academicJournals

Vol. 14(27), pp. 2227-2233, 8 July, 2015
DOI:10.5897/AJB2014.13895
Article Number: 132CA8B54090
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Phenotypic characters of yeasts isolated from *kpete-kpete*, a traditional starter of a Benin opaque sorghum beer

Kadjogbé Yves Djêgui[1], Adéchola Pierre Polycarpe Kayodé[1]*, Emile Sèdjro Tokpohozin[1], Emma W. Gachomo[2], Simeon O. Kotchoni[2], and Joseph Djidjoho Hounhouigan[3]

[1]Laboratoire de Valorisation et de Gestion de la Qualité de Bio ingrédients Alimentaires, Université d'Abomey-Calavi, 01 BP 526 Cotonou, Bénin.
[2]Department of Biology and Center for Computational and Integrative Biology, Rutgers University, 315 Penn St., Camden, NJ 08102, USA.
[3]Laboratoire de Biochimie Microbienne et de Biotechnologie Alimentaires (LMBA), Faculté des Sciences Agronomiques, Université d'Abomey-Calavi; 01 BP 526; Cotonou, Benin.

Received 2 May, 2014; Accepted 24 November, 2014

Opaque sorghum beers are the most consumed African alcoholic beverages. T*choukoutou* is one of the Benin opaque sorghum beers. Its fermentation process is carried out using a traditional starter called *kpete-kpete*. The present study characterized and identified the yeasts isolated from *kpete-kpete.* A total of 24 samples of *kpete-kpete* were collected from eight different commercial processing sites in Northern Benin. The mean values of the pH, titrable acidity, dry matter content and refractive index for all samples were respectively 3.58; 0.07% as lactic acid; 16.61% and 7.0. The mean counts of yeasts was 9.24 log cfu/ml. Based on their phenotypic characters and their assimilation profiles, 49 yeasts were isolated and found to belong to five genera with seven species. Seventy one percent (71%) of the isolates were identified as *Saccharomyces cerevisiae*.

Key words: Sorghum beer, *tchoukoutou*, *kpete-kpete*, yeast, *Saccharomyces cerevisiae*.

INTRODUCTION

Fermented beverages play a major role in the diet of African people. The most studied African alcoholic beverages are opaque beers often produced from sorghum (Chamunorwa et al., 2002; Jespersen, 2003; Maoura et al., 2005; Lyumugabe et al., 2010; Lyumugabe et al., 2012). Opaque sorghum beers are consumed at various festivals and African ceremonies (for example, marriage, birth, the handing over of a dowry, etc.) and constitute a source of economic return for the women beer producers. They are known as *tchoukoutou* in Benin, *dolo* in Burkina-Faso, *pito* in Ghana and *burukutu*, *otika* or *sekete* in Nigeria, Impeke in Burundi (Odunfa, 1985; Sanni and Lönner, 1993; Kayode et al., 2005). These beers are very rich in calories, B-group vitamins

*Corresponding author. E-mail: polykap@yahoo.fr. Tel: +229 9787073.

and essential amino acids such as lysine (Lyumugabe et al., 2012) and inexpensive. Therefore, they are largely consumed by the poorest people and contribute to their dietary needs (Kayodé et al., 2012). Due to their low alcohol content (2-3% v/v) and the large quantity of suspended solids (5-7%), many consumers consider fermented sorghum beers to be more a food than a beverage (Pattison et al., 1998). The beers are mostly produced at household level or at small industrial scale with varying quality and stability (Sanni and Lönner, 1993; Zulu et al., 1997). Basically, the processing of African opaque sorghum beers involves malting, souring, boiling, mashing, straining and alcoholic fermentation (Kayode et al. 2005; Odunfa, 1985; Haggblade and Holzapfel, 1989). Depending on country and region, variations occur in the beer process (Jespersen, 2003). The fermentation remains a critical step in the process (Kayode et al., 2012). Beneficial effects of fermentation include improvement of flavor and texture, reduced loss of raw materials, reduced cooking time, improved bio-availability of micronutrients and elimination of toxic and anti-nutritional factors (Sanni and Lönner, 1993; Iwuoha and Eke, 1996; Padmaja, 1995; Sindhu and Khetarpaul, 2001).

