

## Technological characteristics of wild non-Saccharomyces sourced from banana fruit and honey

### ABSTRACT

This study is aimed at evaluating the technological characteristics of wild non-Saccharomyces sourced from banana fruit and wild honey. The isolation of yeasts was done according to standard microbiological procedures. Technological traits screened for are as follows: fermentation ability, alcohol production, flocculation ability, organic acid production, and hydrogen sulphide production. Five yeast isolates were identified as B10 (*Candida tropicalis*), B7 (*Candida tropicalis*), H4 (*Candida tropicalis*), H7 (*Clavisporalutitanae*), and CY (*Candida tropicalis*), which are sugar fermenters. The percentage of alcohol produced from each sugar fermented by the yeast isolates are as follows: sucrose - B7(11.50%) > H7(8.62%) > CY (7.80%) > H4(4.88%) > B10 (4.11%); Glucose - B7(9.82%) > CY (6.28%) > B10(4.56%) > H7(4.03%) > H4(2.19%) and Fructose - H7(13.11%) > CY (9.40%) > B10(7.03%) > H4(4.41%) > B7(3.70%). Yeast isolate CY demonstrated high flocculation of 28.55 and 44.75 (%) at 5 and 15 (minutes). The organic acid produced by the yeast isolates B10, B7, CY, H4 and H7 are as follows 1.90±0.41, 3.10±0.41, 1.25±0.07, 3.90±0.41 and 2.40±0.41 (AU) respectively and Yeast isolates B7, CY, H4, and H7 produced low hydrogen sulphite concentration. Wild non-Saccharomyces could be the hope of the wine microbiologist to ease the challenges in the wine industry, as they competed flavourably with the commercial wine yeast.

**Keywords:** Non-Saccharomyces, Fermentation ability, flocculation, hydrogen sulphide production.

### Introduction

The *Saccharomyces cerevisiae* yeast has been the most widely accepted microorganism industrially (Dorota, 2008; Nandy and Srivastava, 2018), due to its ability to complete sugar fermentation, high alcohol yield, positive influence on the sensorial features of wines, long history of domestication by man (Brice *et al.*, 2018; Rainier and Pretorius, 2000), outstanding in the processing of substrates into wine, dominates the alcoholic fermentation (Di Paola *et al.*, 2020; Mills *et al.*, 2008) and well researched and understood eukaryotic cell (Knop, 2011).

Review and harmonization of the microbial taxonomy employed in food fermentation (Bourdichon *et al.*, 2012), have led to the adoption of yeast varieties that aim to satisfy consumer longings for wines with reduced alcohol content (Lee *et al.*, 2021) and organic acid (Bely *et al.*, 2008) using commercially tailored wine yeast strains (Ambroset *et al.*, 2011) as standard (Nogami *et al.*, 2007) to identify low alcohol-producing yeast (Mateo and Maicas, 2016; Úbeda *et al.*, 2014). The natural obtainability of yeast strains that possess an ideal blend of oenological properties is decidedly improbable: current interest in non-Saccharomyces yeasts revealed strains possessing interesting oenological properties (Rainier and Pretorius, 2000), thus revealing a new world of improved fermentation of complex and differentiated sensory profiles in wines (Morata, 2019) which have gained importance lately in the biotechnological setting (Varize *et al.*, 2019).

For eons, wine production has been by spontaneous fermentation carried out by yeasts originating from the grapes; and the contribution of non-Saccharomyces yeasts in the wine production has been made known as the backbone of wine quality (Sorrentino *et al.*, 2013). Biotechnologically, yeast biodiversity, especially for oenology, is underutilized, such as the potential benefits of non-Saccharomyces yeasts in wine production that unavoidably have a different list of desired properties (Mateo and Maicas, 2016). The characteristics of yeasts without Saccharomyces (*Candida*, *Kloeckera*, *Hanseniaspora*, *Zygosaccharomyces*, *Schizosaccharomyces*, *Torulaspora*, *Brettanomyces*, *Saccharomycodes*, *Pichia* and *Williopsis* genera) in vinification are receiving increasing attention from wine microbiologists in countries producing Old and New World wines (Jolly *et al.*, 2016) and indigenous yeasts involved in the production of indigenous honey wine (Ogol) have proven to possess the basic oenological properties (Teramoto *et al.*, 2005).

