ARTICLE N°10

Determination of yeast diversity in ogi, mawè, gowé and tchoukoutou by using culture-dependent and -independent methods. (**2013**). *International Journal Food Microbiology* Vol. 165, 84 – 88. Greppi, A., Rantsiou, K., **Padonou, W.,** Hounhouigan, J., Jespersen, L., Jakobsen, M., Cocolin, L.

International Journal of Food Microbiology 165 (2013) 84–88

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Determination of yeast diversity in ogi, mawè, gowé and tchoukoutou by using culture-dependent and -independent methods

Anna Greppi [a], Kalliopi Rantsiou [a], Wilfrid Padonou [b], Joseph Hounhouigan [b], Lene Jespersen [c], Mogens Jakobsen [c], Luca Cocolin [a,*]

[a] Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Via Leonardo da Vinci 44, 10095, Grugliasco, Torino, Italy
[b] Département de Nutrition et Sciences Alimentaires, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Benin
[c] Department of Food Science, Food Microbiology, Faculty of Life Sciences, University of Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form 28 April 2013
Accepted 3 May 2013
Available online 13 May 2013

Keywords:
Yeasts
Predominant species
Candida krusei
Biodiversity
Fermented foods
Culture dependent and independent analysis

ABSTRACT

The maize based ogi and mawè and the sorghum based gowé and tchoukoutou are traditional, spontaneously fermented products widely consumed by the population of Benin (West Africa). Yeast occurrence in the products, as sold on local markets at different locations, was studied using a combination of culture-dependent and independent methods. Number of yeasts is varied from 3.75 $\log_{10}$ colony forming units (cfu)/g for ogi to 5.60 $\log_{10}$ cfu/g for tchoukoutou. Isolated yeasts (236) were identified based on different migration profiles on denaturing gradient gel electrophoresis (DGGE) and 26S rRNA gene sequencing. *Candida krusei* was the yeast most frequently isolated with strongest predominance in the maize based products. Other predominant yeast present at equal or lower incidence were *Clavispora lusitaniae* and *Saccharomyces cerevisiae* in ogi and mawè, *Cl. lusitaniae*, *Candida tropicalis* and *Kluyveromyces marxianus* in gowè and *Cl. lusitaniae*, *S. cerevisiae* and *Candida rugosa* in tchoukoutou. Grouping of *C. krusei* isolates (164) by rep-PCR analysis indicated that several biotypes were involved in fermentation of the four products. The DGGE analysis on the DNA directly extracted from the food matrices demonstrated the presence of *Dekkera bruxellensis* and *Debaryomyces hansenii*, not detected by the culture-based approach. This is the first study combining culture-dependent and independent methods to reveal predominant yeast species and biotypes in traditional foods from Benin.

1. Introduction

Yeast occurs in significant numbers ranging from 10^5 to 10^8 cfu/g in several traditional African fermented food constituting a major dietary component in different regions of Africa, including West Africa (Jespersen et al., 1994; Gadaga et al., 2000; Oyewole, 2001; Van der Aa Kuhle et al., 2001; Naumova et al., 2003; Jespersen et al., 2005; Omemu et al., 2007; N'guessan et al., 2011). A mixed mycobiota comprising the genera *Candida*, *Debaryomyces*, *Dekkera*, *Geotrichum*, *Hanseniaspora*, *Kodamaea*, *Kluyveromyces*, *Meyerozyma*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Trichosporum* and *Zygosaccharomyces* species has been reported. In general the role of yeast in these foods is not understood. The same applies for the potential interactions of such yeast with the human host, although some theories have been proposed as reviewed by Moslehi-Jenabian et al. (2010).

The four products sampled at different locations in Benin and included in the present study are ogi, a gruel obtained by fermentation of a suspension of maize in water (Oke, 1967; Akinrele, 1970; Dada and Muller, 1983); mawè, a fermented maize dough (Hounhouigan et al., 1993a); gowé, a malted and fermented maize or sorghum-based food (Michodjèhoun-Mestres et al., 2005; Vieira-Dalodé et al., 2007) and tchoukoutou, an opaque sorghum beer largely produced in the North of Benin (Kayodé et al., 2006). Related food products are called in different ways in other countries of West Africa e.g. kenkey is a maize-dough produced from whole maize in Ghana similar to mawè for which predominant yeasts were studied by Jespersen et al. (1994); tchapalo is a sorghum beer from Cote d'Ivoire similar to tchoukoutou, studied by N'guessan et al. (2011). Updated and molecular based studies of the yeast communities in traditional African food based upon fermentation of important raw materials like maize and sorghum are not available except for local beers (Van der Aa Kuhle et al., 2001; N'guessan et al., 2011). According to the information available lactic acid bacteria (LAB) in high number are associated with yeasts in such products (Akinrele, 1970; Odunfa and Adeyele, 1985; Ampe and Miambi, 2000; Teniola and Odunfa, 2001; Omemu et al., 2007; Oguntoyinbo et al., 2011 (ogi); Hounhouigan et al., 1993b,c (mawè); Vieira-Dalodé et al., 2007 (gowé); Kayodé et al., 2006 (tchoukoutou)). However, the majority of these studies focused on LAB and yeast identifications were

