

Full Length Research Paper

***Candida* spp. associated with hot beverages of coffee and tea sold on street in Côte d'Ivoire**

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Some *Candida* species are considered as human opportunistic pathogens and can play an important role in spontaneous fermentations, but also as beverage-spoiling microorganisms. The aim of this pioneering study was to investigate *Candida* spp. in hot beverages, which is consumed mostly on the streets in Côte d'Ivoire. Yeast strains were isolated from 400 hot beverages of tea (200) and coffee (200) samples. Yeast cultures were identified at genus and species level by MALDI-TOF mass spectrometry at the biobank laboratory of the Pasteur Institute of Côte d'Ivoire. A total of 37 *Candida* isolates were clearly identified by MALDI-TOF (MS) and revealed 11 species of *Candida*: *C. krusei* (21.6%), *C. tropicalis* (18.9%), *C. parapsilosis* (16.2%), *C. guilliermondii* (16.2%), *C. pelliculosa* (8.1%), *C. dubliniensis* (5.4%), *C. rugosa* (2.7%), *C. kefyr* (2.7%), *C. silvicola* (2.7%), *C. lusitaniae* (2.7%) and *C. orthopsilosis* (2.7%). The results showed that *C. krusei* and *C. tropicalis* were the dominant yeasts in hot beverages from street vendors. *Candida* species were more isolated in tea (10%) than in coffee (8.5%). *C. tropicalis*, *C. pelliculosa* and *C. krusei* were more isolated in Cocody. *C. guilliermondii* and *C. parapsilosis* were more isolated in Port-Bouët. *C. dubliniensis* was only isolated in Yopougon town. The presence of *Candida* spp. in street hot beverages could cause a sanitary risk to consumers or be used as a novel source for biotechnological uses to be explored in future work.

Key words: Street hot beverages, *Candida* spp., coffee, tea, food safety.

INTRODUCTION

Yeasts are unicellular eukaryotes microorganisms that belong to the Kingdom of Fungi and play various roles in affecting the quality and safety of food products (Khattab

et al., 2016). They are ubiquitous, and commonly spoilage fruits, vegetables and other plant materials, in addition to, an association with soil and insects (Bekatorou et al.,

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2006). Yeasts are the major producer of biotechnology products worldwide, exceeding production in capacity and economic incomes than other groups of industrial microorganisms. Yeasts have wide ranging fundamental and industrial importance in scientific, food, medical and agricultural sciences (Johnson, 2013). Traditionally, yeasts have been used for several fermentations such as alcoholic beverages, biomass production and other fermented food. Fermented foods and beverages play an important role in the diet of African people, and the most often, these foods and beverages are produced at household level or at small industrial scale and are consequently often of varying quality and stability (Mogmenga et al., 2017; Tankoano et al., 2017).

Fruit juices and soft drinks constitute suitable environment for growth of most microorganisms. Actually, beverages are excellent substrates for supporting the growth of yeasts, where the highest amount of nitrogenous compounds and vitamins promote occurrence of yeasts (Khattab et al., 2016).

The genus *Candida* includes around 154 species. Among these, six are most frequently isolated in human infections. While *Candida albicans* is the most abundant and significant species, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida lusitanae* are also isolated as causative agents of *Candida* infections (Aggarwal et al., 2018). Non-*Saccharomyces* yeasts could be used as biocontrol agents against moulds and in the treatment of wastewaters contaminated by heavy metals (Ubeda et al., 2014). Hence, a comprehensive understanding, linking intrinsic and extrinsic factors to microbial diversity and successions is of outmost importance for upgrading indigenous sub-Saharan African fermented food and beverages (Johansen et al. (2019). The suspicion or suggestion that beverage-spoiling *Candida* species can be (opportunistic) pathogens is not well-documented in microbiological tests performed on beverages and as a consequence is not given much attention in the relevant literature (Hutzler et al., 2012).

The aim of this work was to investigate *Candida* spp. of street hot beverages of tea, coffee and to perform phenotypic characterization of the isolated yeasts in order to contribute to the body of knowledge of yeasts in beverage food, and to add new information for a previously unexplored geographical area.

MATERIALS AND METHODS

Sampling procedure

A total of 400 hot beverages of coffee (200) and tea (200) samples were collected from street vendors in Abidjan according to the method of Atobla et al. (2020). This study was conducted from July to December 2020.

