

Full Length Research Paper

Characterisation of yeasts isolated from traditional opaque beer beverages brewed in Zimbabwean households

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Received 27 October, 2014; Accepted 9 February, 2015

Variability exists in raw materials and processing methods used to produce household traditional opaque beers in Zimbabwe, resulting in beers of variable quality, depending on fermenting micro-organisms involved. Yeasts are important in determining the alcohol content, nutrition and organoleptic properties of the beers. This study aimed at determining the diversity and characteristics of the predominant yeasts isolated from a variety of beers collected from rural households in different geographical localizations. Predominant yeasts from 13 beer samples were characterized using morphological, biochemical and physiological tests. A total of 14 morphologically different yeasts were isolated. Yeast counts in the beer samples ranged from 7.87 to 9.56 log colony forming units/ml. From the 14 yeast isolates, a total of 11 yeasts were identified to species level. *Saccharomyces cerevisiae* was the predominant species identified in the beers. Other yeast species identified in the beers were *Issatchenkia occidentalis*, *Kluyveromyces marxianus*, *Candida glabrata* and *Sporobolomyces holsaticus*. Two yeast isolates were identified as belonging to the genus *Rhodotorula*. Ten of the isolates were able to ferment at least one of the fermentation substrates D-glucose, D-galactose, maltose, sucrose and raffinose, while three isolates were incapable of fermenting any of the fermentation substrates used. None of the isolates were able to ferment lactose. Five of the *S. cerevisiae* isolates were able to grow at 40°C while *K. marxianus* was the only isolate capable of growing at 45°C.

Key words: Yeasts, characterisation, traditional opaque beer.

INTRODUCTION

Traditional opaque beers in Zimbabwe are of socio-cultural and nutritional value and are marketed for income generation. A variety of cereals are used in the production of traditional opaque beers at household and

village levels in Zimbabwe (Sanni, 1993; Mugocha et al., 2000). Most beers are prepared mainly from bulrush millet (*Pennisetum typhoideum*) and finger millet (*Eleusine coracana*) malts, although sorghum (*Sorghum*

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bicolor) malt and sprouted maize are occasionally used (Gadaga et al., 1999). Preparation of traditional opaque beer varies in the different regions of Zimbabwe and various methods have been cited by several reviewers (Benhura and Chingombe, 1989; Chamunorwa et al., 2002; Lyumugabe et al., 2012). The method used to prepare opaque beer is a tradition preserved by the brewers and passed down to the next generation (Liyumugabe et al., 2012). Generally, the preparation of traditional opaque beer includes the cooking of a cereal meal, souring, mashing, straining and alcoholic fermentation for seven days (Beta et al., 1997; Bvochora et al., 2001; Chamunorwa et al., 2002). The beer is consumed in an active state of fermentation and has a short shelf life.

Traditional opaque beer is produced through an uncontrolled, spontaneous fermentation process and the micro-organisms responsible for the fermentation are believed to include yeasts and bacteria from the malt, and from fermentation pots passed from previous brews (Gadaga et al., 1999). Based on the use of various raw materials and chance inoculation, varied yeasts and bacteria are likely to be involved in the production of the traditional beer, resulting in beers with varied organoleptic properties, shelf life and safety. Microbiological and biochemical characteristics of traditional sorghum beers have been studied in many African countries, targeting strain isolation and identification, development of starter cultures and improvement of quality and safety (Maoura et al., 2005; Lyumugabe et al., 2012). Very varied yeasts and bacteria have been found in some African sorghum beers, with *Saccharomyces cerevisiae* and *Lactobacillus* sp. usually predominating (Maoura et al., 2005; Lyumugabe et al., 2010; Kayode et al., 2011). Chamunorwa and co-workers (2002) identified a variety of lactic acid bacteria from sorghum opaque beer brewed in Zimbabwe.