* Corresponding author at: Via Leonardo da Vinci 44, 10095, Grugliasco, Torino, Italy. Tel.: +39 011 670 8553; fax: +39 011 670 8549.
E-mail address: lucasimone.cocolin@unito.it (L. Cocolin).

0168-1605/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijfoodmicro.2013.05.005

mainly based on morphological, physiological and biochemical characteristics of isolates. These methods have draw-backs in the way that they cannot differentiate isolates at a level suitable in biodiversity studies and they do not allow detection of viable but non-culturable microorganisms (Head et al., 1998). Further, they are no more accepted as the sole basis of taxonomic classification. The present work compares yeast populations in marketed ogi, mawè, gowé and tchoukoutou using a combination of culture-dependent and -independent molecular methods to create a new and better-suited platform for selection of cultures for further studies of specific yeast characteristics to understand their significance for fermentation and product quality.

2. Materials and methods

2.1. Samples collection

Samples of ogi, mawè, gowé and tchoukoutou were collected aseptically in sterile stomacher bags (Seward, West Sussex, UK) from local villages and urban markets in Benin. For each product, three different sites were chosen. Ogi samples were collected in two villages in Bohicon and Abomey-Calavi, respectively, and at the local market of Porto-Novo; mawè samples came from local markets in Cotonou, Godomey and Porto-Novo; gowé samples were obtained from a producer's house in Cotonou, a street seller of Cotonou and the local market of Godomey; and tchoukoutou samples were collected at the local market in Cotonou, a producer's house in Abomey-Calavi and a street producer at Cotonou. All samples were stored at 4 °C and subjected to microbiological analysis within 48 h.

2.2. Determination of pH

To determine the pH, each sample was diluted five times in sterile water. The pH determinations for each diluted sample were performed in duplicate, using a digital pH meter (inoLab pH 730, WTW GmbH, Weilheim, Germany), calibrated with buffer at pH 4.0 and 7.0. For each product, mean and standard deviation of the three sample sites were calculated.

2.3. Enumeration and isolation of yeasts

Ten (10) grams of each ogi, mawè and gowé samples and 10 ml of tchoukoutou samples were suspended in 40 ml of sterile diluent [0.1% bactopeptone (Oxoid, Milan, Italy), 0.85% NaCl (Merck, Darmstadt, Germany), all w/v, pH adjusted to 7.0] and homogenized for 30 s at 230 rpm with a Stomacher (Lab Blender, Model 400, Seward Medical, London, England). From appropriate 10-fold dilutions, yeasts were enumerated on MYGP agar [3 g of yeast extract (Oxoid), 3 g of malt extract (Oxoid), 5 g of bactopeptone (Oxoid), 10 g of glucose (Sigma, Milan, Italy) and 20 g of agar (Oxoid) per liter of distilled water, all w/v, added of 50 mg chloramphenicol (Fisher chemicals, Milan, Italy) and 25 mg of chlortetracycline (Sigma)] and incubated at 24 °C for 3 days. Plates showing between 30 and 300 colony forming units (cfu)/g were counted and results were expressed as $\log_{10}$. For each product, mean and standard deviation among the three sample sites were calculated. From the appropriate dilution, a sector of the agar plate was selected, from which 20 yeast colonies were sub-cultured on new plates and purified by repeated streaking. Colonies on MYPG were examined by microscopy (HBO 50/AC 100×/1.25 oil pH 3, Zeiss, Jena, Germany). A total of 236 isolates were collected. All of them were maintained in glycerol (30 v/v) at −20 °C.

2.4. DNA extraction from pure cultures

Genomic DNA of each isolate was extracted from 1 ml of 24 h MYPG culture, 30 °C, centrifuged at 14,000 ×*g* for 10 min at 4 °C. The pellet of yeast cells was subjected to DNA extraction according to the procedures described by Cocolin et al. (2000). DNA was then quantified by using the NanoDrop instrument (Spectrophotometer ND-1000, Thermo Fisher Scientific, Milan, Italy) and diluted to a concentration of 100 ng/ml.