In Benin, Lactic acid bacteria and yeasts have been reported (Kayodé et al., 2007) to be the major microorganisms involved in the fermentation of *tchoukoutou*. Kpete-kpete is the traditional starter used for the fermentation of *tchoukoutou*. Based on its fermenting properties, producers use it to ferment the sorghum wort during the manufacturing process. It is generally harvested from the bottom of a previous fermenting beer resulting from 13 to 14 h overnight fermentation. However, the microorganisms contained in *kpete-kpete* used for the fermentation of *tchoukoutou* have not yet been investigated. Especially, works reporting on the species of yeasts contained in such starter are hard to come by. The increasing interest for moving from uncontrolled conditions towards regulated processing conditions, and thus ensuring quality safety and product stability, makes the application of starter cultures and thereby identification and classification of the strains involved necessary (Van der Aa Kühle et al., 2001).

The present study was conducted to determine the physicochemical and microbiological characteristics of the traditional starter *kpete-kpete*, and to identify the different species of yeasts involved using phenotypic analysis tools.

MATERIALS AND METHODS

Sampling

Twenty four (24) samples (500 mL) of *kpete-kpete*, the traditional starter of *tchoukoutou*, were collected from eight of the most important production sites of *tchoukoutou* in northern Benin. The processors (one per site) were selected on the basis of their rich beer brewing tradition. The samples were collected in screw-capped bottles, packed in an insulated icebox, transported to the laboratory and analyzed immediately for microbiological analysis (Hounhouigan et al., 1993).

Physico-chemical analysis

Dry matter was determined according to the AACC method (AACC, 1984). The pH was determined using a digital pH meter (HI 8418; Hanna instruments, Limena, Italy) calibrated with buffers at pH 4.0 and 7.0 (WTW, Weilheim, Germany). The titratable acidity, expressed as lactic acid, was performed by using the method described by Nout et al. (1989). The refractive index was measured using a refracto meter (Sopelem 9596, France).

Enumeration of yeasts

Duplicate samples of "*kpete-kpete*" (10 mL) were diluted in 90 mL sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1000 mL distilled water, pH = 7.0) and homogenized with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total count of yeasts was determined on oxytetracyclin glucose yeast extract agar (OGYA, Oxoid CM 0545, Basingstoke, Hampshire, England) containing oxytetracycline (Hounhouigan et al., 1993). Forty nine (49) yeast strains were obtained and subjected to morphological, fermentation and assimilation tests. Prior to these tests, a preliminary microscopic confirmation was performed.

Identification of yeast

The identification of yeast strains was performed according to the method described by Yarrow, (1998) and Kurtzman et al. (2011). The isolates from eight representative sites were purified by successive sub-culturing on oxytetracycline glucose yeast agar (OGYA, CM0545, Basingstoke Hampshire, England) made selective by addition of oxytetracycline. Preliminary confirmation was based on microscopic observation. The isolates were tested for the fermentation of sucrose, lactose, glucose and raffinose, as well as the assimilation of selected nitrogen sources that is, nitrate, ethylamine, L-lysine, cadaverine and creatine. The assimilation of carbon sources was performed using API 20 C AUXstrips (BioMérieux, Lyon, France) according to the manufacturer's instructions. The diazonium blue B reaction, a test to differentiate between ascomycetous and basidiomycetous yeasts, was performed as described by Kurtzman et al. (2003).

Data analysis

For the analytical data, mean values as well as standard deviation are reported. The data were analysed using the statistical program, SPSS 11.0. The on-line available software (http:www.cbs.knaw.nl) of Centraal bureauvoor Schimmel cultures (Central Bureau of Fungal Cultures), Utrecht, the Netherlands was used for identification of yeasts.

RESULTS AND DISCUSSION

Physico-chemical characteristics and yeast content of *kpete-kpete*

The mean value of yeast counts was 9.24 log cfu/mL

Table 1. Physicochemical and microbiological characteristics of the traditional starter kpete-kpete.