While traditional opaque beer forms a very important part of the Zimbabwean culture, there are no published reports of studies of the varied yeasts involved in the fermentations. Yeasts play an important role in determining the alcoholic content of the beers as well as overall product quality and nutrition. Isolation and characterisation of the diverse yeast microflora is therefore important in potential improvement of the efficiency of fermentation and production of consistent quality and safe beers.

The present study therefore involved isolating, characterising and identifying, where possible, the yeasts responsible for the fermentation to produce traditional opaque beers prepared from various raw materials in various households and villages.

MATERIALS AND METHODS

Collection of traditional opaque beer samples

Thirteen (13) samples of traditional opaque beer were randomly

collected from 12 rural households in various regions of Zimbabwe, namely Domboshava, Musana, Chihota, Chipinge and Masvingo. All the beer samples were collected at a stage when the beer was ready for consumption, in an actively fermenting state. Beer samples were collected from the traditional earthenware beer pots and placed in plastic screw-capped bottles. The samples were analyzed within 3 h after sampling.

Microbial analysis of traditional opaque beer samples

Aliquots of the beers (1 ml) were serially diluted using sterile peptone water (Oxoid) and 0.1 ml quantities were plated out on Wort agar (Oxoid) plates to determine yeast counts, respectively. Plates were incubated at room temperature (approximately 26-27°C) for five days then colony counts were carried out using a colony counter (Stuart Scientific). Microbial load was expressed as log colony forming units per millilitre (log cfu / ml) of opaque beer.

Isolation and identification of yeasts

Predominant yeast colonies with distinct morphological differences were picked and purified by streaking three times on Wort agar. Cellular morphology was examined using a Zeiss phase contrast microscope. The sources of the isolates used in the study are shown in Table 2.

The formation of ascospores was examined according to the method described by Van der Walt and Yarrow (1984). The Dalmat plate technique (Kurtzman and Fell, 1998; Barnett et al., 2000) was used to characterise the formation of pseudo- and true hyphae.

Yeast isolates were identified using the conventional methods described by Van der Walt and Yarrow (1984), Kurtzman and Fell (1998) and Barnett et al. (2000). Characterisation of the yeasts was carried out by subjecting the isolates to various physiological and biochemical tests which included fermentation of sugars, liquid assimilation of carbon compounds, assimilation of nitrogen compounds, growth at 25, 30, 37, 40, 42 and 45°C, growth in vitamin free media, cycloheximide resistance, urease test and growth at high sugar concentrations. All tests were carried out in duplicate.

Determination of yeast lipid profiles

Lipids in the yeast cells were extracted using chloroform/methanol (2:1) following the method described by Mpofu et al. (2008). Fatty acids in the lipids were determined by an HP5890 Series II Gas Chromatograph equipped with a Supelcowax 10 column (30 m x 0.55 mm) (Mpofu et al., 2008). Fatty acid profiles detected for the yeast strains were compared with known fatty acid profiles of yeast strains for their identification.

RESULTS AND DISCUSSION

Yeast and lactic acid bacteria counts

Table 1 shows the yeast counts obtained for the opaque beer samples obtained from various Zimbabwean rural households. Yeast counts ranged from 7.87 to 9.56 log cfu/ml.

The range for yeast counts is similar to the range reported by other workers for the Bulgarian cereal-based beverage, *boza* (Gotcheva et al., 2000). Yeast counts in

Table 1. Yeast counts of opaque beer samples from rural Zimbabwean households.

Beer sample number	Site of collection	Raw materials used in beer preparation	Yeast count (log cfu/ml)
1	Domboshava	Maize meal and bulrush millet malt	7.87
2	Domboshava	Maize meal and finger millet malt	8.71
3	Musana	Maize meal and bulrush millet malt	8.91
4	Chihota	Maize meal and sorghum malt	9.22
5	Chipinge	Maize meal, bulrush millet malt and sorghum malt	8.76
6	Chipinge	Maize meal and bulrush millet malt	8.11
7	Chipinge	Maize meal and bulrush millet malt	9.11
8	Chipinge	Maize meal, bulrush millet malt and sorghum malt	9.22
9	Chipinge	Maize meal and sorghum malt	9.38
10	Chipinge	Maize meal and sorghum malt	8.78
11	Masvingo	Finger millet meal and malt	9.25
12	Masvingo	Finger millet meal and malt	9.44
13	Masvingo	Finger millet meal and malt	9.56

Results represent the mean of duplicate sample determinations.