Isolation, purification and storage of yeasts

According to the prescriptions of the standards used, one milliliter

(1 mL) of beverage sample is aseptically transferred to a Petri dish. Enumeration of yeasts on Sabouraud Chloramphenicol agar (SCA, Biokar Diagnostics, France) was carried out according to the NF/ISO 16212: 2011 standard. All the Petri dishes were then incubated in an incubator at 30°C for 48 h for the enumeration of yeasts. Colonies were firstly selected based on colony morphology, aiming at selecting colonies of varying morphology, and colonies were randomly selected. The appearance of white to yellowish colonies would indicate the presence of yeasts. Colonies identified as yeasts by their macroscopic aspects and their microscopic observations in the fresh state were purified by striae on Sabouraud agar (SA, Biokar Diagnostics, France). One hundred and twenty-one (121) purified isolates were obtained and stored at -20°C in MYPG broth supplemented with glycerol 20% (v/v). Colonies were identified as yeasts by their macroscopic aspects on agar medium and their microscopic observations in the fresh state on an optical microscope at magnification 40X then at 100X.

Identification of yeasts by MALDI-TOF mass spectrometry

Yeast cultures were identified at genus and species level by MALDI-TOF MS at the biobank laboratory of the Pasteur Institute of Côte d'Ivoire. The identification of yeasts was done at the genus and species level based on mass of ribosomal proteins by the MALDI-TOF (Vitek MS BioMerieux) MS, which is a spectrometer using a matrix-assisted laser ionization source and a time-of-flight analyzer. MALDI-TOF identification was done in three steps: sample preparation followed by sample analysis and data processing (Lo et al., 2017). For samples preparation, yeasts isolated from street hot beverages were cultured in Sabouraud agar for 48 h at 30°C. Afterwards, a colony of the calibrating strain of *Escherichia coli* ATCC 8739 (positive control), was put on the MALDI-TOF plate with 1 µL of CHCA matrix (α-cyano-4-hydroxycinnamic acid, BioMerieux SA, ref 411071). Then, using a sterile oese calibrated at 1 µL, a colony of yeast to be tested was collected and put on the target wells. The sample was tested in duplicate. On both deposits, 0.5 µL of 25% formic acid (vitek MS-FA, BioMerieux SA, ref 411072) was added. After air drying, 1 µL of CHCA matrix was put on each spot and dried again. Subsequently, the deposition plate was introduced into the Vitek MS for sample analysis after transferring the data from the Prep Station, which is a module consisting of a computer and an optical scanner used to introduce the sample data and their location on the slide to the Vitek MS. The interpretation of the results involved two important parameters: the percentage confidence degree and the confidence level displayed by different colours.

Data analysis

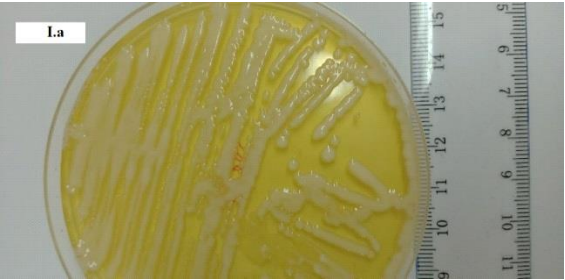
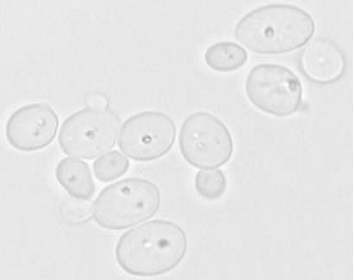
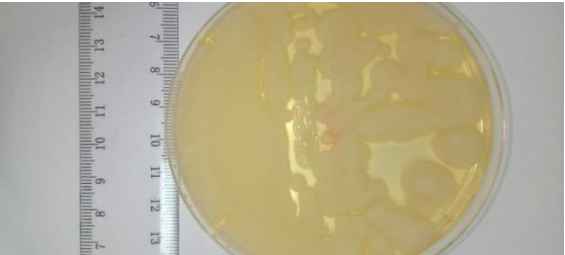
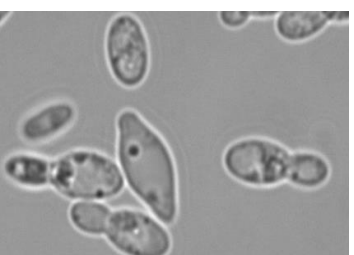