2.5. Direct extraction of DNA from samples

Total DNA from the fermented samples was extracted using the MasterPure™ Complete DNA and RNA Purification kit (Epicentre, Madison, WI, USA) by following the supplier's instructions (Rantsiou et al., 2012).

2.6. PCR-DGGE protocol

One microliter of the yeast DNA and of the DNA extracted directly from the samples (100 ng) were used for the PCR assays in a total volume of 25 μl containing 1× PCR Buffer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphospates (dNTP), 0.75 U of *Taq* Polymerase (Sigma), and 0.2 μM of each primer. The D1 region of the 26S rRNA gene was amplified using the primers NL1GC and LS2 (Cocolin et al., 2000). The amplification cycle of denaturation at 95 °C for 1 min, annealing at 40 °C for 45 s and extension at 72 °C for 2 min was repeated 35 times. The cycle was preceded by an initial denaturation at 95 °C for 5 min and followed by a final extension at 72 °C for 7 min. The amplicons were analyzed on 2% (w/v) agarose gels using a GeneRuler DNA Ladder of 100 bp as molecular weight standard (Promega, Milan, Italy). The gels were run at 100 V/cm (constant voltage) in 1× TAE (45 mM Tris, 45 mM boric acid, 1 mM EDTA pH 8) and stained with ethidium bromide. Digital images of the gels were captured using UVI pro platinun 1.1 Gel Software (Eppendorf, Hamburg, Germany).

The PCR products were analyzed by DGGE using the DCode apparatus (Bio-Rad, Hercules, CA, USA) and were electrophoresed in a 0.8 mm polyacrilamide gel (8% [w/v]) acrylamide-bisacrylamide (37.5:1), as described by Cocolin et al. (2001), using a denaturing gradient from 30 to 60%.

2.7. Identification by sequencing

DGGE profiles of the isolates were visually grouped and the partial 26S rRNA gene of representatives of each group was amplified with primers NL1/NL4 (Kurtzman and Robnett, 1998). The PCR products were sent to MWG Biotech (Edersberg, Germany) for sequencing.

For the culture-independent analysis, selected DGGE bands were excised from the gels, checked by PCR-DGGE, amplified with yeast primers (NL1 without GC clamp and LS2) and sent for sequencing as described by Cocolin et al. (2001).

Alignments in GenBank with the BLAST program (Altschul et al., 1997) were performed to determine the closest known relatives of the partial 26S rDNA sequences obtained.

2.8. Grouping of Candida krusei isolates by rep-PCR

One hundred sixty-four isolates of *C. krusei*, which is the most abundant species, were grouped by rep-PCR. One hundred nanograms of the DNA extracted from pure cultures of the isolates was subjected to rep-PCR analysis using primer $(GTG)_5$ according to Nielsen et al. (2007). Reactions were carried out in a final volume of 25 μl containing: 1× PCR Gold Buffer, 1.5 mM MgCl2, 0.2 mM of each of dNTP, 2 μM primer $(GTG)_5$ and 1.25 U/μl *Taq* polymerase (Applied Biosystem, Milan, Italy). The PCR reaction consisted of 30 cycles of denaturation at 90 °C for 30 s, annealing at 40 °C for 60 s and extension at 65 °C for 8 min. The initial denaturation was at 95 °C for 5 min and the final extension at 65 °C for 16 min. Amplicons were separated by 1.5% agarose gel electrophoresis in 1× TBE (150 min, 120 V) using a Generuler 1 kb DNA ladder as reference (Promega). The rep-PCR profiles were normalized and cluster analyses were performed using Bionumerics software (version 6.1, Applied

Maths, Sint-Martens-Latem, Belgium). The dendograms were calculated on the basis of the Pearson's coefficient of similarity with the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm (Vauterin and Vauterin, 1992).

3. Results

3.1. Yeast counts and pH

The number of yeast expressed as $\log_{10}$ cfu/g ± standard deviation for ogi, mawè, gowé and tchoukoutou samples was 3.75 ± 1.07, 5.52 ± 0.41, 4.04 ± 1.03 and 5.60 ± 0.88, respectively. The corresponding pH values were 3.76 ± 0.10, 5.04 ± 1.46, 3.68 ± 0.32 and 3.32 ± 0.47.