Samples origin	Yeasts (log cfu/g)	pH	Titratable acidity (% lactic acid)	Dry matter (%)	Refractive index
Boukoumbé	9.53±0.05^{ac}*	3.22±0.09^{b}	0.09±0.01^{a}	13.18±1.93^{a}	8.0±1.0^{a}
Djougou	9.51±0.59^{ac}	3.44±0.16^{ab}	0.07±0.01^{a}	16.50±4.18^{a}	8.0±2.0^{a}
Natitingou	8.64±0.04^{b}	3.56±0.11^{ab}	0.08±0.02^{a}	15.78±4.97^{a}	7.0±2.0^{a}
Toucountouna	9.88±0.19^{c}	3.71±0.16^{a}	0.06±0.02^{a}	12.95±3.46^{a}	7.0±2.0^{a}
Tchaourou	9.35±0.09^{ac}	3.54±0.18^{ab}	0.06±0.06^{a}	20.18±2.03^{a}	5.0±2.0^{a}
Parakou	8.92±0.30^{ab}	3.77±0.26^{a}	0.07±0.02^{a}	18.10±2.37^{a}	9.0±2.0^{a}
Pèrèrè	9.11±0.13^{ab}	3.58±0.12^{ab}	0.08±0.01^{a}	15.39±1.95^{a}	5.0±1.0^{a}
N'Dali	8.95±0.33^{ab}	3.79±0.22^{a}	0.07±0.03^{a}	18.61±4.38^{a}	8.0±1.0^{a}
Means	9.24	3.58	0.07	16.61	7.0
^{a}CV (%)	4.37	5.26	14.79	15.64	20.44

^{a}Coefficient of variation, *Values with the same letter in the same column are not significantly different (P< 0.05)

(Table 1). The yeast concentration in *kpete-kpete* is higher than the counts of yeast (7.8-8.5 log ufc/g) in the sorghum beer *tchoukoutou* as reported by Kayodé et al. (2006). That difference could be due to the fact that there is a significant difference between the dry matter of both products: 16.6% for the kpete-kpete and 10.0% for *tchoukoutou* (Kayodé, 2006). Data from Table 1 show there is a significant difference ($p < 0.05$) between the counts of yeasts from the eight communities. Previous studies performed on African opaque sorghum beers established that the frequencies of microbial species vary according to the region and the ingredients used for the brewing (Demuyakor and Ohta, 1991; Ekundayo, 1969; Faparusi et al., 1973; Nout, 1980; Odunfa, 1985; Sanni and Lönner, 1993; Sefa-Dedeh et al., 1999).

Mean values of pH was 3.58. Data analysis showed there is a significant ($p < 0.05$) difference between samples from the various production sites. However, there is no significant difference ($p<0.05$) for the titratable acidity, dry matter and refractive index between the samples from the eight areas. On the basis of the titratable acidity, the dry matter and the refractive index, the starters collected from various sites appear to be similar. However, the different starters seem different on the basis of their yeasts content and their pH values.

Phenotypic characteristics of yeasts isolates

Results (Table 2) show that a minority of the strain could ferment raffinose (24.5%), whereas the great majority fermented sucrose (85.7%) and glucose (100%); but none of the isolates could ferment lactose. These results are in close agreement with data reported by Kayode et al. (2011) for yeast strains isolated from tchoukoutou. The nitrogen assimilation test revealed that a minority of the isolates assimilated ethylamine (16.3%), L-lysine (32.7%) and cadaverine (42.9%) whereas the majority assimilated sulfate of ammonium (67.3 %) and nitrate (55.1%). The diazonium blue B test (Table 2) revealed that 22.4% of the isolates were basidiomycetous whereas 77.6% were ascomycetous. On the basis of their fermentation profile and the nitrogen assimilation pattern, the 49 yeasts could be grouped into 18 distinct clusters. 14.3% were in the first cluster, 8.2% were in the second cluster, 8.2% in the fourth cluster and the rest are distributed in the 15 other clusters.