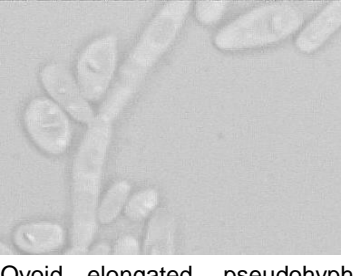
The data were exported to Microsoft Excel to calculate the various scores. Descriptive statistics were used to summarise the variables of interest and determine relationships between them. The Chi-square test was used to test the relationships between the variables. The difference between the variables was considered significant at $p < 0.05$.

RESULTS

Morphologies of strains and cell of yeasts

Yeast strains were isolated from hot beverages of tea and coffee. The aspect of colonies on Sabouraud agar showed creamy or dry, smooth or rough colonies. Microscopic morphology of the fresh isolates revealed

Table 1. Morphologies of strains and yeast cell of isolates.

Group (%)	Aspect of colonies on Sabouraud agar	Microscopic morphology of yeast cell
I (25.5%)	 Creamy white colonies, bright and smooth	 spherical and budding cell
II (38.1%)	 Creamy white colonies smooth and dull surface of regular contour	 Ovoid and budding cell
III (10.7%)	 Creamy white colonies, rough surface, regular contour	 Ovoid elongated and budding cell
IV (2.5%)	 Dry white colonies, with a rough surface	 Ovoid elongated, pseudohypha and budding cell

Group I, II, III and IV: morphotype groups of isolated yeast strains. Magnification: M x100.

spherical, ovoid, elongated cells, single or connected in pairs or chains, and propagates by budding. Based on the macroscopic and microscopic characters, four morphotypes yeast were isolated. Creamy white colonies, smooth, dull surface and regular contour (group II) with a rate of 38.1%, which were the most prevalent, followed by the creamy white colonies, bright and smooth (group I)

with the rate of 25.5% (Table 1).

Identification of *Candida* species

Candida species were identified by MALDI-TOF MS (Table 2). A total of 37 *Candida* isolates were clearly

Table 2. *Candida* species identified by MALDI-TOF MS.

<i>Candida</i> species	Laboratory code	Score	Rate of isolation : n (%)
<i>C. tropicalis</i>	I7C2, IV3T1, IV7T2, IV11T3, IV12T2, IV14T2, IV14T4	99.9	7 (18.9)
<i>C. parapsilosis</i>	II1T4, II5T3, V5T4, V5T5, V8C2, V8T4	99.9	6 (16.2)
<i>C. pelliculosa</i>	IV2T1, IV2T1, IV8T4	99.9	3 (8.1)
<i>C. krusei</i>	I7C1, I7C5, III3T1, IV1C2, IV10C1, IV10C2, IV13T2, V1C2	99.9	8 (21.6)
<i>C. guilliermondii</i>	V8C4, V9C2, V11C1, V12C2, V15C1, V15C2	99.9	6 (16.2)
<i>C. dubliniensis</i>	III13T2, III13T3	99.9	2 (5.4)
<i>C. rugosa</i>	IV5C2	88.9	1 (2.7)
<i>C. kefyri</i>	V13T2	99.9	1 (2.7)
<i>C. silvicola</i>	V9C5	99.5	1 (2.7)
<i>C. lusitanae</i>	IV11C1	85.8	1 (2.7)
<i>C. orthopsilosis</i>	V5T1	88.2	1 (2.7)
Total			37 (100)

Table 3. Presence of *Candida* species in hot beverages on street.

Hot beverages	Number	Presence of <i>Candida</i> species in hot beverages: n (%)	Prevalence of <i>Candida</i> species:n (%)
Coffee	200	17 (8.5)	17 (45.9)
Tea	200	20 (10.0)	20 (54.1)
Total	400	37 (9.2)	37 (100)

identified and showed 11 species: *C. tropicalis*, *C. parapsilosis*, *C. pelliculosa*, *C. krusei*, *C. guilliermondii*, *C. dubliniensis*, *C. rugosa*, *C. kefyri*, *C. silvicola*, *C. lusitanae* and *C. orthopsilosis*. The major species included *C. krusei* (21.6%), *C. tropicalis* (18.9%), *C. parapsilosis* and *C. guilliermondii* (16.2 %) followed by a low proportion of *C. pelliculosa* (8.1%), *C. dubliniensis* (5.4%), *C. rugosa* (2.7 %), *C. kefyri* (2.7 %), *C. silvicola*, *C. lusitanae* and *C. orthopsilosis* (2.7%).

