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Diversity of yeasts involved in the fermentation of tchoukoutou, an opaque sorghum beer from Benin

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Opaque sorghum beers are traditional alcoholic beverages in several African countries. Known as tchoukoutou in Benin, the beer is often obtained from an uncontrolled fermentation. It is consumed in an actively fermenting state and has a sour taste. The present study characterized and identified the yeasts involved in the fermentation process of this type of beer using the phenotypical approach. Of 12 beers from 4 different locations, the mean values of the pH, titratable acidity, dry matter content and refractive index were respectively 3.67, 0.70 (% as lactic acid) 18.08% and 7.00. Lactic acid bacteria and yeasts were the predominant microorganisms involved in the fermentation of tchoukoutou. Their counts were respectively 9.1 log cfu/ml and 9.1 logcfu/g. Enterobacteriaceae were not detectable in the beer. Based on the phenotypic characters and the assimilation profiles of 40 isolated yeasts, four genera with seven species of yeasts were identified. The yeast species predominant in the Benin opaque sorghum beer tchoukoutou was *Saccharomyces cerevisiae*.

Key words: Sorghum, beer, tchoukoutou, *Saccharomyces cerevisiae*, yeast,

INTRODUCTION

Traditional alcoholic beverages are obtained in sub-Saharan Africa from carbohydrate-rich products (Sefa-Dedeh et al., 1999). Opaque sorghum beers are popular alcoholic beverages in Africa. The fermentation is often spontaneous and uncontrolled. The beverages often consumed in an actively fermenting state have short shelf-lives (Odufa, 1985). In the West Africa region they are known as tchoukoutou in Benin, dolo in Burkina-Faso, pito in Ghana, and burukutu or otika in Nigeria

(Odufa, 1985; Glover et al., 2005; Kayodé et al., 2005). The beers have a sour taste, a relatively high dry matter content (5-13 g 100 ml⁻¹) and low alcohol content (2-3 ml 100 ml⁻¹), which make them suitable beverages for adults and teenagers (Agu and Palmer, 1998; Briggs et al., 2004). They are largely consumed by the poorest people and significantly contribute to the diet of millions of consumers. The beers are mostly prepared with Guinea corn (*Sorghum bicolor*) but other cereals such as millet or maize can be used as adjunct or as substitutes (Kayode et al., 2005). The manufacturing process consists of malting (soaking, germination, sun drying), brewing (mashing, boiling, filtration) and fermentation (Haggblade and Holzappel, 1989). Depending on the

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geographic location, variations may occur in the process (Odunfa, 1985; Haggblade and Holzapfel, 1989).

Lactic acid bacteria and yeasts were identified during the fermentation of several cereal products in Africa (Jespersen et al., 1994; Muyanja et al., 2002; Mugula et al., 2003; Vieira-Dalodé et al., 2007; Muyanja et al., 2010). The yeast species most often reported in African traditional food and beverages belong to the species *Saccharomyces cerevisiae*. (Jespersen, 2002). Few studies investigated the micro-organisms involved in African opaque beers. The microorganisms isolated from the beer consist essentially of yeasts and lactic acid bacteria (LAB) (Van der Aa Kühle et al., 2001; Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999; Sanni and Lönner, 1993; Faparusi et al., 1973). However, the species isolated and their frequency distributions in the product vary according to the regional location. *Saccharomyces cerevisiae* (33%), *Kluyveromyces* spp, *Candida* spp., and *Torulaspora delbrueckii* are predominant yeasts in Ghanaian opaque beer (Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999). In Burukutu Nigeria, Sanni and Lönner (1993) found the predominance of *Candida* spp., *Geotrichum candidum*, *S. cerevisiae*, *Kloeckera apiculata* or *Torulaspora delbrueckii*. Van der Aa Kühle et al. (2001) demonstrated the predominance of *Saccharomyces* spp (of which 45% were identified as *Saccharomyces cerevisiae*) in the fermentation of sorghum beer from Ghana and Burkina-Faso. Very limited information exists on the microbiological attributes of the Beninese opaque beers. The objectives of this study are two-fold. Firstly, to characterise the Benin opaque sorghum beer in terms of its physical and microbiological attributes. Secondly, to characterise and identify the yeasts involved in the fermentation of the beer.

MATERIALS AND METHODS

Sampling

Samples of actively fermenting (about 12 h of fermentation) opaque sorghum beer were collected from twelve commercial processing sites 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 analysed immediately for microbiological analysis (Hounhouigan et al., 1993).

Physico-chemical analysis

Dry matter was determined according to American Association of Cereal Chemistry (AACC) methods (AACC, 1984). Titratable acidity pH and were determined as described by Nout et al. (1989). The refractive index was measured using a refracto meter (Sopelem 9596, France).

Enumeration of micro organisms

Duplicate aliquots of *tchoukoutou* (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 homogenised with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total counts of aerobic mesophilic bacteria (TC), lactic acid bacteria (LAB), yeasts and Enterobacteriaceae were enumerated as described by Hounhouigan et al. (1993).