Understanding the metabolic activity of safe wild yeasts could proffer solutions to the challenge of high alcohol production in wine (Viana *et al.*, 2017), as non-Saccharomyces possess good fermentation attributes of industrial importance (Ebabhi *et al.*, 2013; Vicente *et al.*, 2021). Currently, indigenous yeasts involved in spontaneous fermentation of alcoholic beverages are being studied (Bourdichon *et al.*, 2012; Teramoto *et al.*, 2005), for the expression of essential enzymes required for quality winemaking, and these qualities aroused the use of non-Saccharomyces in winemaking (Cocolin *et al.*, 2000; Mateo and Maicas, 2016) and essential components of human production of fermented food (Di Paola *et al.*, 2020).

Flocculation is a natural, reversible active assemblage of cells into flocs. Aggregation of microorganisms is common among bacteria, filamentous fungi, algae, and yeasts. Dominant flocculation genes (FLO genes) FLO1, FLO5, and FLO8 were proposed to be the structural genes encoding proteins (lectins) involved in flocculation (Straver *et al.*, 1993), FLO1, FLO2 and FLO4 (Domingues *et al.*, 2000). The presence of glycoproteins on cells surfaces of the flocculents is due to the ionic or lectin-like binding force which is influenced by pH, sugar concentration, and flocculation inducing substances (Kamada and Murata, 1984), dissolved oxygen, pH, fermentation temperature, and yeast handling, storage conditions and cell wall composition (Verstrepen *et al.*, 2003). The FLO11-encoding flocculin is required for flocculation, adhesion to agar and plastic, invasive growth, pseudohyphae formation and biofilm development (Bayly *et al.*, 2005; Nayyar *et al.*, 2014; Van Mulders *et al.*, 2010).

Hydrogen sulfide (H<sub>2</sub>S) production during fermentation is common and a substantial problem in the global wine industry as it imparts undesirable off-flavors at low concentrations. The production of H<sub>2</sub>S is a necessary intermediate compound in wine, resulting from the assimilation of sulfur through the sulfate reduction pathway using sulfite reductase (Cordente *et al.*, 2009). The gene leading to reduced H<sub>2</sub>S formation as an allele of MET10 (MET10-932), which encodes a catalytic subunit of sulfite reductase, MET1, MET5, MET8, or MET10, and loss of sulfite reductase activity is inversely

correlated with H<sub>2</sub>S formation (Linderholm *et al.*, 2010). However, Good Manufacturing Practices must be strengthened to deal with the problem of volatile sulphur production in wines (Hine *et al.*, 2015; C.-W. Huang *et al.*, 2016; Li *et al.*, 2019; Malfeito-Ferreira, 2011; Ugliano *et al.*, 2011; Winter *et al.*, 2014; Zara and Nardi, 2021).

The organic acids content of fermented alcoholic beverages is largely associated with the substrate, but the concentrations of some of the organic acid are formed by yeasts as by-products of the main metabolic pathways during fermentation (Whiting, 1976). The non-conventional yeast *Yarrowialipolytica* degrades hydrophobic substrates efficiently to produce organic acids (Darvishi *et al.*, 2009), and organic acid production from glucose by yeast at neutral and low pH (Klinke *et al.*, 2009). The regulation of organic acidis concurrently achieved by activation or inactivation of single genes like GTR1, GTR2, LIP5, LSM1, PHO85, PLM2, RTG1, RTG2, and UBP3 genes (Yoshida and Yokoyama, 2012). Wine-related yeast, *Candida zemplinina*, and *Saccharomyces cerevisiae* produce organic acidic (malic, fumaric and succinic acid) at a level comparable to the production of acid by *Saccharomyces* species(Magyar *et al.*, 2014, Khan *et al.*, 2021, Sahni *et al.*, 2021). Yeast strains have different patterns of consumption and production of organic acids and organic acid management during fermentation (Chidi *et al.*, 2015), which contributes significantly to the perception of wine quality by consumers(Chidi *et al.*, 2018). This study aims to evaluatethe technological characteristic of non-*Saccharomyces* wild bananas sourced from the wild.

## Materials and Methods

### Isolation of yeast from samples

About 10g of the ripe banana fruit and 10ml of the wild honey was transferred aseptically into 250ml conical flasks containing 90ml sterile peptone broth and incubated for 24-48 hours at 30 °C. After incubation, an aliquot (0.1ml) of the broth was transferred to prepared yeast extract peptone dextrose agar plates(YEPDA) supplemented with chloramphenicol and evenly spread using a sterile bent glass rod. Plates were also incubated at 30 °C for 48 hours ( Hong and Park, 2013),observed growth was sub-cultured on YEPDA plates. The morphology of the yeasts was confirmed macroscopically and microscopically (under the light microscope at X40 and X100 magnification) (Ali and Latif, 2016) after staining.

