0001
Salmonella sp. and *Shigella* sp.	nd	nd	nd	nd	nd	nd	nd	<0.0001
Enterococci	4.17	4.32	3.61	4.1	3.92	3.47	4.31	<0.0001
Sulphite reducing bacteria	<1	<1	<1	<1	<1	<1	<1	<0.0001

nd: Not detected, p <0.0001 indicated statistical significant difference. CFU: colony forming unit.

Table 3. Pearson correlation matrix of prevalence of microorganism's distribution.

Microorganism	Mesophil total bacteria	Yeast and mold	*S. aureus*	Total coliform	*Enterococci*	Sulphite reducing bacteria
Mesophil total bacteria	1					
Yeast and mold	-0.167	1				
Staphylococcus aureus	-0.342	0.683*	1			
Total coliform	-0.420	0.560	0.861*	1		
Enterococci	0.232	0.526	0.647*	0.584	1	
Sulphite reducing bacteria	0.235	0.741*	0.704*	0.486	0.888*	1

*Correlation is significant at the 0.05 level.

$\log_{10}$ (CFU.g^{-1}); *S. aureus* from 4.08 to 4.76 $\log_{10}$ (CFU.g^{-1}) and *Enterococci* from 3.47 to 4.31 $\log_{10}$ (CFU.g^{-1}). Total coliform was enumerated in the samples of Fada N'Gourma, Ouahigouya and Garango with ranging value of 2.14 to 2.32 $\log_{10}$ (CFU.g^{-1}). The presence of sulphite reducing bacteria was observed in all the samples analyzed. The results of microbial control of samples have shown that it did not exceed the critical limit of AFNOR (2009). The local starter is obtained after wort of sorghum fermentation. It is collected as residues in the bottom of jar and dried under the sun.

Numeration of the aerobic mesophilic bacteria on local starter samples showed microbial contamination. A similar result was determined by Atter et al. (2014) who counted 8.41 ± 0.38 $\log_{10}$ (UFC.g^{-1}) after the wort fermentation. There was a large number of aerobic mesophilic bacteria due to lack of hygienic conditions. The presence of yeast in the samples is because it is alcohol fermentation. Indeed, Lyumugabe et al. (2014) counted 8.51 $\log_{10}$ (CUF.g^{-1}) in traditional beer of Rwanda. Djegui et al. (2014), Djêgui et al. (2015), Keita et al. (2016) and N'tcha et al. (2016) showed that yeast and lactic acid bacteria were principal microorganisms in local beer starter. They numerated respectively 8.71, 9.53 and 10.35 $\log_{10}$ (UFC.g^{-1}) of yeast in ferment of Benin and Burkina Faso. The presence of mold in the samples is due to contamination caused by the spores disseminated in the environment.

Total coliform isolated in the samples of Ouahigouya, Garango and Fada N'Gourma was 2.14, 2.22 and 2.32 $\log_{10}$ (CUF.g^{-1}), respectively. Pathogenic germs such as *S. aureus*, *Enterococci* and sulphite reducing bacteria in the samples can induce food toxi-infection. Moreover, sulphite reducing bacteria regroup sporulated bacteria, including *Clostridium* which can cause food damage. *Enterococcus* sp. and *Enterococcus faecium* presence in the samples indicates fecal contamination (Dromigny, 2011).

The results are not in concordance with those of Atter et al. (2014) and Konfo et al. (2014). These did not find total coliform and *Staphylococcus* sp. in traditional beer after fermentation. This is due to the probiotic properties of yeast and lactic bacteria, according to Sawadogo et al. (2008). Moreover, traditional beer is acidic (pH 3 to 4), as mentioned by Glover et al. (2009) and Freire et al. (2014). Consequently, total coliform, *S. aureus*, sulphite reducing bacteria and *Enterococci* presence in the samples pointed to exogenous contamination due to handling during processing and drying.

Similar results of contamination of local food were observed by certain authors in others countries. Indeed, Adjrah et al. (2013) detected total coliform in the ready-to-eat salads in Lomé. Somda et al. (2014) identified *Staphylococcus* sp., *Micrococcus* sp. sulphite reducing bacteria and total coliform in *Soumbala*, a cooked condiment in Burkina Faso. Valenzuela et al. (2016) isolated *Enterococcus faecalis* and *E. faecium* in food fermented in Spain.

Correlation of prevalence of microorganisms

The results of correlation are presented in Table 3. The distribution of microorganisms in the ferments showed positive and negative correlation (p=0.005) among microbial parameters (Table 3). The highest positive correlation was observed between *Enterococci* and sulphite reducing bacteria (r=0.888). SRB also was positively correlated with yeast and mold (r=0.741) and also *S. aureus* (r=0.704). Moreover, *S. aureus* and total coliform were positive correlated (r=0.861). They were also negatively correlated with aerobic mesophil bacteria (r= -0.420).

Principal components analysis

The results of Table 4 show that analysis of principal component has demonstrated a variability of coordinate of microbial parameters found throughout the local ferment analyzed. The cumulative values of the variance of the three principal components (F1, F2 and F3) for the microbial group were 94.312%, with Eigen values ranging between 0.492 and 3.695. Principal component F1 had an Eigen value of 3.695 and contributed to 61.587% of the variation of the parameters. This principal component

Table 4. Coordinate of 7 microbial parameters and their contribution to identification of hygienic quality of the ferment.