Distribution of *Candida* species in hot beverages

From total of 400 hot beverage samples analyzed, *Candida* species were more isolated in tea than in coffee. The rate of the presence of *Candida* species in tea was 10%, whereas in coffee it was 8.5%. The prevalence of *Candida* species in tea was 54.1% (Table 3). The Chi-square test ($\chi^2 = 0.268$) showed that the presence of *Candida* species in hot beverage of coffee and tea was not significantly linked to the sample analyzed (p -value = 0.605). *C. krusei* is mostly isolated in coffee than in tea, while *C. tropicalis* is more isolated in tea than in coffee. *C. guilliermondii*, *C. rugosa*, *C. silvicola* and *C. lusitanae* were only isolated in coffee while *C. pelliculosa*, *C. dubliniensis*, *C. kefyri* and *C. orthopsilosis* were isolated only in tea (Figure 1).

Distribution of *Candida* spp. according to the location

C. tropicalis (n = 6), *C. pelliculosa* (n = 3) and *C. krusei*

(n = 4) were more isolated in Cocody. *C. guilliermondii* (n = 6) and *C. parapsilosis* (n = 4) were more isolated in Port-Bouët. *C. dubliniensis* was only isolated in Yopougon town (Figure 2).

DISCUSSION

The aspect of colonies on Sabouraud agar showed creamy or dry colonies, smooth or rough. Microscopic observation of the fresh isolates revealed spherical, ovoid, elongated cells. In the study of Sipiczki (2011), *Candida citri* vegetative cells are ovoid or nearly spherical, single or connected in pairs or chains, and propagated by multipolar budding. It is important to bear in mind that while pseudohyphae may appear physically more similar to hyphae, they actually share far more properties with yeasts and might be better described as elongated, attached yeast cells (Sudbery et al., 2004; Delma et al., 2011). In addition, these results suggest that in the case of *Candida* species, morphology may have evolved in a stepwise fashion from yeast to pseudohyphae to hyphae (Bastidas and Heitman, 2009). Consistent with this hypothesis, while many *Candida* species are capable of forming yeast and pseudohyphae, only three species, which are phylogenetically closely related (*C. tropicalis*, *C. dubliniensis*, and *C. albicans*), are known to form hyphae as well (Moran et al., 2002; Delma et al., 2011). It is therefore likely that the earliest *Candida* species also possessed a very weak ability to form pseudohyphae and over time evolved to undergo this morphological transition more frequently in response

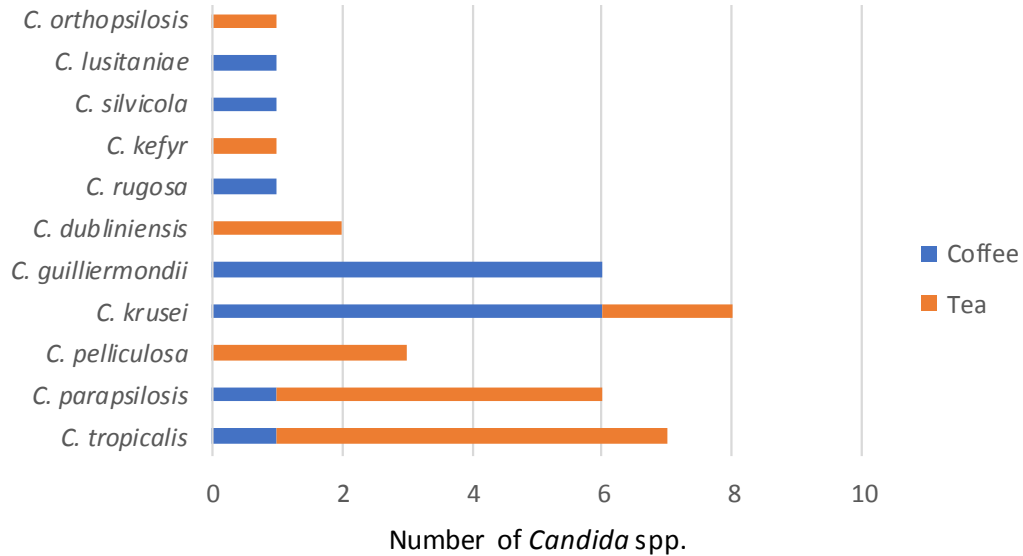


Figure 1. Distribution of *Candida* spp. in beverages of tea and coffee.

