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Screening local cereal-based beverages in Tanzania for yeast contaminants

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Abstract

During spontaneous fermentation of cereals; yeasts ferment carbohydrates to produce alcohol and they also provide vitamins, amino acids, peptides, and nucleotides needed by lactic acid bacteria as well as produce flavour compounds. Nevertheless, spontaneous fermentation is prone to yeast contamination from the surroundings which pose a health risk of opportunistic yeast infection. A study was carried out involving culturing, isolation and identification of yeast contaminants present in the local cereal-based beverages namely Kindi, Kimpumu, Togwa and Mbege purposively sampled and collected from Morogoro, Mbeya and Kilimanjaro regions in Tanzania between February and May 2019. The results disclosed 24% of the yeasts actively involved in the fermentation were opportunistic and identified as *Candida zeylanoides, Candida albicans, Cryptococcus gattii, Rhodotorula minuta, Candida ciferrii, and Candida dubliniensis*. Such contamination levels from the studied samples sets a base for further research to establish mechanisms of reducing exposure of cereal-based beverage consumers to pathogenic effects of the opportunistic yeasts which may include infections by *Candida* spp.

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Introduction

Yeasts play an important role during spontaneous fermentation of traditional cereal-based foods and beverages in most African countries (Pedersen *et al.*, 2012). Despite being the major producer of alcoholic cereal beverages; they are also important in the development of taste and flavors of such drinks (Aka *et al.*, 2014; Mukisa *et al.*, 2017). Yeast contaminants in most traditional cereal-based fermented beverages in Africa (Tanzania inclusive) are inevitable owing to the fact that preparation of such beverages is achieved through spontaneous fermentation which involves a variety of competing microbes (Aka *et al.*, 2014; Achi and Asamudo, 2019). As such screening of microbes for use as starter culture before fermentation is rarely practiced (Enujiugha and Badejo, 2017).

Yeasts are widely spread in the world and are found in various materials such as soil, water, plants and plant products, animals, skin, gastro-intestinal tract of animals and fermented foods (Lara-Hidalgo *et al.*, 2017). They are unicellular fungi in contrast to molds which are multicellular fungi. They differ from bacteria by having oval, elongated, elliptical or spherical cell shapes which are larger in size ranging from 5-8 µm in diameter (James *et al.*, 2005). They grow in acidic conditions; over a wide range of acid pH as well as in ethanol (up to 18%). Many of them grow when there is 55 - 60% sucrose. They produce a variety of colors such as creamy, pink and red (James *et al.*, 2005). Most yeasts found in foods divide by fission or budding. The fungal kingdom to which they belong is the second largest group of eukaryotic organisms on earth and their number is estimated at 1.5-5.1 million species *(Raja et al.*, 2017; Schoch *et al.*, 2012).

Yeasts are economically important to human life. Their major role in the production of fermented foods and alcoholic beverages cannot be overlooked (Hierro *et al.*, 2004; Raja *et al.*, 2017). They produce beneficial secondary metabolites like vitamins and antibiotics (Hierro *et al.*, 2004; Raja *et al.*, 2017). Despite their positive role to human and environment, yeasts are harmful as they can cause spoilage which results to huge economic losses in the food chain (Hierro *et al.*, 2004). Some Yeast spp. are opportunistic pathogens and thus caution should be taken when selecting yeast strains for use as starter cultures. Such yeasts include *C. albicans, Candida parapsilosis, Candida glabrata, C. dubliniensis, C. ciferrii* and some other Candida spp.(Silva *et al.*, 2012). When cultured on Sabouraud dextrose agar (SDA); colonies of *Candida* spp. appear creamy or yellow. The yeast *R. minuta* grow as yellow creamy colonies on SDA while *C. gattii* grow as butyrous glistening colonies. *Candida* spp. can also be identified by use of CHROMagar® *Candida* (CHROMagar®, Paris France) which is a differential medium (Silva *et al.*, 2012). Generally, most *Candida* spp. are innocuous but some of them may proliferate in the host and induce Candidiasis diseases particularly in individuals with weakened immune system (Johansen *et al.*, 2019). To date, almost 41 - 47% of total yeast infections globally is caused by *C. albicans* (Johansen *et al.*, 2019).