Table 2. Table showing the opaque beer samples from which the characterized yeasts were isolated and the yeast identities.

Yeast reference Number	Beer sample number	Raw materials used in preparation of beer	Site of collection	Yeast isolate identity
1	1	Maize meal and bulrush millet malt	Domboshava	<i>Saccharomyces cerevisiae</i>
2	11	Finger millet meal and finger millet malt	Masvingo	<i>Saccharomyces cerevisiae</i>
3	2	Maize meal and bulrush millet malt	Domboshava	<i>Saccharomyces cerevisiae</i>
4	5	Maize meal and sorghum malt	Chipinge	<i>Saccharomyces cerevisiae</i>
5	6	Maize meal, bulrush millet malt and sorghum malt	Chipinge	<i>Saccharomyces cerevisiae</i>
6	2	Maize meal and bulrush millet malt	Domboshava	Unidentified
7	2	Maize meal and bulrush millet malt	Domboshava	<i>Issatchenkia occidentalis</i>
8	7	Maize meal, bulrush millet malt and sorghum malt	Chipinge	<i>Kluyveromyces marxianus</i>
9	6	Maize meal and sorghum malt	Chipinge	<i>Sporobolomyces holsaticus</i>
10	8	Maize meal and sorghum malt	Chipinge	<i>Saccharomyces cerevisiae</i>
11	11	Finger millet meal and malt	Masvingo	<i>Rhodotorula</i>
12	8	Finger millet meal and malt	Masvingo	<i>Rhodotorula</i>
13	12	Finger millet meal and malt	Masvingo	<i>Issatchenkia occidentalis</i>
14	13	Maize meal, finger millet malt and bulrush millet malt	Masvingo	<i>Candida glabrata</i>

the range of 7.41 to 7.50 log cfu/ml were reported for *boza*. The range of yeast counts of 7.78 to 9.56 log cfu/ml recorded for the beers was higher than the range of 4.9 to 6.79 log cfu/g, recorded in Nigerian traditional fermented beverages (Sanni and Lonner, 1993). Microflora involved in many cereal based fermentations is mainly a mixture of lactic acid bacteria and yeasts (Chamunorwa et al., 2002; Kayode et al., 2011; Luymugabe et al., 2012).

Identification of yeasts

Fourteen morphologically different yeasts were isolated from the 13 traditional opaque beer samples. The isolates were coded 1 to 14. Table 2 shows the yeast isolates and the opaque beer samples from which the studied yeasts were isolated.

From the 14 yeasts isolated, 11 were identified to species level, 2 were identified to the genus level and 1

Table 3. Fermentation of different sugars by yeasts isolated from opaque beer.

Sugar	Yeast reference number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D-Glucose	+	+	+	+	+	+	+	+	-	+	-	-	+	+
D-Galactose	+	+	+	+	+	-	-	+	-	+	-	-	-	-
Maltose	+	+	+	+	+	+	-	+	-	+	-	-	-	-
Sucrose	+	+	+	+	+	-	-	+	-	+	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+	+	+	+	+	-	-	+	-	+	-	-	-	-