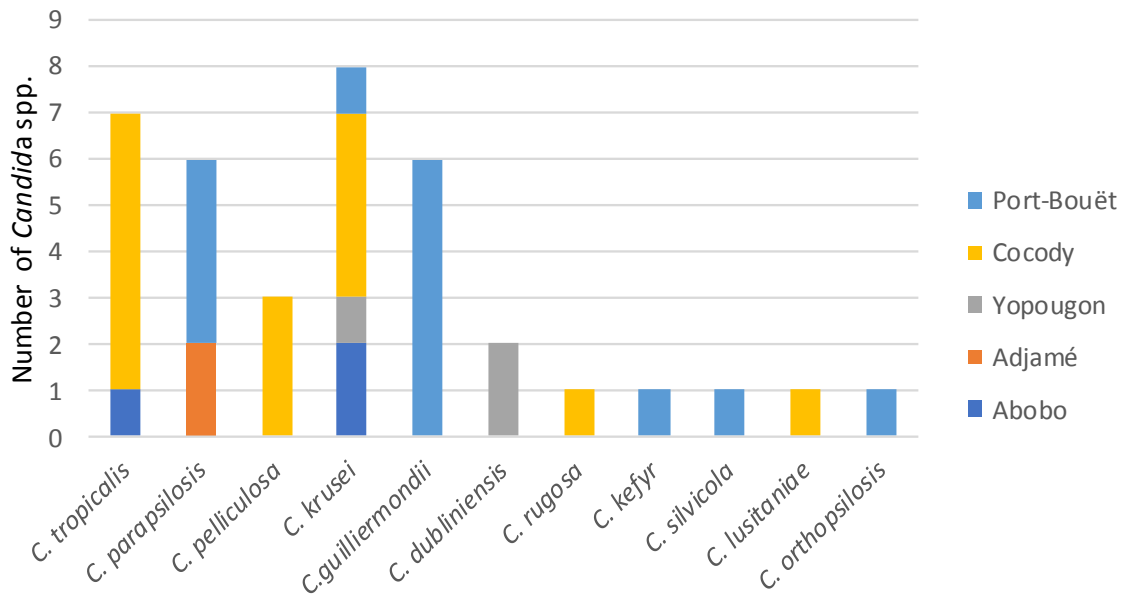


Figure 2. Distribution of *Candida* spp. according to the location.

to a broader array of host environmental conditions and niches (Delma et al., 2011). Over evolution, *Candida* species are believed to have acquired the ability to form pseudohyphae more frequently and in response to a broader range of conditions in the host environment (Delma et al., 2011). In contrast, hypha-forming *C. albicans*, *C. dubliniensis*, and *C. tropicalis* appear to have an increased ability to adhere to host cells and secrete proteases relative to other *Candida* species, which lack the ability to form hyphae (Moran et al., 2002). The stressful microbial environments in fermented food and

beverages result in a high selection pressure, which can lead to development of new strains better adapted to the fermentation process. As a consequence, species occurring in spontaneously fermented food and beverages might, over time, differentiate into populations of strains of the same species (Suzzi, 2011).

