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Diversity of yeasts in otchè, a traditional starter used in fermentation of an opaque sorghum beer "chakpalo"

Yves Kadjogbé Djegui[1], Adéchola Pierre Polycarpe Kayodé[1]*, Raymond A. Atchadé[1], Emma W. Gachomo[2], Simeon O. Kotchoni[2] and Joseph Djidjoho Hounhouigan[1]

[1]Département de Nutrition et Sciences Alimentaires, Faculté des Sciences Agronomiques, 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.

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Sorghum beer is a significant diet component of millions of poor rural communities in sub-Saharan Africa. In this study, we identified and biochemically characterized yeast strains isolated from otchè, a traditional starter used in fermentation of chakpalo, an opaque sorghum beer in Benin. 12 samples of otchè were collected from 12 different commercial processing sites. The mean values of pH, titratable acidity, dry matter content and refractive index of the starters analyzed were 3.37, 0.17 (% as lactic acid), 7.15 and 7.0%, respectively. The mean yeast count per sample was 8.72 log cfu/ml. Based on phenotypical and biochemical characterization (carbon and nitrogen assimilation) profile, 50 yeast strains were identified and found to belong to five genera and ten species. *Saccharomyces cerevisiae* was found to be the most predominant yeasts species of otchè.

Key words: Sorghum, beer, chakpalo, otchè, yeast, *Saccharomyces cerevisiae*.

INTRODUCTION

Opaque beers are mostly prepared from Guinea corn (*Sorghum bicolor*) and sometimes from other cereals such as millet and maize (generally used as adjunct or substitutes) (Kayode et al., 2005). Opaque beers are popular alcoholic beverage in the northern Guinea Savannah region of West Africa. They are known as tchoukoutou and chakpalo in Benin, dolo in Burkina-Faso, pito in Ghana and burukutu or otika in Nigeria (Kayodé et al., 2005; Odunfa, 1985). Largely consumed by the poorest rural community in these regions, the beverage plays a significant role in the diet of millions of the consumers (Kühle et al., 2001; Jespersen, 2003). The nutritional attributes of several commercial sorghum beers have been reported to contain significant amount of protein, ash, carbohydrate, iron (Fe) and zinc (Zn) (Novellie and De Schaepdrijver, 1986). This justifies a renewed interest for the beverage by the community. It is generally viewed as a significant source of dietary nutrients, considering the large quantity of daily consumption by the people (Briggs et al., 2004).

*Corresponding author. E-mail: polykap@yahoo.fr. Tel: +229 97870734.

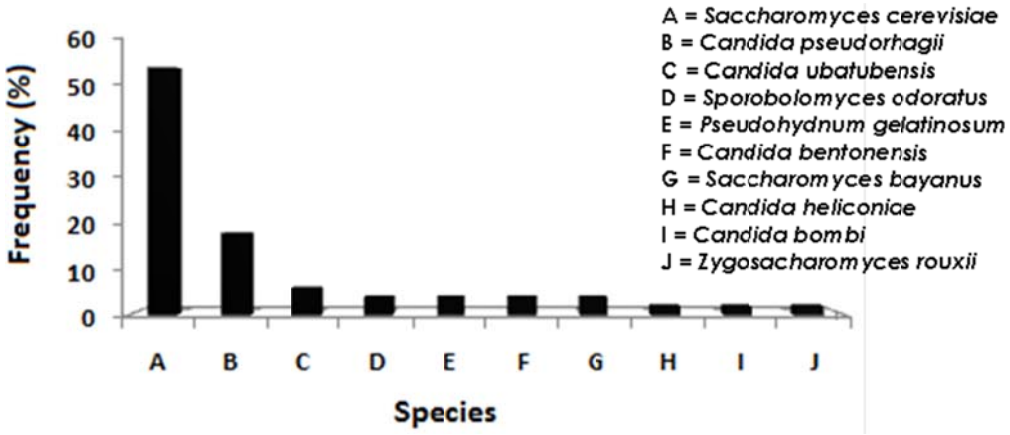

Figure 1. Frequency distribution of yeast species involved in otchè.