isolate was not identified. Yeast isolates identified were *S. cerevisiae*, *Issatchenkia occidentalis*, *Kluyveromyces marxianus*, *Sporobolomyces holsaticus* and *Candida glabrata*. Isolates 11 and 12 were identified as belonging to the genus *Rhodotorula*, the nearest species being *Rhodotorula mucilaginosa* and *Rhodotorula minuta*. *K. marxianus* species was previously isolated from sorghum grain and sorghum beer in South Africa and from *pozol*, fermented maize dough in Mexico (Barnett et al., 2000). In this study, isolate *K. marxianus* was isolated from traditional opaque beer brewed using maize meal, bulrush millet malt and sorghum malt. The *S. holsaticus* species is commonly found in leaves (Kurtzman and Fell, 1998) and may be originating from leaves used for sieving the beer in some beer-brewing households. Isolates 11 and 12 were identified up to genus level as *Rhodotorula*. The nearest species may be *R. mucilaginosa* or *R. minuta*, according to the identification keys used (Barnett et al., 2000). *Rhodotorula* yeasts were isolated from traditional opaque beer brewed using finger millet meal and finger millet malt in Masvingo. *R. mucilaginosa* is reported to have been previously isolated from pasteurized beer in Germany and from malt syrup (Kurtzman and Fell, 1998). Isolate 14 was identified as belonging to the genus *Candida*. Isolate 14 was identified as *C. glabrata*, which was previously isolated from sorghum malt (Kurtzman and Fell, 1998) and was isolated in a brew using finger millet malt and bulrush millet malt in this study.

Morphological characteristics of yeasts

S. cerevisiae isolates were characterized by cream to pale brown colonies, round to ovoid cells, multipolar budding and ascospore formation. *I. occidentalis* isolates had pale-cream, butyrous colonies with margins fringed with pseudohyphae and ovoid ascospores. *K. marxianus* was characterized by cream, flat butyrous colonies, cylindrical, highly branched cells and cylindrical asci. Yeast cells of the species *S. holsaticus* were characterized by pink, mucoid colonies, blastospores in chains and kidney-shaped ballistoconidia borne on stalks. Yeasts belonging to the genus *Rhodotorula* had pink,

butyrous colonies and spheroidal cells, often in chains. The morphological and cultural characteristics of the *Candida* species showed that the species had smooth, flat, cream colonies, round to ovoid cells and no ascospores were observed in this species.

Fermentation of sugars

None of the *S. cerevisiae* isolates could ferment lactose while the majority of the *S. cerevisiae* isolates were able to ferment D-glucose, D-galactose, sucrose and raffinose (Table 3). Due to its ability to ferment a wide range of sugars, *S. cerevisiae* has been found to predominate in most traditional opaque beers and other fermented beverages (Mugula et al., 2003; Lyumugabe et al., 2010) and is used in commercial production of beer, wine and bread. Most of the yeast isolates were able to ferment D-glucose, D-galactose, sucrose and raffinose. *K. marxianus* was capable of fermenting glucose, galactose, sucrose and raffinose. *K. marxianus* and its anamorph, *Candida kefir* are important yeast species in sorghum fermented beverages because of their ability to ferment a wide range of sugars. *C. glabrata* could ferment glucose only out of the sugars studied. *I. occidentalis* species is capable of fermenting glucose and has been isolated before from bread and bakers' yeast (Barnett et al., 2000).

Assimilation of carbon compounds

Table 4 shows the carbon assimilation pattern of the various yeast isolates. The majority of *S. cerevisiae* yeast isolates were capable of assimilating D-glucose, D-galactose, sucrose, maltose, α , α -trehalose and raffinose. Assimilation of lactate is a variable characteristic in *S. cerevisiae* (Kurtzman and Fell, 1998) and isolates 4 and 5 were incapable of assimilating lactate. Isolate 6, which was not identified in this study, had most physiological characteristics similar to *S. cerevisiae* but differed in its ability to assimilate L-arabinose, L-arabinitol and D-mannitol. *I. occidentalis* yeast species were able to assimilate D-glucose, glycerol, lactate, succinate and

Table 4. Assimilation of carbon compounds.