Yeasts in spontaneously fermented cereal-based beverages, may originate from the raw materials (cereals, water), beverage handlers, processing equipment and surrounding environment (Todorov and Holzapfel, 2014; Lara-Hidalgo *et al.*, 2017; Johansen *et al.*, 2019). The functional properties of yeasts in African fermented food and beverages comprise stimulation of lactic acid bacteria, carbohydrate fermentation, production of flavor compounds, production of tissue-degrading enzymes, removal of mycotoxins and imparting probiotic health benefits (Johansen *et al.*, 2019). In a study by Johansen *et al.* (2019), *Saccharomyces cerevisiae* was found to be the major yeast species isolated from 43 indigenous sub-Saharan African fermented food and beverages, *Pichia kudriavzevii* being the second and *Kluyveromyces marxianus* the third. It has been reported that *S. cerevisiae* occurs most frequently in all indigenous African fermented food and beverages and where it occurs dominate all other yeast species. It dominates fermentation in 93% of all alcoholic beverages in Africa (Johansen *et al.*, 2019). Similarly, the fermentation of non-alcoholic beverages is

dominated by *P. kudriavzevii* whereas *Candida tropicalis* and *K. marxianus* are invariably found in African fermented food and beverages. Most of the African spontaneously fermented food and beverages are recognized for their natural flavors and tastes. In these beverages; yeasts are recognized for their role of development of flavor compounds from carbohydrates (Johansen *et al.*, 2019; Mukisa *et al.*, 2017).

The major flavor compounds released by yeasts during fermentation of cereal beverages comprise organic acids, aldehydes, alcohols, and esters (Johansen *et al.*, 2019; Lara-Hidalgo *et al.*, 2017; Mukisa *et al.*, 2017). In one of common cereal-based beverage in Tanzania namely Togwa; the flavour compounds that yeasts produce include acetaldehyde, ethanol, acetoin, diacetyl, 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, 2-methylpropan-1-ol, 2-methylbutan-1-ol, and 3-methylbutan-1-ol (Johansen *et al.*, 2019; Mugula *et al.*, 2003a). In synergistic action with LAB, some yeasts have been found to carry out biological decontamination by reducing the hazardous effects of mycotoxins in African fermented cereal foods and beverages. Strains of *S. cerevisiae* have been reported to reduce mycotoxins in African fermented cereal beverages (Johansen *et al.*, 2019) and there is possibility for antagonistic yeasts to be used to inhibit the growth of pathogenic bacteria (Lara-Hidalgo *et al.*, 2017). *S. cerevisiae* and *S. cerevisiae* subsp. *boulardii* are the only known probiotic yeasts to date (Pedersen *et al.*, 2012; Ogunremi *et al.*, 2015; Lara-Hidalgo *et al.*, 2017). In Tanzania and many other African countries; screening of traditional cereal-based beverages for contaminants particularly pathogenic yeasts is rarely done (Enujiugha and Badejo, 2017). And since the beverages are consumed in the fermenting state, consumers of such drinks are exposed to pathogenic microbes particularly bacteria and yeasts which may lead to toxic diseases (Aka *et al.*, 2014; Franz *et al.*, 2014). Consequently, this study focused on screening, isolation and identification of yeast contaminants present in fermented cereal-based beverages namely Kindi, Kimpumu, Togwa and Mbege with the objective of establishing a base for further studies on potential health hazards such drinks might pose to consumers.

Materials and methods

Sampling and collection of the samples

Purposive sampling was used to collect four samples of Kindi and Kimpumu at Kalobe and Nzovwe villages respectively in Mbeya region, Togwa at Vituli village in Morogoro region and Mbege at Boro village in Kilimanjaro region. Samples were collected aseptically using sterile syringes and put in labelled glass bottles, placed in a cool box and taken to the laboratory for preservation at 4 °C.

Enrichment and isolation of yeast species

Samples (1mL) of the collected cereal-based beverages were enriched in 20 mL Sabourad Dextrose Agar (SDA) broths (Biomérieux SA, Marcy - l'Etoile, France) at 37 °C for 24 h and then 0.5µl of each enrichment media was inoculated on SDA agar (Oxoid, UK) by the method of streaking and incubated at 37 °C for 48 – 72 h. Colonies which appeared dome-shaped, mucoid or creamy and glittering were isolated and slanted on SDA agar.