Assimilation profile and identification of yeasts isolates

On the basis of their assimilation of carbon compounds, seventeen assimilation profiles were distinguished (Table 3). The majority of yeasts assimilated glucose (100%), galactose (93.9%) and maltose (79.6%); many assimilated acetyl-glucosamine (51%), saccharose (46.9%), palatinose (30%), glycerol (32.7%), xylose (36.7%), lactic acid (38.8%), xylose (36.7%), glycerol (32.7%), palatinose (30.6%); some of yeasts assimilated potassium gluconate (26.5%) methyl-αD-glucopyranoside (22.4%), lactose (20.4%), sodium glucuronate (18.7%), trehalose (18.4%), mannitol (18.4%), levulinic acid (18.4%); only a few of yeasts could assimilate erythritol (2%), sorbose (4.1%), potassium-2- cetogluconate (6.1%), arabinose (8.2%), glucosamine (8.2%). None of them assimilated actidione, cellobiose, rhamnose (Table 3). Based on their phenotypic characteristics, the 49 yeasts were found to belong to five genera and seven species of yeasts. These are *Saccharomyces cerevisiae* (71.4%), *Sporobolomyces odoratus* (12.2%), *Candida pseudorhagii* (6.1%), *Candida heliconiae (4.1%)*, *Schizosaccharomyces octosporus* (2%), *Schizosaccharomyces pombe* (2%) et *Zygosaccharomyces rouxii* (2%) (Figure 1). The diversity of yeast strains contained in "*kpete-kpete*" can be

Table 2. Phenotypic characters of yeasts isolated from traditional starter kpete-kpete.

Cluster	Isolates numbers	Fermentation				Assimilation of nitrogen source					DBB test[2]
		Glu	Lac	Suc	Raf	Nit	Eth	Lys	Cad	SAM	
I	27, 40, 41, 43, 45, 47, 49	+	-	+	-	-	-	-	-	-	+
II	1, 26, 34, 37	+	-	+	+	+	+	+	+	+	-
III	6,7, 14, 18	+	-	+	-	+	-	-	-	+	-
IV	23, 38, 46, 48	+	-	+	-	-	-	-	-	-	-
V	5, 17, 25	+	-	-	-	-	-	-	-	-	-
VI	12, 13, 39	+	-	+	-	+	-	-	+	+	-
VII	4,20, 22	+	-	+	-	+	-	+	-	+	-
VIII	31,32, 36	+	-	+	+	+	-	-	+	+	-
IX	2,15	+	-	-	-	+	+	+	+	+	-
X	3,11	+	-	+	+	-	-	+	+	-	+
XI	8, 9	+	-	+	-	+	-	-	+	+	-
XII	10, 16	+	-	-	-	+	-	+	-	+	-
XIII	21, 42	+	-	+	-	-	-	-	+	+	-
XIV	28, 35	+	-	+	-	+	+	+	+	+	+
XV	24, 44	+	-	+	-	-	-	-	-	+	-
XVI	30, 33	+	-	+	+	+	-	-	-	+	-
XVII	19	+	-	+	-	-	-	-	+	+	-
XVIII	29	+	-	+	+	-	-	+	-	+	-
Frequency (%)		100	0	85.7	24.5	55.1	16.3	32.7	42.9	67.3	22.4

[1]Glu = glucose, Lac = lactose, Suc = sucrose, Raf = rafinose, Nit = nitrate, Eth = ethylamine, Lys = L-lysine, Cad = cadaverine, SAM = sulfate of ammonium [2]DBB = diazonium blue B.

Table 3. Assimilation profiles of yeasts isolated from traditional starter *kpete-kpete*.

Parameter	a*	b	c	d	E	f	g	h	i	j	k	l	m	n	o	p	q	Total (%)
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Glycerol	-	-	-	-	+	-	+	+	-	+	+	+	-	+	+	+	-	32.7
Potassium 2-cétogluconate	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	6.1
L-arabinose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	8.2
D - xylose	-	-	+	-	+	+	-	-	-	+	+	+	-	+	-	+	+	36.7
D - galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	93.9
Actidione (Cycloheximide)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
D - sacharrose	-	-	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	46.9
N-Acétyl - Glucosamine	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	51.0
Lactic acid	-	-	+	-	-	-	+	+	-	+	+	+	-	+	+	+	+	38.8
D - cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
D - Raffinose	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	14.3
D - maltose	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	-	79.6
D - trehalose	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	-	-	18.4
Methyl - αD-glucopyranoside	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	+	-	22.4
D -mannitol	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	18.4
D - lactose	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	20.4
Inositol	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.2
D - sorbose	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	12.2

Table 3. Contd.