3.2. Identification of yeast isolates by PCR-DGGE

As shown in Table 1, a total of six different species were identified. All of them have been correctly identified with sequences in the GenBank database. *C. krusei* was the species most frequently isolated in all the products analyzed. In the maize-based products (ogi and mawè) it represented more than 90% of the isolates. Concerning other yeast species, ogi and mawè were almost identical, with *Clavispora lusitaniae* and *Saccharomyces cerevisiae* detected at lower incidence. The biota of tchoukoutou samples showed less isolates of *C. krusei* but higher levels of *Cl. lusitaniae* and *S. cerevisiae* and a presence of *Candida rugosa*. Gowé samples differed from the others with the presence of *Candida tropicalis* and *Kluyveromyces marxianus* occurring in similar levels as *C. krusei* (Table 1). Sample sites for ogi and mawè were very similar. Minor differences were observed among tchoukoutou sample sites whereas pronounced differences were observed among the gowé sample sites (Table 1).

3.3. Direct food matrix DGGE analysis

The DGGE fingerprints obtained are shown in Fig. 1. A total of 6 bands (a–f) were sequenced. The GenBank database allowed the identification of the bands with 100% identity and the species names and accession numbers are shown in Fig. 1. Bands in the middle of the DGGE gel were also cut and sequenced and they were determined to be heteroduplex (data not shown). For ogi, *Pichia kudriavzevii*, teleomorph of *C. krusei* (band a) and *Cl. lusitaniae* (c) were detected. Both species were also detected by culturing. *Dekkera bruxellensis* (b) was detected but not by culturing. *S. cerevisiae* (e) was not detected by direct DGGE and so was *C. krusei* (a) in two samples of ogi. For mawè, *C. krusei* (a), *Cl. lusitaniae* (c) and *S. cerevisiae* (e) were detected and the corresponding species were detected by culturing as well. *K. marxianus* (d) was detected by direct DGGE but not by culturing. Contrary to cultural data, *C. krusei* (a) was absent in one mawè sample (lane 5). For gowé *K. marxianus* (d) was detected by direct DGGE and by culturing whereas the band of *Debaryomyces hansenii* (f) was detected only by direct DGGE. *C. krusei*, *Cl. lusitaniae* and *C. tropicalis* were not detected by direct DGGE, contrary to culturing. In tchoukoutou samples only *S. cerevisiae* (e) was detected by culturing. *C. krusei*, *Cl. lusitaniae* and *C. rugosa* were not detected by direct DGGE but by culturing.

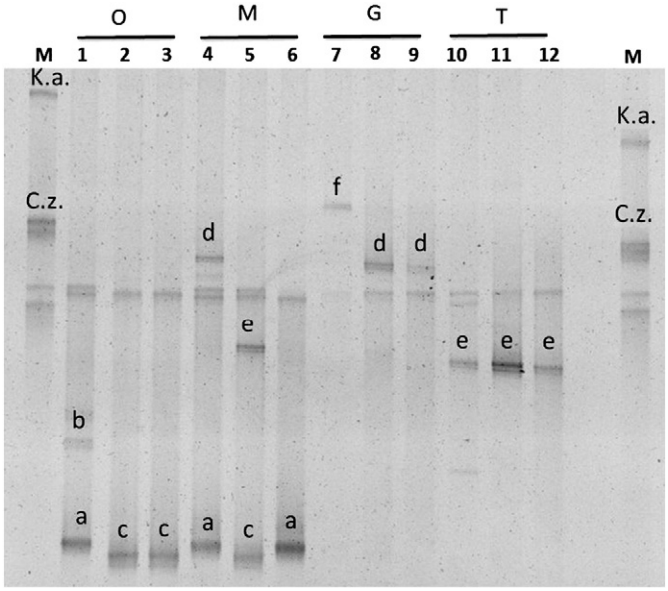


Fig. 1. DGGE profiles of the partial 26S rRNA gene fragment of the yeasts present in the food matrices. M: marker: K.a.: *Kloeckera apiculata*, C.a.: *Candida zemplinina*; lines 1–2–3: ogi samples; lines 4–5–6: mawè samples; lines 7–8–9: gowé samples; lines 10–11–12: thoukoutou samples. O = ogi samples, M = mawè samples, G = gowé samples and T = tchoukoutou samples. Band a: *Pichia kudriavzevii* (formerly named as *Issatchenkia orientalis*, anamorph of *Candida krusei*. Accession No. JQ585732), band b: *Dekkera bruxellensis* (FJ805785), band c: *Clavispora lusitaniae* (EF694616), band d: *Kluyveromyces marxianus* (FJ896141), band e: *Saccharomyces cerevisiae* (JF427814), and band f: *Debaryomyces hansenii* (HQ641266).