Carbon compound	Yeast reference number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	-	-
L-Sorbose	-	-	-	-	-	-	-	-	+	-	+	+	-	-
D-Glucosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Ribose	-	-	-	-	-	-	-	-	+	-	+	+	-	-
D-Xylose	-	-	-	-	-	-	-	+	-	+	+	-	-	-
L-Arabinose	-	-	-	-	-	+	-	-	+	-	+	+	-	-
D-Arabinose	-	-	-	-	-	-	-	-	+	-	+	+	-	-
L-Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	-	-	+	+	+	+	+	-	-
Maltose	+	+	+	+	+	+	-	-	-	+	+	+	-	-
α,α -Trehalose	+	+	+	+	+	+	-	-	-	-	+	+	-	+
Methyl α Glucoside	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Salicin	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+	+	+	+	+	-	-	+	+	+	+	+	-	-
Melezitose	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Soluble starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	+	+	+	-	-	-	+	-
Meso erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	+	-	+	+	-	-
Xylitol	-	-	-	-	-	-	-	+	-	-	+	+	-	-
L-Arabinitol	-	-	-	-	-	+	-	-	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 keto D-Gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-Gluconate	-	-	-	-	-	-	-	-	+	-	-	-	-	-
DL-Lactate	-	+	+	-	-	-	+	+	-	-	-	-	+	-
Succinate	-	-	-	-	-	-	+	+	+	-	+	+	+	-
Citrate	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Propan 1,2 diol	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Butan 2,3 diol	-	-	-	-	-	-	-	+	-	-	-	-	-	-

ethanol as sole sources of carbon. *K. marxianus*, *S. holsaticus* and *Rhodotorula* isolates were able to assimilate most of the carbon compounds tested while *C. glabrata* managed to assimilate D-glucose; α,α -trehalose; D-gluconate and ethanol only, of the carbon sources tested. Further studies need to be carried out to determine the role of *C. glabrata* in beer.

Assimilation of nitrogen compounds

All the yeast isolates were able to assimilate ammonium

sulphate (Table 5). *S. cerevisiae* and *C. glabrata* species were unable to use nitrate, nitrite, ethylamine, L-lysine, creatine and creatinine as sole nitrogen sources. *I. occidentalis* and *K. marxianus* were able to use ethylamine and L-lysine as nitrogen sources while *S. holsaticus* and the *Rhodotorula* genus yeast isolates were able to assimilate ethylamine.

Growth at different temperatures

All the *S. cerevisiae* yeast strains and *I. occidentalis* were

Table 5. Assimilation of nitrogen compounds.

Nitrogen compound	Yeast reference number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ammonium sulphate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrite	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethylamine	-	-	-	-	-	-	+	+	+	-	+	+	+	-
L-lysine	-	-	-	-	-	-	+	+	+	-	+	+	+	-
Creatine	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6. Growth at different temperatures.

Temperature	Yeast reference number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+	+	-	+	-	-	+	+
40°C	-	-	+	+	+	+	+	+	-	+	-	-	+	+
42°C	-	-	-	-	-	-	-	+	-	-	-	-	-	-
45°C	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Table 7a. Fatty acid ratios (%) of *Saccharomyces cerevisiae* yeasts and isolate 6 from opaque beer.

Fatty acid	Yeast reference number						
	1	2	3	4	5	6	10
16:0	16.90	9.18	16.16	16.13	14.71	15.78	15.65
16:1	34.18	47.95	39.17	34.70	39.33	34.15	35.55
18:0	11.82	6.95	12.06	13.44	10.66	12.36	12.22
18:1	31.71	34.45	28.90	31.86	33.28	35.81	34.03
18:2	5.38	1.46	3.72	3.87	2.06	1.90	2.54
18:3 ω 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18:3 ω 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00