D - ribose	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+	16.3
L - Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Palatinose	-	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	-	30.6
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	2.0
D - melibiose	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	10.2
Sodium glucuronate	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	18.7
D - melezitose	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	14.3
Potassium gluconate	+	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	26.5
Levulinic acid	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	18.4
L - sorbose	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	4.1
Glucosamine	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	8.2
Sulfate of ammonium	-	-	-	-	-	-	+	-	-	+	+	-	-	+	-	-	-	14.3
Nb of isolate (%)	18.4	16.3	10.2	8.2	6.1	6.1	4.1	4.1	4.1	4.1	4.1	4.1	2.0	2.0	2.0	2.0	2.0	

***a** = isolates 9, 20, 27, 40, 41, 44, 45, 47, 49; **b** = 15, 26, 28, 29, 34, 35, 37, 38; **c** = 7, 11, 12, 13, 39; **d** = 30, 31, 32, 33 ; **e** = 3, 36, 43 ; **f** = 42, 46, 48; **g** = 1, 14 ; **h** = 2, 4 ;**i** = 5, 8; **j** = 6,17; **k** = 10, 6; **l** = 18, 19; **m** = 21; **n** = 22; **o** = 23; **p**=24; **q** = 25; Nb = number.

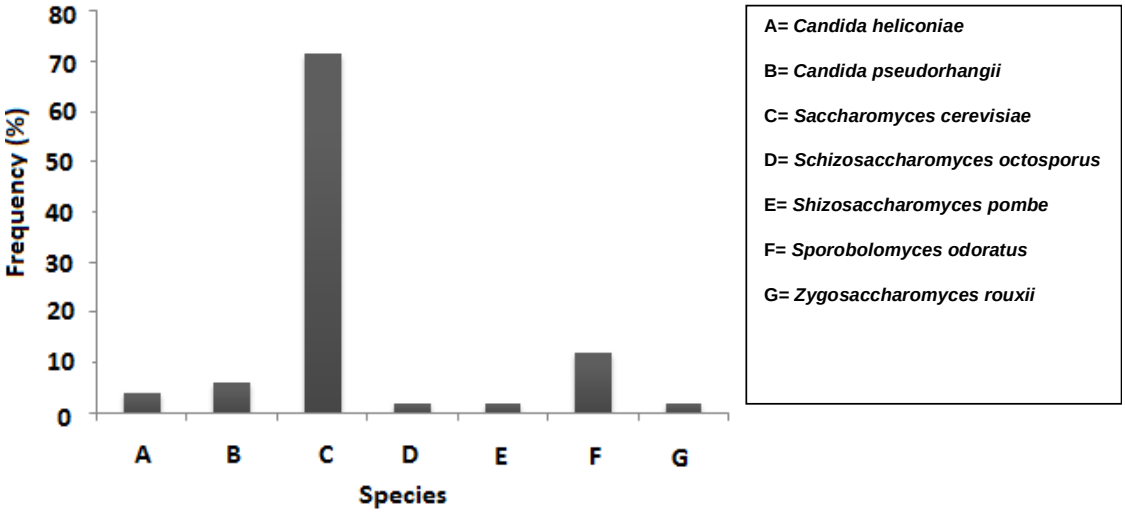

Figure 1. Frequency distribution of yeast species contained in the traditional starter kpete-kpete

explained by the fact that in Africa, traditional fermented products result from spontaneous fermentation and as a result, both desirable and non-desirable strains are present in the product (Lyumugabe et al., 2012).

S. cerevisiae was found as being the predominant yeast strain in the traditional starter *kpete-kpete*. These findings are in accordance with previous studies. Konlani et al. (1996) found that *S. cerevisiae* accounted for 55-90% of yeast population in samples of sorghum beer originated from Togo and Burkina Faso. Likewise, Demuyakor and Ohta (1991) ; Van der Aa Kühle et al. (2001); Sanni and Lönner (1993), have respectively reported the predominance of *S. cerevisiae* in the traditional sorghum beers from Nigeria, Burkina-Faso and Ghana. Also, in an opaque sorghum beer from Northen Ghana, Glover et al. (2005) identified 72% of 247 isolates as *S. cerevisiae* on the basis of their carbon and nitrogen compounds assimilation profiles. Moreover, N'guessan et al. (2011) investigated 240 yeast strains isolated from fermenting sorghum wort inoculated with yeast. In this study 87.36 % of strains are found to be *S. cerevisiae*. To be accepted as *S. cerevisiae*, the isolate should be able to assimilate glucose, sucrose, maltose, raffinose and ethanol (Vaughan-Martini and Martini, 1998). In the present study, some of the isolates could not assimilate all of these sugars. In spite of that, they were identified as *S. cerevisiae*. Many isolates from Ghanain and Burkina-Faso sorghum beers were identified as *S. cerevisiae* by Demuyakor and Ohta (1991) and Van der Aa Kühle et al. (2001), and yet, these microorganisms showed carbon assimilation profiles different from the taxonomical key proposed by Vaughan-Martini and Martini (1998). Like in our study, some of the isolates analyzed by theses authors could not assimilate sucrose, raffinose and

trehalose.