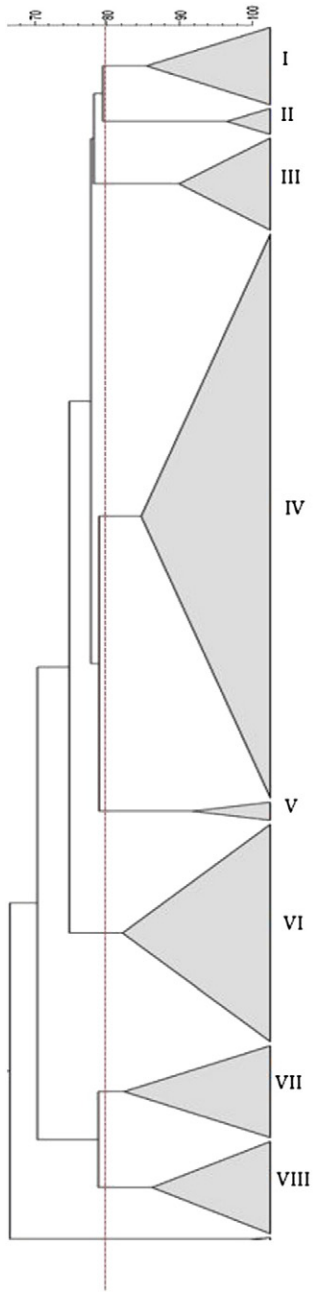
It was noticed that no samples contained band a as with bands c, f and e indicating an apparent disagreement of the DGGE analysis with the cultural data as shall be discussed below.

3.4. Grouping of C. krusei isolates

All 164 isolates of *C. krusei* were analyzed by rep-PCR for grouping and product specificity. The dendrogram obtained after cluster analysis of rep-PCR patterns is shown in Fig. 2. Using a coefficient of similarity of 80%, eight different clusters were observed. Clusters IV and VIII were common to all products and included a total of 89 (54%) of the isolates; cluster IV was the biggest one, containing a total of 76 isolates (46%) (Fig. 2). For each product this cluster accounted for roughly 50% of the isolates except for tchoukoutou (30%). Ogi isolates were distributed in all the clusters. Clusters II and III only contained ogi isolates. In general, in gowè and tchoukoutou samples less biodiversity was observed.

Table 1
Identification of yeasts from marketed samples of ogi, mawè, gowé and thoukoutou by PCR-DGGE. For each product, three different sample sites in Benin were chosen, indicated in roman numbers. The number of isolates for each species and the total number (in %in brackets) are shown for each product.

Yeast species	Ogi				Mawè				Gowé				Tchoukoutou				All products
	I	II	III	Tot	I	II	III	Tot	I	II	III	Tot	I	II	III	Tot	Tot
Candida krusei	19	19	19	57 (96)	20	16	18	54 (92)	19			19 (32)	10	18	6	34 (59)	164 (69)
Clavispora lusitaniae			1	1 (2)			1	1 (2)	1		3	4 (6)		1	11	12 (20)	18 (8)
Saccharomyces cerevisiae	1			1 (2)		4		4 (6)					8	1		9 (15)	14 (6)
Candida tropicalis										3	16	19 (32)					19 (8)
Kluyveromyces marxianus										17	1	18 (30)					18 (8)
Candida rugosa															3	3 (5)	3 (1)
Tot	*20*	*19*	*20*	*59*	*20*	*20*	*19*	*59*	*20*	*20*	*20*	*60*	*18*	*20*	*20*	*58*	*236*



Cluster	O	M	G	T	tot
I	1		2	8	11
II	4				4
III	13				13
IV	**30**	**23**	**12**	**11**	**76**
V	1	2			3
VI	4	14		12	30
VII	3	10			13
VIII	1	5	5	2	13

Fig. 2. Cluster analysis of rep-PCR fingerprints of *Candida krusei* isolates (164). The dendrogram was generated after cluster analysis of the digitized fingerprints and was derived from UPGMA linkage of Pearson correlation coefficients. A similarity coefficient of 80% was chosen to guarantee differentiation. The origin of the isolates is shown as O = ogi samples, M = mawè samples, G = gowé samples and T = tchoukoutou samples. In bold, the bigger common cluster.