Identification of yeast

For identification of yeasts, isolates from three representative production sites were purified by successive sub-culturing on malt extract agar (MEA, CM 59, Oxoid). 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 i.e. 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

Mean values and 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 Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands was used for identification of yeasts.

RESULTS AND DISCUSSION

Physico-chemical characteristics of the Benin opaque beer

The mean value of the pH was 3.67 and the titratable acidity of the beer averaged to 0.7 (% as lactic acid). The dry matter content of the beer is high but variable (15-20%) and averaged to 18.1%. The refractive index averaged to 7.0 (Table 1). It has been demonstrated that at pH < 4.0 in food products, the growth of diarrhea causing pathogens is inhibited (Motarjemi and Nout, 1996). Thus it can be expected that the *tchoukoutou* beer is inherently safe from a microbiological point of view. The Benin opaque beer resembles *pito*, the Ghanaian opaque beer, in terms of acidity content (Sefa-Dedeh et al., 1999) but its refractive index is higher.

Microbial content

The mean counts of lactic acid bacteria and yeasts were respectively 9.1 and 9.1 log cfu/ml. The Enterobacteriaceae were not detectable in the beer (log cfu/ml < 1) (Table 2). The yeast concentration in *tchoukoutou* is relatively higher than reported in *pito* beer (Glover et al., 2005) but close to values reported for *burukutu*, another opaque sorghum beer from Nigeria

Table 1. Physico-chemical characteristics of the Benin opaque sorghum beer *tchoukoutou*.

Sample origin	pH	Titrateable acidity (%lactic acid)	Dry matter (%)	Refractive index
Tchaourou (n=3)	3.5±0.19a	0.8±0.3a	20.2±2.03a	5.0±2a
Parakou (n=3)	3.7±0.26a	0.6±0.3ab	18.1±2.37a	10.0±2b
Perere (n=3)	3.6±0.12a	0.8±0.2a	15.4±1.95ab	5.0±1a
N'dali (n=3)	3.8±0.22a	0.6±0.03ab	18.6±4.39a	8.0±1ab
Mean	3.7	0.7	18.1	7.0
CV (%)	3.5	16.5	11.1	35.0

Table 2. Microbiological characteristics of the Benin traditional opaque sorghum beer *tchoukoutou*.

Sample origin (Northern Est of Benin)	Lactic acid bacteria (log cfu/g)	Yeasts (log cfu/g)	Total mesophilic aerobic bacteria (log cfu/g)	Enterobacteriaceae (log cfu/g)
Tchaourou (n=3)	9.5	9.3	9.9	< 1
Parakou (n=3)	8.6	8.9	9.0	< 1
Perere (n=3)	9.6	9.1	9.7	< 1
N'dali (n=3)	8.6	8.9	9.6	< 1
Mean	9.1	9.1	9.67	-
CV(%)	6.2	2.2	4.3	-

(Faparusi et al., 1973). Indeed, lactic acid bacteria and yeasts are the predominant microorganisms involved in most African fermented beverages and food (Ogunfa, 1985; Mugula et al., 2003; Muyanja et al., 2002).

Phenotypic characters of yeast isolates

After preliminary microscopic confirmation all 40 yeasts were subjected to morphology, fermentation and assimilation tests. None of the isolates could ferment lactose whereas the majority fermented glucose (100%), sucrose (95%) and raffinose (90%). Only 5% of the isolates assimilated nitrate, ethylamine or creatine, whereas 15% assimilated L-lysine. All isolates were ascomycetous yeasts as revealed by Diazonium Blue B test (Table 3). On the basis of their fermentation profile and their nitrogen assimilation pattern the 40 yeasts could be grouped into 5 distinct clusters with the majority (63%) present in one group which showed a metabolism profile typical of *Saccharomyces* spp.

Assimilation profile and identification of yeasts isolates

Based on their assimilation of carbon compounds, sixteen assimilation profiles were distinguished. All yeasts assimilated glucose and maltose (100%), 42.5% assimilated sucrose, 23% assimilated raffinose and only 2.5% could assimilate trehalose. None of them

assimilated arabinose, sorbitol and methyl- α D-glucopyranoside (Table 4). On the basis of their phenotypic characteristics, the 40 yeasts were found to belong to four genera and seven species of yeast (Figure 1). Clearly, *Saccharomyces cerevisiae* (68%) predominates in the Benin opaque sorghum beer. Our result resembles findings by Konlani et al. (1996) who reported a prevalence of 55-90% for *S. cerevisiae* in sorghum beer from Togo and Burkina-Faso, two regions close to our study area. Van der Aa Kühle et al., 2001 also identified a large number (45%) of the yeasts involved in the fermentation of opaque beers from Burkina-Faso and Ghana as *S. cerevisiae*. From a study carried out on *pito* in Northern Ghana, Glover et al. (2005) identified 72% of 247 isolates as *S. cerevisiae* based on their assimilation profiles. Twenty seven percent of isolates had narrow assimilation profiles atypical of the specie and could not be clearly identified.