capable of growing at temperatures from 25 to 37°C (Table 6). *S. cerevisiae* isolates 3, 4, 5 and 10 and *C. glabrata* were able to grow at 40°C. Thermotolerant yeast isolates are very useful in industrial fermentation plants where high fermentation temperatures are used. There are many advantages associated with producing ethanol at higher than conventional temperatures (25-30°C) and these include reduced running costs with respect to maintaining growth temperatures in large scale systems, reduced risk of contamination and increased productivity (Singh et al., 1998). There are several reports on thermotolerant *Kluyveromyces* yeast strains (Banat et al., 1995; Singh et al., 1998), but few reports describe *S. cerevisiae* isolates capable of growth and ethanol production at elevated temperatures (Banat et al., 1995; Abdel-Fattah et al., 2000). The *K. marxianus* species are also thermotolerant, being able to grow at temperatures

as high as 45°C, thus making them important for industrial production of fuel ethanol.

Fatty acid ratios

Fatty acid ratios of 16:1 and 18:1 fatty acids were characteristically high in *S. cerevisiae* (Table 7a). Table 7b shows the fatty acid ratios of isolates 7 and 13 and other yeasts besides the *S. cerevisiae* species. The potential use of lipid profiles for yeast identification is under current investigation.

Additional tests

All the *S. cerevisiae* isolates were incapable of growth in

Table 7b. Fatty acid ratios (%) of yeasts isolated from opaque beer.

Fatty acid	Yeast reference number						
	7	8	9	11	12	13	14
16:0	18.50	21.64	18.62	11.74	13.03	20.16	16.26
16:1	19.80	25.13	2.60	2.99	2.16	11.55	31.78
18:0	8.84	5.35	4.64	2.33	3.42	9.96	11.75
18:1	39.42	34.09	39.05	71.80	64.31	49.71	36.53
18:2	10.05	12.64	30.51	9.98	15.48	8.61	3.68
18:3 ω 3	3.38	1.05	4.57	1.15	1.60	0.00	0.00
18:3 ω 6	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 8. Results of additional characterisation tests carried out on yeasts isolated from opaque beer.

Growth in/on	Yeast reference number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
0.01 % Cycloheximide	-	-	-	-	-	-	-	+	+	-	+	+	+	+	
0.1% Cycloheximide	-	-	-	-	-	-	-	+	+	-	+	+	-	-	
1% Acetic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
50% Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
60% Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Arbutin	-	-	-	-	-	-	-	+	-	+	+	-	-	-	
Vitamin free medium	-	-	-	-	-	-	+	-	+	-	+	+	+	-	
Test															
Diazonium Blue B test	-	-	-	-	-	-	-	-	+	-	+	+	-	-	
Starch test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Urease test	-	-	-	-	-	-	-	-	+	-	+	+	-	-	
Ascospore/Basisdiospore	+	+	+	+	+	+	+	+	-	+	-	-	+	-	
Pseudo/true hyphae	-	-	-	-	-	-	+	-	-	-	-	-	+	-	

vitamin free media and in 0.01 and 0.1% cycloheximide concentrations (Table 8). None of the *S. cerevisiae* isolates displayed urease activity or were able to split arbutin. *R. mucilaginosa* isolates displayed urease activity and revealed β glucosidase activity by their ability split arbutin.

Conclusion

A wide diversity of yeasts with different characteristics was isolated and identified in traditional opaque beer samples, *S. cerevisiae* being predominant. All the yeast isolates were capable of using glucose as a carbon source. The non-fermenting yeast isolates may contribute to the flavor characteristics of the beer or may be spoilage microbes. Further studies will involve molecular characterization of the yeast isolates in an attempt to better characterize them and determine the role of the various yeast species in determining the overall product quality of the beers. Studies are underway to determine the biochemical characteristics of traditional opaque

beers. Several thermo tolerant yeasts were isolated and it is necessary to further research on their fermentation characteristics at the elevated temperatures for potential use in industrial ethanol production.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

We thank UNESCO-MIRCEN for sponsorship, Andri van Wyk and Pete Botes for technical assistance and Norbert Tsanyiwa for assistance during the collection of the traditional opaque beer samples.

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