### Molecular Characterization

The molecular characterization was carried out in the Bioinformatics Service Laboratory, Ibadan, Nigeria. The CTAB method as described by Ali and Latif(2016) was adoptedfortheextraction of DNA from yeast strains. In this method, 24 hours yeast cultures in YEPD broth were centrifuged at maximum speed. Approximately 10 mg of yeast cells for each strain were taken and pre-warmed in 200 µl of solution I at 65 ° C containing 1.4M NaCl, 2% CTAB, 20mM EDTA (pH 8.0), 0.2% β-mercaptoalcohol, and 100mM Tris-HCl (pH 8.0) was introduced, mixed well and incubated at 65 °

Cfor 15-20 minutes in the water bath. After incubation, all tubes were cooled for 3-5 minutes and the same volume of solution II (Chloroform: Isoamyl alcohol, 24:1) was added, thoroughly mixed and centrifuged at 14,000 rpm for 10 minutes at room temperature. The aqueous phase (upper) was taken from each Eppendorf separately and 3M Na acetate (1/10) was introduced into each Eppendorf along with an equal volume of cold isopropanol or a double volume of cold absolute alcohol, gently mixed and placed on ice for 10 minutes. All tubes after incubation were centrifuged at 12,000 rpm at 4 °C for 15 minutes and the supernatant was discarded. About 500µl of chilled 70% alcohol (solution III) was added directly to washed pellet and then centrifuged at 14000 at 4°C for 2 minutes. The pellet was air-dried after the supernatant was removed from each tube. The pellet was resuspended in 50µl double deionized water or TE buffer and stored at -20 °C. The yield of DNA was quantified by a spectrophotometer. The ribosomal DNA internal transcribed spacer region: ITS1 (GTAGGTGAACCTGCGG) and ITS4 (TCC GCTTATTGATATGC) was used to amplify the DNA (Oliveira *et al.*, 2008). The reaction mixture contained 100ng of DNA, 5µl of 10pmol of each oligonucleotide primer, 3µl of 25mM MgCl<sub>2</sub>, 3µl of 250mM dNTP mixture and Taq DNA polymerase (5units) in a total volume of 50 µl. PCR conditions were as follows: 3 min. at 94 °C followed by 35 cycles (45 s at 94°C, 45 s. at 55 °C (annealing temperature), 1 min. at 72°C, and final extension for 7 min. at 72°C. The amplified product was determined by running on 0.8% agarose gel and visualized using a UV illuminator and photographed. More so, PCR products of the partially amplified-ITS region were subjected to restriction fragment length polymorphism (RFLP) for two restriction endonucleases TaqI and HaeIII. The reaction mixture contained 3.0 µl of 1X buffer (R-buffer for BsuRI (HaeIII) and unique-buffer for TaqI), 15.0 µl PCR products (approximately 1.0 µg), 1µl of specific endonuclease, and 11µl of deionized water with a total volume of 30µl. The reaction mixtures were incubated at their specific temperatures as recommended by the manufacturer's instructions (Fermentas) The restriction fragments were separated along with a DNA 100bp ladder on 1.5% w/v agarose gel and photographed after visualization under UV light. Finally, 2.5µl of the purified PCR products were sequenced using the Applied Biosystems ABI PRISM™ 3100 DNA sequence Analyzers with the BigDye® Terminator v3.1 Cycle Sequencing kit and protocols (Shittu *et al.*, 2016). The DNA sequence obtained was blasted onto the NCBI gene bank to confirm the identities of the various yeasts.

### **Screening of yeast isolates for sugar fermentation ability**

The method of Alabere *et al.* (2020) was adopted. Yeast extract peptone dextrose (YEPD) broth was compounded by transferring 15g of peptone water, 10g of yeast extract, and 20 g of fructose, glucose, sucrose, galactose, maltose, and lactose respectively into 2litres conical flasks containing 1litre of distilled water, then 10ml of broth was dispensed into test tubes containing inverted Durham tube sterilized by autoclaving at 121°C and 15 Psi for 15 minutes. At cooling, yeast isolates were

inoculated into the broth and incubated for 48 hours at 28-30 (°C). The presence of gas (space) in the Durham tube was a confirmation of sugar fermentation.