4. Discussion

Samples of ogi, mawè, gowé and tchoukoutou were collected at different locations in Benin including markets, villages or street sellers. Details were not known with regard to the way the products were processed, time of fermentation, when and how the women decided to sell them and how they were transported and stored. Even though the oral transmission of the techniques appears to be quite precise, every producer creates her own suitable technique and decides to sell the product at her preferred time. This can explain the high value of pH obtained for the mawè samples (5.04 ± 1.46). Hounhouigan et al. (1993a) reported pH values of approximately 3.5 in commercial mawè. The same range of pH was reported by Jespersen et al. (1994) on kenkey. On the other hand, the pH measured for ogi, gowé and tchoukoutou samples were in accordance with the results found in literature (Omemu et al., 2007 (ogi); Vieira-Dalodé et al., 2007 (gowé); Kayodé et al., 2006 (tchoukoutou)). The yeast counts did not always match with those previously reported for ogi (Omemu et al., 2007), mawè (Hounhouigan et al., 1993b; Jespersen et al., 1994), gowé (Vieira-Dalodé et al., 2007) and tchoukoutou (Kayodé et al., 2006) and this is consistent with a high inter-product variability, but the order of magnitude was similar. By comparing concentrations of yeast with the reported counts of LAB (Teniola and Odunfa, 2001 (ogi); Hounhouigan et al., 1993c (mawè); Vieira-Dalodé et al., 2007 (gowé); Kayodé et al., 2006 (tchoukoutou)) it seems that, in terms of cfu/g, LAB occur in 3–4 tenfold higher in number. However, the larger size of yeast cells i.e. the total yeast biomass indicates a significant bioactivity despite the much lower cfu/g.

In the four products studied identification of yeast isolates revealed that *C. krusei* was the predominant culturable species. In ogi and mawè samples, this species was almost the only one isolated. These products are maize-based fermented products and it can be speculated that the composition of the food matrix supports growth and survival of this specific species. The predominance of *C. krusei* in the later stages of African fermentation of maize dough has also been reported by Jespersen et al. (1994). To find a possible explanation for its predominance, Halm et al. (2004) measured the intracellular pH of single cells of *C. krusei* and *S. cerevisiae* isolated from fermented maize dough. The authors wanted to investigate if the high predominance of the yeast in the late stages of the fermentation was due to a particular tolerance to high lactic acid concentration. Their results showed how *C. krusei* is more resistant to short-term pH changes caused by lactic acid than *S. cerevisiae*. Concerning the tchoukoutou, the fermentation is sometimes interrupted by the producers after approximately 12 h to avoid a final product with too high level of ethanol as consumers in Cotonou and Abomey-Calavi often prefer lighter i.e. a lower ethanol beverage contrary to the traditional recipe from the North of Benin. This could explain the predominance of *C. krusei* instead of *S. cerevisiae*, which is normally reported as being predominant in local beers with higher level of ethanol (Demuyakor and Ohta, 1991; Sefa-Deheh et al., 1999; N'guessan et al., 2011). Concerning the overall predominance of the yeast *C. krusei*, cluster analysis based on rep-PCR profiles did not indicate an apparent link between the four products and the clustering. Apart from two clusters specific for ogi, isolates from the four different matrices clustered together, underlining a homogeneous distribution of the *C. krusei* biotypes in the products. However, the usefulness of the rep-PCR was demonstrated by the presence of eight distinct clusters. It is assumed that isolates of different clusters have different roles in the fermentation. It is realized that other typing techniques could have indicated different clustering and relationships with the products. For bacteria, rep-PCR is a powerful, rapid, reproducible and highly discriminatory tool (Versalovic et al., 1994). While typing of yeasts is so far very limited, Redkar et al. (1996) has been successful in biotyping *Candida* spp. by the use of rep-PCR.

The presence of *C. krusei* was also detected in previous studies on ogi from Nigeria (Omemu et al., 2007), kenkey from Ghana (Jespersen et al., 1994), gowé from Benin (Vieira-Dalodé et al., 2007) and sorghum beers from Togo and Burkina Faso (Konlani et al., 1996).