In Benin, the beverage is produced by women through a three-phases based process: malting, mashing and fermentation (Pattison et al., 1998; Kayode et al., 2012). The fermentation step is the most important step of the process. The fermentation aspect of the process has several beneficial effects, which include reduced loss of raw materials, improvement of protein quality and carbohydrate digestibility, improved bioavailability of micronutrients and elimination of toxic and anti-nutritional factors such as cyanogenic glycosides (Sanni and Lönner, 1993; Iwuoha and Eke, 1996; Padmaja, 1995; Sindhu and Khetarpaul, 2001). The success of the fermentation depends, among other factors, on the quality of the fermentation starter. Traditional starters of African opaque beers have been reported to contain mainly yeasts and lactic acid bacteria (Van der Aa Kühle et al., 2001; Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999; Sanni and Lönner, 1993). In addition, the yeast strains have been reported to be involved in several different types of beverage processes (Zulu et al., 1997; Torner et al., 1992; Gadaga et al., 1999). To date, no study on “otchè”, the traditional starter of chakpalo, has been carried out. Therefore, it is necessary to assess physicochemical, biochemical and microbial characteristics of this traditional starter.

In order to establish the microbial diversity population of otchè, we carried out in this study a detail microbial screening of different geographically collected otchè from Benin and further determined their physicochemical properties, fermentation ability and carbon assimilation sources.

MATERIALS AND METHODS

Sample collection

Twelve samples of otchè were collected from twelve commercial processing sites in the center regions of Benin (Figure 1). The processors (beverage processing personnel; one per site) were selected based on their well-established brewing traditional skills. The samples were collected and kept in sterile screw-capped bottles, packed in insulated iceboxes, transported to the laboratory and immediately screened for microbial diversity population (Hounhouigan et al., 1993).

Physicochemical property analysis of ochè

In order to characterize the physicochemical properties of the starter, we first determined the dried matter of the samples using 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 acidity titration, expressed as lactic acid, was carried out using the method described by Nout et al. (1989). The refractive index was measured using a refractometer (Sopelem 9596, France).

Determination of yeast population density

To quantitatively determine the microbial community, duplicate samples of “otchè” (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 as described previously (Hounhouigan et al., 1993). Total counts of yeast population were determined as described previously (Hounhouigan et al., 1993).

Identification of yeast strains

The identification of microbial (yeast) strains was performed according to the current guidelines (Yarrow, 1998; Kurtzman et al., 2011). The isolates from twelve representative collection sites were purified using successive sub-culturing method on oxytetracycline glucose yeast agar (OGYA, CM0545, Basingstoke Hampshire, England), where oxytetracycline was used as selection marker. Additionally, microscopic observation was carried out to ascertain the identification of microbial strains. The isolates were then tested for fermentation ability on sucrose, lactose, glucose and raffinose. They were also tested for nitrogen assimilation properties on selected nitrogen sources such as nitrate, ethylamine, L-lysine, cadaverine and creatine. The assimilation of carbon sources was performed using API 20 C AUX strips (BioMérieux, Lyon, France) according to the manufacturer’s instructions. The Diazonium Blue B

Table 1. Physico-chemical characteristics and yeasts content of the starter of chakpalo.

Samples origin	pH	Titratable acidity (% lact. acid)	Dry matter (%)	Refractive index	Yeasts (log cfu/g)
Bantè (n= 3)	3.58 ± 0.08b*	0.07 ± 0.02a	9.68 ± 0.76b	11 ± 1b	8.76 ± 1.1a
Dassa (n= 3)	3.32 ± 0.14a	0.12 ± 0.02a	5.34 ± 0.99a	5 ± 0a	9.41 ± 0.48a
Glazoué (n= 3)	3.34 ± 0.18a	0.09 ± 0.03a	9.48 ± 1.77b	5 ± 1a	9.21 ± 0.07a
Savè (n = 3)	3.24 ± 0.15a	0.41 ± 0.35b	4.08 ± 0.68a	5 ± 0a	7.51 ± 0.06b
Mean	3.37 ± 0.18	0,17 ± 0,22	7.15 ± 2.74	7 ± 3	8.72± 0.92
[a]CV (%)	4.4	92.6	40.0	46.2	9.8

[a]Coefficient of variation.*Values with the same letter in the same column are not significantly different (P < 0.05).

(DBB) reaction, a test to differentiate between ascomycetous and basidiomycetous yeasts, was performed as described by Kurtzman et al. (2011).