Identification of yeast contaminants

Identification of yeast contaminants was achieved by subculturing of the isolates on slants employing SDA agar and then performing Gram Stain tests. Isolates were Gram positive if they appeared purple and Gram negative if they appeared red or pink. Microbial shapes (Spherical, ovoid or elliptical) and arrangements (Single, pair, triple, tetrad, or chains) were used to characterize the yeast cells and tentatively identified based on morphological characterization as described by Kurtzman *et al.*, (2011). Isolates were tentatively identified as yeast cells if they were spherical or ovoid in shape, Gram positive, grow on SDA as dome-like colonies with entire edge; mucoid, creamy and glittering. Affirmation of the identity of yeast spp. was done by employing the API® 20 C AUX (Biomérieux SA, Marcy - l'Etoile, France) following the manufacturer's procedure. This was only done with a focus on yeast

cells singled out to be contaminants. Yeast contaminants in the fermented cereal-based beverages were recognized based on outcomes of previous studies by Pereira *et al.*, 2010; Wirth and Goldani, 2012; Khosravi *et al.*, 2013; Chen *et al.*, 2014; and Polvi *et al.*, 2015.

Results

Yeast isolates

Twenty-five (25) colonies presumed to be yeasts were isolated from the collected samples of cereal-based beverages namely Kindi, Kimpumu, Togwa and Mbege (Table1).

Table 1. Isolates characterized as yeast contaminants in local cereal-based beverages.

Isolate No.	Source	Morphological characterization	Morphological selection (presumed contaminant/not contaminant)	API Identification	Isolate No.	Source	Morphological characterization	Morphological selection (presumed yeast contaminant/not contaminant)	API Identification
1	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	14	Kimpumu	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
2	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	15	Mbege	Creamy yeast-like colonies; G+ve budding cells with pseudo hyphae	contaminant	*Candida ciferrii*
3	Kindi	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	16	Kimpumu	Large, round, creamy colonies; G+ve ovoid budding cells, pseudo hyphae	contaminant	*Candida albicans*
4	Kimpumu	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	17	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
5	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	18	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
6	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	19	Mbege	Smooth, glistening, whitish colonies; G+ve ovoid cells, pseudo hyphae	contaminant	*Candida dubliniensis*
7	Kimpumu	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	20	Kindi	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
8	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	21	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
9	Kindi	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	22	Kindi	Yellow, creamy, round colonies; G+ve yeast-like cells	contaminant	*Rhodotorula minuta*
10	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	23	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
11	Togwa	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.	24	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
12	Kimpumu	Very small, whitish creamy yeast-like colonies; G+ve yeast-like cells in clusters, curving pseudo hyphae	Contaminant	*Candida zeylanoides*	25	Mbege	Large, Smooth, creamy, sparkling colonies; G+ve yeast cells with pseudo hyphae	not contaminant	n.d.
13	Kindi	Butyrous colonies; G+ve spherical budding cells	Contaminant	*Cryptococcus gattii*					

Note: n.d. means not done (isolate not presumed yeast contaminant based on morphological and microscopy features); G+ve = Gram positive.

Yeast spp. identified as contaminants

Among the 25 isolates, only 6 (24%) isolates were recognized as yeast contaminants in locally prepared cereal-based beverages in Tanzania. These were *C. zeylanoides, C. albicans, C. gattii, R. minuta, C. ciferrii,* and *C. dubliniensis* (Table 1).

Discussion

The screening results for yeast contaminants in locally prepared cereal-based beverages; Kindi, Kimpumu, Togwa and Mbege suggest that most of these beverages are also proliferated with non-beneficial yeasts (24% of yeasts taking part in fermentation) which appear in such beverages as opportunistic yeast pathogens. Such yeasts normally appear harmless in healthy humans but become most infective in immunocompromised persons (Satyanarayana and Kunze, 2009; Polvi *et al.*, 2015). The extent to which yeasts cause infection varies in different geographical regions and largely hinge on the nature of immune suppression of the patients and predominant yeast in the environment (Satyanarayana and Kunze, 2009). The opportunistic *C. zeylanoides* was found in the cereal-based beverages mostly in Kimpumu and based on a study by Khosravi *et al.* (2013); this yeast is rarely present in humans and animals. However, its presence has been confirmed on the skin, blood and nails of humans as an opportunistic yeast pathogen. It is thus an emerging opportunistic yeast pathogen which requires clinical strategy for its management (Khosravi *et al.*, 2013). It was present in the locally prepared fermented cereal beverages as a wild yeast contaminant.