The culture-independent approach identified *D. bruxellensis* and *D. hansenii*, but they were not detected by the culture-dependent method. They may be present in the habitat in viable but not-culturable states (Head et al., 1998; Ercolini, 2004) or as dead cells. On the other hand, *C. tropicalis* and *C. rugosa* were only detected by culture-dependent analysis and this can be explained by the detection limit of 10^3 cfu/ml for the DGGE analysis (Cocolin et al., 2000). Unexpectedly, the band identified as *C. krusei* (the anamorph of *P. kudriavzevii*) was absent in the direct DGGE analysis for two samples of ogi, one of mawè and all gowé and tchoukoutou samples, despite its presence according to the cultural

data. This could be due to PCR-bias in the food matrices where different yeast species are present at high levels and interfering with the specific binding to *C. krusei* of the primers applied. The same could apply for *Cl. lusitaniae* in ogi and mawè samples, *D. hansenii* in gowé sample and *S. cerevisiae* in tchoukoutou samples. In all cases these species were clearly amplified by the primer set, possibly masking the presence of *C. krusei*. As support of this explanation it was noticed that none of the samples showed a profile with the simultaneous amplification of *C. krusei* and *Cl. lusitaniae*, *D. hansenii* or *S. cerevisiae*. Such results indicate the need for simultaneous use of culture-dependent and -independent techniques for studying yeast populations in traditional fermented products with unknown mycobiota.

It is realized that few samples of the same products were analyzed. However, this work is considered as a first attempt to compare the mycobiota between different African fermented foods that have not been investigated by the combined use of culture dependent and -independent methods. Further studies are required to investigate the dynamics of yeast population during fermentation to assess whether the dominance of *C. krusei* was preceded by other yeast species in the previous stages of fermentation and with the purpose of selecting starter cultures with specific functional characteristics for controlled fermentations.

Acknowledgments

This study was funded by the Danish International Development Assistance (DANIDA). The Department of Nutrition and Food Science in Benin and the Department of Agricultural, Forestry and Food Sciences in Italy provided the technical and scientific support.

References

Akinrele, I.A., 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. Journal of the Science of Food and Agriculture 21, 619–625.

Altschul, S.F., Madden, T.L., Shaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389–3402.

Ampe, F., Miambi, E., 2000. Cluster analysis, richness and biodiversity indexes derived from denaturing gradient gel electrophoresis fingerprints of bacterial communities demonstrates that traditional maize fermentations are driven by the transformation process. International Journal of Food Microbiology 60, 91–97.

Cocolin, L., Bisson, L.F., Mills, D.A., 2000. Direct profiling of the yeast dynamics in wine fermentations. FEMS Microbiology Letters 189, 81–87.

Cocolin, L., Manzano, M., Cantoni, C., Comi, G., 2001. Denaturing gradient gel electrophoresis analysis of the 16 S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. Applied and Environmental Microbiology 67, 5113–5121.

Dada, L.O., Muller, H.G., 1983. The fate of aflatoxin B1 in the production of ogi, a Nigerian fermented sorghum porridge. Journal of Cereal Science 1, 63–70.

Demuyakor, B., Ohta, Y., 1991. Characteristics of pito yeasts from Ghana. Food Microbiology 8, 183–193.

Ercolini, D., 2004. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. Journal of Microbiological Methods 56, 297–314.

Gadaga, T.H., Mutukumira, A.N., Narvhus, J.A., 2000. Enumeration and identification of yeasts isolated from Zimbabwean traditional fermented milk. International Dairy Journal 10, 459–466.

Halm, M., Hornbaek, T., Arneborg, N., Sefa-Dedeh, S., Jespersen, L., 2004. Lactic acid tolerance determined by measurement of intracellular pH of single cells of *Candida krusei* and *Saccharomyces cerevisiae* isolated from fermented maize dough. International Journal of Food Microbiology 94, 97–103.

Head, I.M., Saunders, J.R., Pickup, R.W., 1998. Microbial evolution, diversity, and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial Ecology 35, 1–21.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H.M., Rombouts, F.M., 1993. Changes in the physico-chemical properties of maize during natural fermentation of Mawè. Journal of Cereal Science 17, 291–300.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H., Rombouts, F.M., 1993. Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of mawe', a fermented maize dough from Benin. International Journal of Food Microbiology 18, 279–287.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M., Houben, J.H., Rombouts, F.M., 1993. Composition and microbiological and physical attributes of mawè, a fermented maize dough from Benin. International Journal of Food Science and Technology 28, 513–517.

Jespersen, L., Halm, M., Kpodo, K., Jakobsen, M., 1994. Significance of yeasts and moulds in maize dough fermentation for 'kenkey' production. International Journal of Food Microbiology 24, 239–248.

Jespersen, L., Nielsen, D.S., Honholt, S., Jakobsen, M., 2005. Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. FEMS Yeast Research 5, 441–453.

Kayodé, A.P.P., Hounhouigan, D.J., Nout, M.J.R., Niehof, A., 2006. Household production of sorghum beer in Benin: technological and socio-economic aspects. International Journal of Consumer Studies 1470–6431.