Statistical data analysis

For statistical data analysis, mean values and standard deviation are reported. The data were analyzed using the SPSS 11.0 statistical program. The on-line available software (http:www.cbs.knaw.nl) of Central bureau voor Schimmelcutures, Utrecht, the Netherlands was used for identification of yeasts.

RESULTS

Properties of otchè and number of yeast

Table 1 shows physico-chemical characteristics of a number of yeasts found in otchè collected from different sampling sites. The mean pH value of the samples analyzed was 3.37 ± 0.18. Data analysis showed that there is no significant difference between samples from Dassa, Glazoué and Savè. Samples collected from Bantè had the highest ($p<0.05$) pH value. The average titratable acidity value of otchè was 0.17 (% as lactic acid). The acidity of otchè collected from Savè was significantly ($p<0.05$) higher than those of the samples collected from the other sites. The average dry matter of the traditional starter used for the fermentation of chakpalo was 7.15%. The average refractive index was 7.0 (Table 1). The lowest values of the refractive index were obtained from samples collected from Dassa, Glazoué and Savè, while the highest was from samples collected from Bantè.

The mean yeast count per traditional starter was 8.72 $\log_{10}$ cfu/ml. There was no significant ($p>0.05$) difference between the yeast content of otchè from Dassa, Bantè and Glazoué (Table 1). But the yeast content of otchè from Savè is significantly ($p<0.05$) lower than those from other sites.

Phenotypic characteristics of yeast isolates

All the 50 yeast strains were subjected to microscopic observation to further confirm the microbial identity before carrying out the fermentation and assimilation tests. According to Table 2, only a few isolates could ferment lactose (4%) and raffinose (28%) whereas the majority of the strains fermented glucose (98%) and sucrose (88%). Most of the isolates assimilated ethylamine (82%), nitrate (80%), creatine (80%), L-Lysine (76%), cadaverine (66%). The Diazonium Blue B test revealed that 10% of the isolates were basidiomycetous, whereas 90% were ascomycetous (Table 2). On the basis of their fermentation profile and their nitrogen assimilation pattern, the yeast strains were grouped into 25 distinct clusters based on their biochemical characteristics. 32% of the yeast strains were in the first cluster, 6% were in the second cluster, while the remaining strains were diversely grouped into the 23 other clusters as shown in Table 2.

Assimilation of carbon compounds by the yeast isolates

Based on the carbon utilization, thirty three assimilation profiles were distinguished. All yeasts assimilated glucose (100%), the majority of strains assimilated galactose (94%), saccharose (90%) and maltose (88%). A relative majority of the strains assimilated D-raffinose (68%) and palatinose (54%). Fourty eight percent assimilated D-trehalose and N-aceltyl-glucopyranoside, 44% assimilated L-arabinose and potassium 2-cétogluconate, 40% assimilated Cycloheximide (Actidione), Lactic Acid and D-mannitol; 38% assimilated Methyl-αD-glucopyranoside D-Xylose and D-ribose; 36% assimilated D-Melesitose; 34% assimilated Glycerol, 32% assimilated D-cellobiose and 28% assimilated D-sorbose. A relatively small number of yeasts assimilated L-sorbose (18%), Glucosamine (18%), D-Melibiose (12%) and Rhamnose (12%). None of the yeasts tested assimilated levulinic acid, potassium gluconate, sodium glucuronate, erythriotol, inositol and D-lactose (Table 3).

Identification of yeasts isolates

The yeast strains identified were found to belong to five genera and ten species of yeasts (Figure 1). These were: *Saccharomyces cerevisiae* (54%), *Candida pseudorhagii*

Table 2. Phenotypic characters of yeasts isolated from chakpalo.