Researches on improvement of traditional sorghum beers revealed that *S. cerevisiae* is of a vital importance for making an effective starter culture. Sefa-Dedeh et al. (1999) used a pure culture of *S. cerevisiae* and a mixture culture consisting of *S. cerevisiae* and other strains such as Kloeckera apiculata and Candida tropicalis, to produce a pito beer containing high ethanol content. Also, Orji et al. (2003) found that *S. cerevisiae* in combination with Lactobacillus plantarum, as a starter culture, also led to the satisfactory production of a pito beer. N'Guessan et al. (2010) successfully used *S. cerevisiae* in combination with C. tropicalis as starter cultures for the alcoholic fermentation of the tchapalo beer.

Conclusion

S. cerevisiae was identified as the predominant specie of yeast in the traditional starter *kpete-kpete* on the basis of the phenotypic characterization. In order to refine the identification process, the molecular characterization is found to be necessary. This would be an important step towards the elaboration of a starter culture for the fermentation of African opaque sorghum beers. Such approach would lead to an improvement of the fermentation process and the quality of local African beers.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

AACC (1984). Approved methods of the American Association of cereal chemistry. American Association of Cereal Chemistry, St. Paul, MN.

Chamunorwa AT, Feresu SB, Mutukumira AN (2002). Identification of lactic acid bacteria isolated from opaque beer (*Chibuku*) for potential use as a starter culture. J. Food Technol. Afr. 7: 93-97.

Demuyakor B, Ohta Y (1991). Characteristics of pito yeasts from Ghana. Food Microbiol. 8:183-193.

Ekundayo JA (1969).The production of pito, a Nigerian fermented beverage. J. Food Technol. 4:217-225.

Faparusi SI, Olofinboba FO, Ekundayo JA (1973).The microbiology of burukutu beer. Z. Allg. Mikrobiol. 13: 563-568.

Glover RLK, Abaidoo RC, Jackobsen M, Jespersen L (2005). Biodiversity of Saccharomyces cerevisae isolated from a survey of pito production sites in various parts of Ghana. Syst. Appl. Microbiol. 28:755-761.

Haggblade S, Holzapfel H (1989).Industrialization of indigenous fermented foods. Steinkraus K.H. Ed. Marcel Dekker In. New York. p. 91.

Hounhouigan DJ, Nout MJR, Nago CM, Houben JH, Rombouts FM (1993). Microbiological changes in mawe during natural fermentation. World J. Microbiol. Biotechnol. 10:410-413.

Iwuoha CI, Eke OS (1996). Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. Food Res. Int. 29:527-540.

Jespersen L (2003).Occurrence and taxonomic characteristics of strains of Saccharomyces cerevisiae predominant in African indigenous fermented foods and beverages. FEMS Yeast Res. 3: 191-200.

Kayode APP, Adegbidi A, Linnemann AR, Nout MJR, Hounhouigan DJ (2005).Quality of farmer's varieties of sorghum and derived foods as perceived by consumers in Benin. Ecol. Food Nutr. 44:271-294.

Kayode APP, Deh DC, Baba-Moussa L, Kotchoni SO, Hounhouigan JD (2012).Stabilisation and preservation of probiotic properties of the traditional starter of African opaque sorghum beers. Afr. J. Biotechnol. 11 (30):7725 -7730.

Kayode APP, Vieira-Dalodé G, Linnemann AR, Kotchoni SO, Hounhouigan AJD, van Boekel MAJS, Nout MJR (2011). Diversity of yeasts involved in the fermentation of tchoukoutou, an opaque sorghum beer from Benin. Afr. J. Microbiol. Res. 5 (18):2737-2742.

Konlani S, Delgenes JP, Moletta R, Traore A, Doh A (1996). Isolation and physiological characteristics of yeasts involved in sorghum beer production. Food Biotechnol.10: 29-40.