Candida species were identified by MALDI-TOF in our study. *Candida* isolates were clearly identified and showed 11 species: *C. tropicalis*, *C. parapsilosis*, *C. pelliculosa*, *C. krusei*, *C. guilliermondii*; *C. dubliniensis*; *C. rugosa*, *C. kefyr*, *C. silvicola*, *C. lusitaniae* and *C.*

orthopsilosis. According to Bader et al. (2011), MALDI-TOF MS analysis was able to differentiate closely related species when conventional biochemical methods were not such as species of the parapsilosis complex (*C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*). Furthermore, MALDI-TOF MS can detect 95.7 – 100% of common *Candida* species like *C. albicans*, *C. glabrata*, *C. dubliniensis*, and *C. tropicalis* (Bader et al., 2011; Bille et al., 2012; Iriart et al., 2012). Accuracy is lower for uncommon species like *C. inconspicua*, *C. rugosa*, and *C. norvegensis* (73.6–88.9%). However, when databases are sufficiently extensive and regularly updated, MALDI-TOF MS could detect these species, whereas the classical identification method could not (Santos et al., 2011; Posteraro et al., 2013). For Johansen et al. (2019), these techniques have so far not been used for identification of yeasts from indigenous sub-Saharan African fermented food and beverages.

Candida species were more isolated in tea (10.0%) than in coffee (8.5%). Atobla et al. (2021) also isolated more yeasts in hot beverages of tea than in coffee. It is well known that final product quality in industries such as wine-making, sausage production, cheese ripening, bakery, and the “fermentations” of cacao and coffee beans is affected directly by the development of spoilage microorganisms (Romano et al., 2006; Viljoen, 2006). *C. krusei* (21.6%) is the most isolated yeast in hot beverages of coffee and tea. Likewise, in the study of Jespersen et al. (2005), *C. krusei* is also the dominant yeast present in chocolate production and cocoa bean fermentation in West African cocoa beans. Heap fermentation in particular is most effective with *C. krusei* (Jespersen et al., 2005).

C. tropicalis with a rate of 18.9% was more isolated in tea and *C. guilliermondii* was only isolated in coffee in our study. Likewise, Johansen et al. (2019) reported that *C. tropicalis* is frequently identified in indigenous sub-Saharan African fermented food and beverages, including akyeke, amasi, attiéké, bandji, fufu, fura, gari, gowé, kaffir, lafun, mawè, mukumbi, nunu, palm wine, pito, sethemi, tchapalo and teffinjera (Coulin et al., 2006; Oyewole, 2001; Greppi et al., 2013). For the production of “fufu”, Oyewole (2001) reported that six different yeast species, *Pichia saitoi*, *Pichia anomala*, *C. krusei*, *C. tropicalis*, *Zygosaccharomyces bailii* and *S. cerevisiae* have been identified. For Johansen et al. (2019), yeasts are most probably occurring as a result of contamination during processing due to improper human handling. As indigenous sub-Saharan African food and beverages predominantly are produced by spontaneous fermentation, the consumers may be exposed to large populations of different yeast species of often unknown origin (Ogunremi et al., 2017).

C. tropicalis, *C. pelliculosa* and *C. krusei* are more isolated in Cocody town. *C. pelliculosa*, *C. guilliermondii*, *C. kefyri*, *C. silvicola* and *C. orthopsilosis* were more isolated in Port-Bouët town. For Turner and Butler (2014), the incidence varies substantially with geographical

location. *C. glabrata* is the highest in Asia-Pacific and the European Union (EU); whereas the incidence of *C. tropicalis* infection in Africa and the Middle East is approaching three times that of the European Union; on the other hand, *C. parapsilosis* is highest in North America and Latin America (Pfaller and Diekema, 2004; Klevay et al. 2008; Alexander et al., 2013). From a local kombucha in Saudi Arabia, Ramadani and Abulreesh (2010) isolated *Candida* sp. in kombucha beverages and identified four yeasts: *C. guilliermondii*, *C. colliculosa*, *C. kefyri*, and *C. krusei*.

According to Johansen et al. (2019), the market size of indigenous sub-Saharan African fermented food and beverages are growing among others due to their ability to be used as convenient food by consumers. Additionally, fermentation is an affordable and sustainable way of processing that can be easily used to improve the quality and safety of food and beverages. Unfortunately, uncontrolled processing, handling and selling on streets may induce contamination and growth of harmful microorganisms.

Conclusion

To our knowledge, this is the first study to assess *Candida* species in hot beverages in Côte d'Ivoire. The present study has proven that a significant number of *Candida* species are involved in hot beverages on street. Consequently, further research is needed to optimize fermentation conditions to eliminate opportunistic pathogenic yeasts during processing and equally important, improved hygienic conditions need to be ensured in order to prevent cross-contamination from human handling during food processing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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