Cluster	Microbial isolates number	Fermentation ability				Assimilation of nitrogen source					DBB test
		Glu[1]	Lac	Suc	Raf	Nit	Eth	Lys	Cad	Ctr	
I	2, 4, 6, 8, 11, 14, 19, 24, 29, 30, 32, 33, 34, 35, 36, 48	+	-	+	-	+	+	+	+	+	–
II	41, 44, 47	+	-	+	+	+	+	+	+	+	–
III	9, 37	+	-	+	-	-	-	-	-	+	-
IV	10, 31	+	+	+	-	+	+	+	+	+	-
V	13, 39	+	-	+	+	+	+	+	+	+	+
VI	15, 17	+	-	+	+	+	+	+	-	+	-
VII	16, 21	+	-	-	-	+	+	+	+	+	-
VIII	18, 20	+	-	+	-	+	+	-	-	-	-
IX	22, 27	+	-	+	-	+	+	+	-	+	-
X	38, 40	+	-	+	-	+	+	-	-	+	-
XI	1	+	-	-	-	+	+	+	-	+	-
XII	3	+	-	+	-	-	-	-	+	+	-
XIII	5	+	-	+	-	-	-	-	-	-	-
XIV	7	+	-	+	-	-	-	+	+	+	-
XV	12	+	-	-	+	+	+	+	+	+	+
XVI	23	+	-	+	+	+	-	+	+	-	-
XVII	25	+	-	+	+	-	+	+	-	+	-
XVIII	26	-	-	+	+	-	+	+	+	-	-
XIX	28	+	-	+	-	-	+	-	-	+	-
XX	42	+	-	+	+	+	+	-	+	-	+
XXI	43	+	-	+	+	-	-	-	-	-	+
XXII	45	+	-	-	-	+	+	+	+	+	-
XXIII	46	+	-	+	+	+	+	-	-	-	-
XXIV	49	+	-	+	-	+	-	-	+	-	-
XXV	50	+	-	-	-	-	-	-	-	-	-
Frequency (%)		98	4	88	28	80	82	76	66	80	10

[1]Glu = glucose, Lac = lactose, Suc = Sucrose, Raf = rafinose, Nit = nitrate, Eth = ethylamine, Lys = L-lysine, Cad = cadaverine, Crt = creatine DBB = diazonium Blue B. (+) Fermented or assimilated; (-) did not ferment or did not assimilate. Frequency (%): represents the ratio of isolates that were able to ferment each sugar or to assimilate nitrogen source or DBB. How it is calculated: for each sugar or nitrogen source or DBB, the number of + was divided by the total number of isolates (50).

(18%), *Candida ubatubensis* (6%), *Candida bentonensis* (4%), *Saccharomyces bayanus* (4 %), *Pseudohydnum gelatinosum* (4%), *Sporobolomyces odoratus* (4%), *Candida heliconiae* (2%), *Candida bombi* (2%) and *Zygosacharomyces rouxii* (2%). *Saccharomyces cerevisiae* was found to be the dominant yeast species in the traditional starter otchè.

DISCUSSION

The highest mean values of pH and refractive index were noticed in samples collected from Bantè. In the same way, the titratable acidity of otchè collected from Savè was significantly ($p<0.05$) higher than that of the samples collected from the other sites. Also, dry matter value varies from a site to another one. As observed in other studies (Demuyakor and Ohta, 1991; Owuama, 1999), traditional starters can easily be distinguished by their specific physicochemical such as pH, dry matter, titratable acidity and refractive index.

The yeast concentration of otchè was relatively lower than that reported in tchoukoutou, an opaque sorghum beer of Benin (Kayodé et al., 2011). Distribution frequencies of microbial species vary according to the localities and ingredients used for the production of African beers (Faparusi et al., 1973; Nout, 1980; Odunfa, 1985; Demuyakor and Ohta, 1991; Sanni and Lönner, 1993; Sefa-Dedeh et al., 1999).

Fermentation and assimilation tests showed that the majority of yeasts assimilated glucose and sucrose whereas only a few strains could ferment lactose and

Table 3. Assimilation profiles of yeasts isolated from Chakpalo.