Kurtzman CP, Boekhout T, Robert V, Fell JW, Deak T (2003). Methods to identify yeasts. In: Boekhout T, Robert V (Eds), Yeasts in Food: Beneficial and detrimental aspects. Hamburg: B. Berhr'sVerlag GmbH and Co. KG. pp. 69-121.

Kurtzman CP, Fell JW, Boekhout T, Robert V (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW & Boekhout T (eds). The Yeasts, a Taxonomic Study, 5th edn. Elsevier. pp. 87-110.

Lyumugabe F, Gros J, Nzungize J, Bajyana E, Thonart PH (2012). Characteristics of African traditional beers brewed with sorghum malt: a review. Biotechnol. Agron. Soc. Environ. 16 (4): 509-530.

Lyumugabe F, Kamaliza G, Bajyana E, Thonart PH (2010). Microbiological and physic-chemical characteristics of Rwandese traditional beer ''Ikigage''. Afr. J. Biotechnol. 9:4241-4246.

Maoura N, Mbaiguinam M, Nguyen HV, Gaillardin C, Pourquie J (2005). Identification and typing of the yeast strains isolated from *bili bili*, a traditional sorghum beer of Chad. Afr. J. Biotechnol. 4: 646-656.

N'guessan FK, Brou K, Jacques N, Casaregola S, Dje KM (2011). Identification of yeasts during alcoholic fermentation of Tchapalo, a traditional sorghum beer from Côte d'Ivoire. Anton Leeuw Int. J. G. 99:855-864.

N'guessan FK, N'diri DY, Camara F, Djè MK (2010). *Saccharomyces cerevisiae* and *Candida tropicalis* as starter cultures for the alcoholic fermentation of *tchapalo*, a traditional sorghum beer. World J. Microbiol. Biotechnol. 26:693-699.

Nout MJR (1980). Microbiological aspects of the traditional manufacture of Busaa, a Kenyan opaque maize beer. Chem. Mikrobiol. Technol. Lebensm. 6: 137 - 142.

Nout MJR, Rombouts FM, Havelaar A (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic micro-organisms. Int. J. Food Microbiol. 8:351-361.

Odunfa SA (1985). African fermented foods. In Wood, BJB.(ed.), Microbiol. Fermented Foods. ELS Appl Sc., London, UK. pp. 167 - 195.

Orji MU, Mbata TI, Anich GN, Ahonkhai I (2003). The use of starter cultures to produce "*pito*", a Nigerian fermented alcoholic beverage. World J. Microbiol. Biotechnol. 19: 733-736.

Padmaja G (1995). Cyanide detoxification in cassava for food and feed uses. Crit. Rev. Food Sci. Nutr. 35: 299 - 339.

Pattison TL, Geornaras I, von Holy A (1998). Microbial populations associated with commercially produced South African sorghum beer as determined by conventional and Petrifilm plating. Int. J. Food Microbiol. 43: 115 -122.

Sanni AI, Lönner C (1993). Identification of yeast isolated from Nigerian traditional alcoholic beverages. Food Microbiol. 10: 517-523.

Sefa-Dedeh S, Sanni AI, Tetteh G, Sakyi-Dawson E (1999). Yeasts

in the traditional brewing of pito in Ghana. World J. Microbiol. 15:593-597.
Sindhu SC, Khetarpaul N (2001). Probiotic fermentation of indigenous food mixture: effects on antinutrients and digestibility of starch and protein. J. Food Comp. Anal. 14: 601-609.
van der Aa Kühle A, Jesperen L, Glover RLK, Diawara B, Jakobsen M (2001).Identification and characterization of Saccharomyces cerevisiae strains isolated from West African sorghum beer. Yeast 18:1069-1079.
Vaughan-Martini A, Martini A (1998).Saccharomyces Meyen ex Reess. In: Kurtzman CP, Fell JW (eds.). The Yeasts, A Taxonomic study. ELS. Sc.: Amsterdamj, pp. 358-371.
Yarrow D (1998). Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP & Fell JW (eds) The Yeasts. A Taxonomic Study, 4th edn. Elsevier, 418 Amsterdam, pp. 77-100.
Zulu RM, Dillon VM, Owens JD (1997).Munkoyo beverage, a traditional Zambian fermented maize gruel using Rhynchosia root as amylase source. Int. J. Food Microbiol. 34:249-258.