For an isolate to be accepted as *S. cerevisiae* it must be able to assimilate glucose, sucrose, maltose, trehalose, raffinose and ethanol (Vaughan-Martini and Martini, 1998). In the present study, even though many isolates could not assimilate all of these sugars, they were identified as *S. cerevisiae*. 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 showed carbon assimilation profiles different from the taxonomical key proposed by Vaughan-Martini and Martini (1998). Like in our result, many of the isolates analysed by these authors were not able to assimilate sucrose, raffinose and trehalose.

Table 3. Phenotypic characters of yeasts isolated from *tchoukoutou*.

Cluster	Isolates Nr	Fermentation				Assimilation of nitrogen source					DBB test ²	
		Glu ¹	Lac	Suc	Raf	Nit	Eth	Lys	Cad	Crt		
I	26, 28, 11, 12, 22, 37, 24, 34, 8, 15, 16, 19, 17, 20, 35, 2, 9, 38, 39, 7, 10, 21, 40, 31, 3	+	-	+	-	-	-	-	-	-	-	-
II	4, 13, 32, 27, 33, 29, 14	+	-	+	+	-	-	-	-	-	-	-
III	25, 30, 5, 6	+	-	+	-	-	-	+	-	-	-	-
IV	1, 18	+	-	-	+	-	-	-	-	-	-	-
V	23, 36	+	-	+	+	+	+	+	-	+	-	-
Frequency (%)		100	0	95	90	5	5	15	0	5	0	0

¹ Glu = glucose, Lac = lactose, Suc = sucrose, Raf = raffinose, Nit = nitrate, Eth = ethylamine, Lys = L-lysine, Cad = cadaverine, Crt = creatine ² DBB= diazonium Blue B.

Table 4. Assimilation profiles of yeasts isolated from *tchoukoutou*.

	a*	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	Total (%)
D-glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Glycerol	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	5
Calcium 2-keto-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	2.5
L-arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
D-xylose	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	10
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	2.5
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
D-galactose	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+	67.5
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	2
D-sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Methyl- α D-Glucopyranoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
N-acetyl-glucosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	2.5
D-cellobiose	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	2.5
D-lactose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	2.5
D-maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
Sucrose	+	+	-	+	-	-	-	+	+	+	-	+	+	-	+	+	57.5
D-trehalose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	2.5
D-melezitose	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	5
D-raffinose	-	+	-	-	-	-	-	+	-	-	-	+	+	-	-	+	22.5
Nb of isolate (%)	15	12.5	12.5	12.5	7.5	5	5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	

*a = isolates 11, 12, 22, 26, 28, 37, b = isolates 8, 24, 27, 33, 34, c = isolates 10, 15, 16, 18, 19, d = isolates 17, 20, 25, 30, 35, e = isolates 4, 13, 32, f = isolates 2, 9, g = isolates 31, 36, h = isolate 23, i = isolate 1, j = isolate 14, k = isolate 5, l = isolate 7, m = isolate 6, n = isolate 21, o = isolate 40, p = isolate 3.

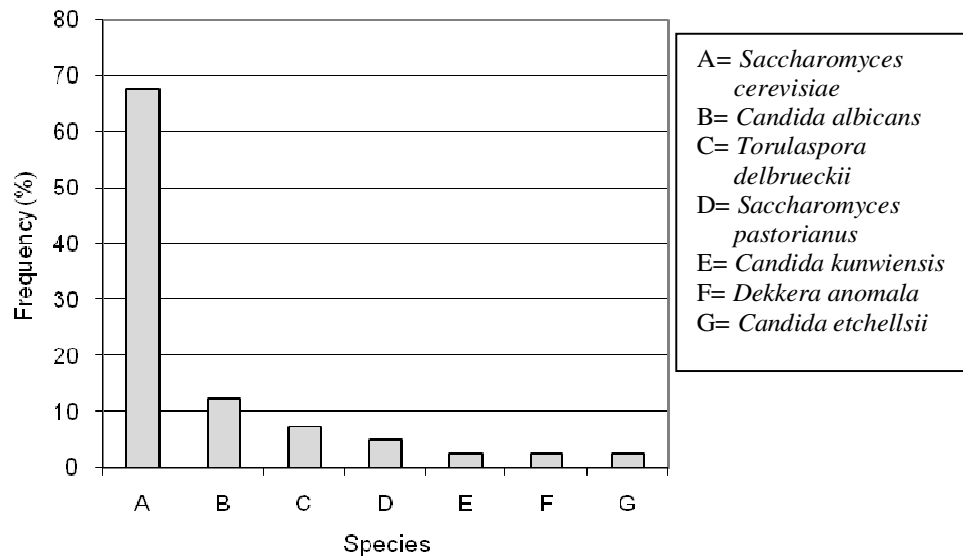


Figure 1. Frequency distribution of yeast species involved in *tchoukoutou* fermentation.

Conclusion

Based on the phenotypic characterisation, *S. cerevisiae* was found to be the predominant yeast species in the fermentation of Benin opaque sorghum beer. There is a need of genotyping the isolates for a best characterisation of the yeasts involved. In view of controlling the quality of *tchoukoutou*, the predominant species identified can be selected and used as single or mixed starter cultures for a more predictable fermentation outcome.

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