	a*	b	c	d	e	f	g	h	I	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	za	zb	zc	zd	ze	zf	zg	Total (%)
*GAL	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	94
ACT	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	40
SAC	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	90
NAG	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	48
LAT	+	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	+	40
ARA	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	44
CEL	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	32
RAF	+	-	+	+	-	-	+	-	+	+	+	-	+	-	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	68
MAL	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	88
TRE	+	-	-	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	-	+	+	48
2KG	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	+	+	44
MDG	+	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	+	38
MAN	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	-	+	+	40
LAC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
INO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
SOR	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	28
XYL	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	+	38
RIB	+	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	38
GLY	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	34
RHA	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	+	12
PLE	+	-	+	+	-	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	-	+	-	-	+	54
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MEL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-	-	+	12
GRT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
MLZ	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	-	-	+	36
GNT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
LVT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
SBE	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	18
GLN	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	18
ESC	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	24
Nb of isolates (%)	16	14	4	8	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	

a* = 2, 6, 11, 14, 29, 18, 20, 30; b = 32,34,35, 36, 31, 43, 42; c = 38, 40; d = 16,21,15,17; e = 3; f = 33; g = 49; h = 12; i = 37; j = 10 ; k = 5; l = 41; m = 47; n = 48; o = 46; p = 44; q = 1; r = 45; s = 50; t = 22; u = 24; v = 13; w = 25; x = 27; y = 4; z= 9; za = 23; zb = 39; zc = 7; zd = 8; ze = 28; zf = 26; zg = 19; GAL = D- Galactose, ACT = Cycloheximide (Actidione), SAC = D- Saccharose, NAG = N- Acety-Glocusamine, LAT = Lactic Acid, ARA = L-arabinose, CEL = D-cellobiose, RAF = D-raffinose, MAL = D-maltose, TRE = D-trehalose, 2KG = Potassium 2-cétogluconate, MDG = Methyl-αD-glucopyranoside, MAN = D-mannitol, LAC = D-lactose, INO = Inositol, SOR = D-sorbose, XYL= D-Xylose, RIB = D-ribose, GLY= Glycerol, RHA = Rhamnose, PLE = Palatinose, ERY = Erythritol, MEL = D-Melibiose, GRT = Sodium glucuronate, MLZ = D-Melesitose, GNT = Potassium Gluconate, LVT = Acide levulinique, GLU = D-Glucose, SBE = L-sorbose, GLN= Glucosamine, ESC = Sulfate of ammonium.

raffinose. These results were quite different from previous data by Sanni and Lönner (1993) on 49 yeasts isolates from Nigerian traditional beers known as burukutu, pito, sekete, agadagidi and palm win. The authors reported that yeasts isolated from these beers fermented glucose (77.55%), galactose (46.94%), maltose (40.82%), sucrose (44.89%) and melibiose (4.08%), but none of them fermented lactose. In this study, *S. cerevisiae* appeared to be the most predominant microbial strain in otchè, the starter used for the fermentation of Chakpalo, the Benin-opaque sorghum beer. This microbial identification data is in agreement with our previously reported findings of the prevalence of *S. cerevisiae* in tchoukoutou, another sorghum beer from Benin (Kayode et al., 2011, Greppi et al., 2013). Similarly, Konlani et al. (1996) reported a prevalence of 55-90% of *S. cerevisiae* in opaque sorghum beer from Togo and Burkina-Faso. Glover et al. (2005) also identified 72% of 247 isolates as *S. cerevisiae* based on their assimilation profiles. This comparative data indicates that the prevalence of *S. cerevisiae* in the starter is lower than in the beer itself. According to Vaughan-Martini and Martini (1998), an isolate is characterized as *S. cerevisiae* if such microbial strain could assimilate glucose, sucrose, maltose, trehalose, raffinose and ethanol. In our study, it has been noticed that many isolates could not assimilate all these sugars. However, we were able to characterize our isolated strains as *S. cereviciae* strains on the basis of additional biochemical characterization. Van der Aa Kühle et al. (2001) and Demuyakor and Ohta (1991) also identified many isolates from Ghanaian and Burkina-Faso sorghum beers as *S. cerevisiae*, even though these microorganisms had a very different carbon assimilation profile from the taxonomical key proposed by Vaughan-Martini and Martini (1998). Our results are in agreement with these findings.

Conclusion

This study suggests that *S. cerevisiae* is the predominant microbial species in the traditional starter used for the fermentation of chakpalo. Moreover, we suggest a more robust microbial identification approach based on genome fingerprinting techniques for the characterization of traditional starters used in the fermentation of opaque sorghum beers in Africa. The current study highlights the acidity profile, dry matter of otchè, a traditional starter for the fermentation of Chakpalo, the Benin-opaque sorghum beer.

Conflict of Interests

The authors have not declared any conflict of interests.

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