Parameters	Principal components		
	F1	F2	F3
Mesophil aerobic bacteria	-0.077	0.785	-0.099
Yeasts and molds	0.426	-0.026	0.787
Staphylococcus aureus	0.479	-0.204	-0.145
Total coliform	0.430	-0.322	-0.430
Enterococci	0.435	0.339	-0.372
Sulphite reducing bacteria	0.457	0.351	0.161
Eigen values	3.695	1.472	0.492
Variance (%)	61.587	24.532	8.194
Cumulative (%)	61.587	86.118	94.312

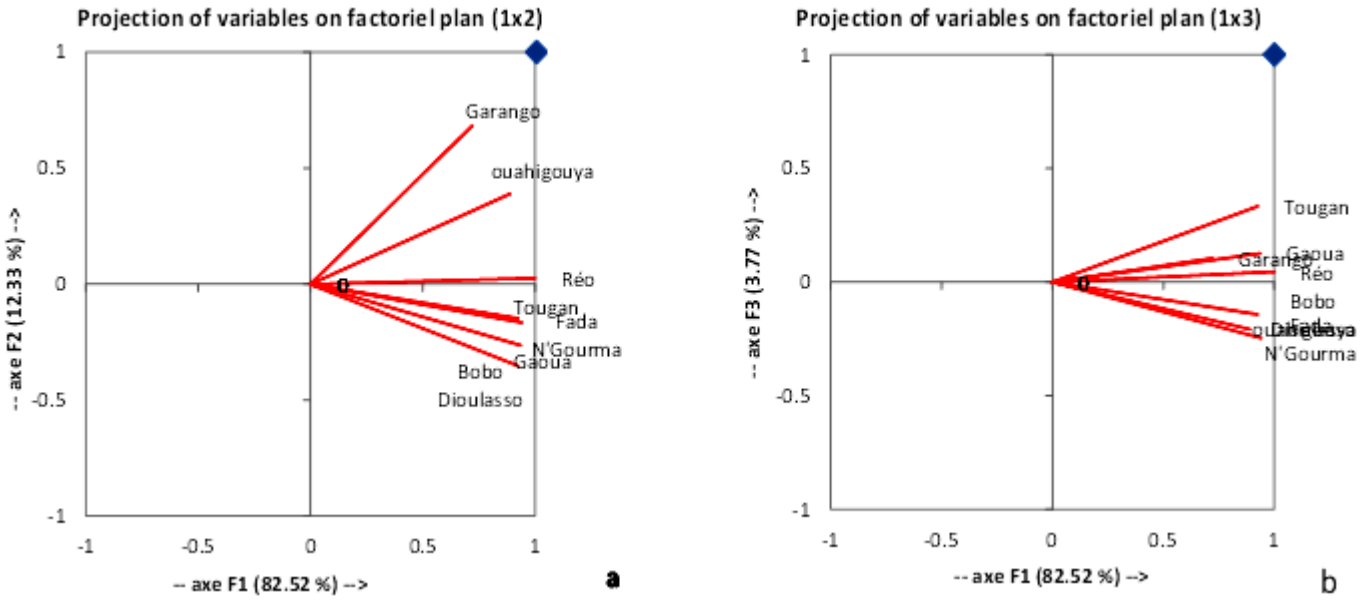

Figure 2. Distribution of locality according to ferments quality on the 1x2 and 1x3 axes of the principal components.

(F1) is associated positively to yeast and mold, *S. aureus*, total coliform, *Enterococci*, sulphite reducing bacteria. Principal components, F2 and F3 had respective Eigen values of 1.472 and 0.492; they contributed 24.532 and 8.194% to the total variation and were associated positively with rate of aerobic mesophilic bacteria, *Enterococci* and sulphite reducing bacteria.

Projection of variables on factorial plan

According to symmetrical scaling of component analysis score, the axis 1×2 and 1×3 explained respectively 94.85 and 86.29% of total inertia. The projection of variables on axis 1x2 showed a regrouping of locality according to samples. The projection plan 1x2 (Figure 2) showed a classification of 2 principal groups according to aerobic mesophilic bacteria and yeasts and molds count in the samples of the different localities. The first group (Ouahigouya, Réo and Garango) correlated positively with the two axes. The second group (Tougan, Gaoua, Bobo Dioulasso and Fada N'Gourma) correlated positively with axis 1 and negatively with axis 2.

The projection plan 1x3 showed two classifications of principal microbial group according to mesophil aerobic bacteria and *S. aureus*. The first group, regrouping (Ouahigouya, Bobo Dioulasso and Fada N'Gourma) correlated positively with axis 1 and negatively with axis 3; and a second group (Réo, Gaoua, Garango and Tougan) correlated positively with the two axes.

Conclusion

The results show that local ferment of “dolo” contained various microorganisms. The plenty number of mesophil total bacteria observed in the ferment samples could be due to lack of hygienic conditions. Pathogenic germs (*S. aureus*, total coliform, sulphite reducing bacteria, etc.) revealed that the process of ferment production need to be ameliorated for suitable and safety exploitation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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