The presence of *C. albicans* in the fermented cereal beverages shows the healthy risk the traditionally prepared cereal beverages pose to consumers. Opportunistic *C. albicans* has been reported as the most common causative agent of invasive yeast infection in humans (Satyanarayana and Kunze, 2009; Johansen *et al.*, 2019) and if it grows uncontrollable, it can penetrate deep into the body such as in the blood stream, the kidney, heart and brain. Most of the opportunistic yeast pathogens reported are *Candida* spp. (which include *albicans, krusei, tropicalis, glabrata, parapsilosis, rugosa, kefyr,* and *dubliniensis*), *Trichosporon* spp., *Cryptococcus* spp. (*C. gattii, C. neoformans, C. grubii*) and some other genera (Satyanarayana and Kunze, 2009; Pereira *et al.*, 2010). This is in line with the results of this study whereby *Candida* spp. were predominant as yeast contaminants. The yeast *C. albicans* is known to be the potent agent of Candidiasis causing almost 47% of total yeast infection worldwide (Johansen *et al.*, 2019). However, *C. albicans* is found in few indigenous African fermented foods and beverages as a contaminant (Johansen *et al.*, 2019). In recent years, non-albicans species of *Candida* have been reported to cause more than 50% of the yeast infections (Pereira *et al.*, 2010; Miceli *et al.*, 2011).

The presence of *C. gattii* in the spontaneously fermented cereal-based beverages may cause yeast infection to consumers that can affect the central nervous system, the lungs, the sight and other parts of the body (Chen *et al.*, 2014). This is because the yeast has comparable virulent properties to *C. neoformans* which belongs to the group of yeasts classified as opportunistic yeast pathogens (Chen *et al.,* 2014).

The yeast *R. minuta* was found among the yeasts present in the fermented cereal-based beverages particularly in Kindi. In the past, *Rhodotorula* spp. were regarded as non-pathogenic; but in recent studies they have been categorized as emerging opportunistic yeast pathogens in humans (Miceli *et al.*, 2011; Wirth and Goldani, 2012). The genus *Rhodotorula* comprises eight species but only three of them; *R. Minuta, R. glutinis,* and *R. mucilaginosa* have been observed to cause disease in humans (Wirth and Goldani, 2012). The infections caused by *Rhodotorula* spp. in humans include prosthetic joint, skin, meningeal, ocular and peritoneal infections (Miceli *et al.*, 2011; Wirth and Goldani, 2012).

Like other *Candida* spp. discussed earlier on, *C. ciferrii* and *C. dubliniensis* are common microflora in the gastro-intestinal tract (GIT) and genitourinary

tract (GUT) of human beings with the tendency to attack and bring about diseases when immuno-imbalances occurs within the human body (Miceli *et al.*, 2011). Immune response of the host is the crucial factor of the kind of infection caused by *Candida* spp. (Miceli *et al.*, 2011). *Candida* spp. as previously stated are opportunistic pathogens with the propensity to bring about various superficial and systemic infections (Miceli *et al.*, 2011). The presence of these two opportunistic yeast pathogens in the locally prepared cereal-based beverages indicates the health risk associated with consumption of the beverages and the extent to which consumers are exposed to fungemia. The yeasts; *C. ciferrii* and *C. dubliniensis* are among the emerging opportunistic yeast pathogens and also referred to as non-albicans *Candida* spp. which have been observed to cause more than 50% of all yeast infections globally (Pereira *et al.*, 2010; Miceli *et al.*, 2011; Polvi *et al.*, 2015).

Based on this study, there is a continually need of ensuring proper screening of the microflora for safety, quality, functionality and reliability of the end products.

Conclusion

This study has indicated that spontaneously fermented cereal-based beverages in Tanzania are prone to contamination by opportunistic yeast pathogens (24% of participating yeasts). The yeast contaminants isolated and identified from the locally prepared cereal-based beverages (Kindi, Kimpumu, Togwa and Mbege) were *C. zeylanoides, C. albicans, C. gattii, R. minuta, C. ciferrii,* and *C. dubliniensis*. *Candida* spp. were the most predominant yeast contaminants in the screened cereal-based beverages. Other yeasts namely *C. gattii* and *R. minuta* were found to be minor yeast contaminants in the beverages. The study has revealed that spontaneously fermented cereal-based beverages in Tanzania are susceptible to opportunistic yeast pathogens and thus exposing the consumers to yeast infections particularly Candidiasis caused by *Candida* spp. Further researches to establish mechanisms of reducing exposure of cereal-based beverage consumers to pathogenic effects of the opportunistic yeasts which may include infections by *Candida* spp. are needed.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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