Konlani, S., Delgenes, J.P., Moletta, R., Traore, A., Doh, A., 1996. Isolation and physiological characterization of yeasts involved in sorghum beer production. Food Biotechnology 10, 29–40.

Kurtzman, C.P., Robnett, C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 73, 331–371.

Michodjèhoun-Mestres, L., Hounhouigan, D.J., Dossou, J., Mestres, C., 2005. Physical, chemical and microbiological changes during natural fermentation of "*gowe*", a fermented beverage from sprouted or non sprouted sorghum (*Sorghum bicolor*) in West-Africa. African Journal of Biotechnology 4, 487–496.

Moslehi-Jenabian, S., Pedersen, L.L., Jespersen, L., 2010. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients 2, 449–473.

Naumova, E.S., Korshunova, I.V., Jespersen, L., MNaumov, G.I., 2003. Molecular genetic identification of *Saccharomyces* sensu stricto strains from African sorghum beer. FEMS Yeast Research 3, 177–185.

N'guessan, K.F., Brou, K., Jacques, N., Casaregola, S., Dje, K.M., 2011. Identification of yeasts during alcoholic fermentation of *tchapalo*, a traditional sorghum beer from Côte d'Ivoire. Antonie Van Leeuwenhoek 99, 855–864.

Nielsen, D.S., Teniola, O.D., Ban-Koffi, L., Owusu, M., Andersson, T.S., Holzapfel, W.H., 2007. The microbiology of Ghanaian cocoa fermentations analyzed using culture-dependent and culture-independent methods. International Journal of Food Microbiology 114, 168–186.

Odunfa, S.A., Adeyele, S., 1985. Microbial changes during traditional production of ogi-baba, a West African fermented sorghum gruel. Journal of Cereal Science 3, 173–180.

Oguntoyinbo, F.A., Tourlomousis, P., Gasson, M.J., Narbad, A., 2011. Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. International Journal of Food Microbiology 145, 205–210.

Oke, O.L., 1967. Chemical changes in the Nigerian foodstuff Ogi. Food Technology 21, 202–204.

Omemu, A.M., Oyewole, O.B., Bankole, M.O., 2007. Significance of yeasts in the fermentation of maize for Ogi production. Food Microbiology 24, 571–576.

Oyewole, O.B., 2001. Characteristics and significance of yeasts' involvement in cassava fermentation for "fufu" production. International Journal of Food Microbiology 65, 213–218.

Rantsiou, K., Greppi, A., Garosi, M., Acquadro, A., Mataragas, M., Cocolin, L., 2012. Strain dependent expression of stress response and virulence genes of *Listeria monocytogenes* in meat juices as determined by micro-array. International Journal of Food Microbiology 152, 116–122.

Redkar, R.J., Dubé, M.P., McCleskey, F.K., Rinaldi, M.G., Del Vecchio, V.G., 1996. DNA fingerprinting of *Candida rugosa* via repetitive sequence-based PCR. Journal of Clinical Microbiology 34, 1677.

Sefa-Deheh, S., Sanni, A.I., Tetteh, G., Sakyi-Dawson, E., 1999. Yeasts in the traditional brewing of pito in Ghana. World Journal of Microbiology and Biotechnology 15, 593–597.

Teniola, O.D., Odunfa, S.A., 2001. The effect of processing methods on the levels of lysine, methionine and the general acceptability of 'ogi' processed using starter cultures. International Journal of Food Microbiology 24, 239–248.

Van der Aa Kuhle, A., Jespersen, L., Glover, R.L.K., Diawara, B., Jakobsen, M., 2001. Identification and characterization of *Saccharomyces cerevisiae* isolated from West African sorghum beer. Yeast 18, 1069–1079.

Vauterin, L., Vauterin, P., 1992. Computer-aided objective comparison of electrophoretic patterns for grouping and identification of microorganisms. European Journal of Clinical Microbiology 33, 633–641.

Versalovic, J., Schneider, M., de Bruijn, F.J., Lupski, J.R., 1994. Genomic fingerprinting of bacteria using repetitive sequence based PCR (rep-PCR). Methods in Molecular and Cellular Biology 5, 25–40.

Vieira-Dalodé, G., Jespersen, L., Hounhouigan, D.J., Moller, P.L., Nago, C.M., Jakobsen, M., 2007. Lactic acid bacteria and yeasts associated with gowé production from sorghum in Benin. Journal of Applied Microbiology 103, 342–349.