### Screening of yeast isolates for alcohol production

Yeast isolates were screened for alcohol production capacity according to the method of Ambadas (2011) and Reddy *et al.* (2009) modified using peptone water containing sucrose, glucose, and fructose (20 % (w/v)) respectively as substrate. The bottles were sterilized and rapidly cooled to room temperature and the yeast inoculum was transferred to the sterilized 50 ml fermentation medium. Test organisms were inoculated into a 100 ml flask containing 50 ml fermentation media and incubated for 48 h. The YEPS broth was adjusted to pH 4.0 before sterilization. Fermentation bottles were kept on a shaker to shake at 120 rpm and aeration for 8 h. Then followed by anaerobic fermentation for 24 h.

Percentage alcohol by volume =  $(OG - FG) \times 131.25$ . Ogu (2011). ..... Equation 1.

where:

OG = Original gravity of the sample.

FG = Final gravity of the sample.

For specific gravity correction, sample temperature below 20 °C 0.0002 is subtracted per degree °C and temperature above 20 °C 0.0002 is added per degree °C (Jean, 2006).

### Flocculation ability

Flocculation properties of yeast isolates were confirmed by using the method described by mill (1964) with slight modification. yeast isolates were cultured for 3 days at 30 °C in a 100 ml conical flask containing 50 ml of Peptone water broth (15 g/l peptone and 5 g/l NaCl) supplemented with 10 g/l yeast extract and 20 g/l glucose under 24 hours shaking (140 rpm). Cells were harvested by centrifugation (4000 x g for 5 minutes) and washed with deionized water. The dispersed yeast was washed three times in a 1% NaCl solution and in deionized water, then the cells were suspended in 10 ml of 50 mM acetate buffer (pH 4.6) enriched with 0.1 % (g/l) CaCl<sub>2</sub>, while the initial OD<sub>600</sub> nm culture for each was determined. After agitation on shaking incubator at 140 rpm for 30 min, 5 ml of the cell suspension was transferred to a new test tube and allowed to stand undisturbed for 0, 5 and 15 minutes in a vertical position, after which, samples (3000 µl) were taken from just below the meniscus and the OD<sub>600</sub> nm determined using spectrophotometric method (Nayyar *et al.*, 2014).

Flocculation ability (F) was determined by the following equation:  $F = (1 - B/A) \times 100\%$ . Where A is the absorbance at 600 nm (OD<sub>600</sub>) immediately before the cells were shaken in flocculation buffer and B is the absorbance at 600 nm (OD<sub>600</sub>) after the flocculation settled for 5 min and 15 minutes.

### Organic acid production

The ability of yeast isolates to produce organic acid was determined using the methods adopted by Uzahet *al.* (2020). After a 3 - 5 days incubation period at 28 – 30 ° C of Czapek-Doxagar medium, supplemented with 0.5 g of Ca<sub>2</sub>CO<sub>3</sub> and bromocresol green as indicator, the cells were then inoculated with each yeast isolate. Positive isolates were identified based on the presence of yellow zones around the colonies. The rate of organic acid production by yeast was determined by measuring the zones of

clearance of each isolate. To ascertain the acid unitage (AU) of each yeast isolate, the diameter of the yellow zone was divided by the diameter of the colonies.

### Hydrogen sulphide production

Hydrogen sulphide (H<sub>2</sub>S) production was evaluated on BiGGY and Lead Acetate agar. The agar plates were streaked with a pure culture of yeast and incubated for 48 hours at 28-30 °C. The qualitative measure for H<sub>2</sub>S production on the medium was decided by the colour of the colonies, which ranged from white through brown to near-black. The white colony means no hydrogen sulfide production and the black colony means high hydrogen sulphide concentration (Cordente *et al.*, 2009).

## Result

### Sugar fermentation ability

Yeast isolates isolated from banana fruit and wild honey were subjected to sugar fermentation to determine their fermenting ability of fructose, glucose and sucrose due to the presence of gas trapped in the Durham tube. Yeast isolates CY a commercial wine yeast adapted for referencing, B7 and B7 are wild yeast isolates from banana, and H4 and H7 are wild yeast isolates from wild honey. and yeast isolates identified as B10 (*Candida tropicalis*), B7 (*Candida tropicalis*), H4 (*Candida tropicalis*), H7 (*Clavisporalutitaniae*), and CY (*Candida tropicalis*) fermented the three sugars as represented in Table 1.

**Table 1.** Sugar fermentation ability of yeast isolates

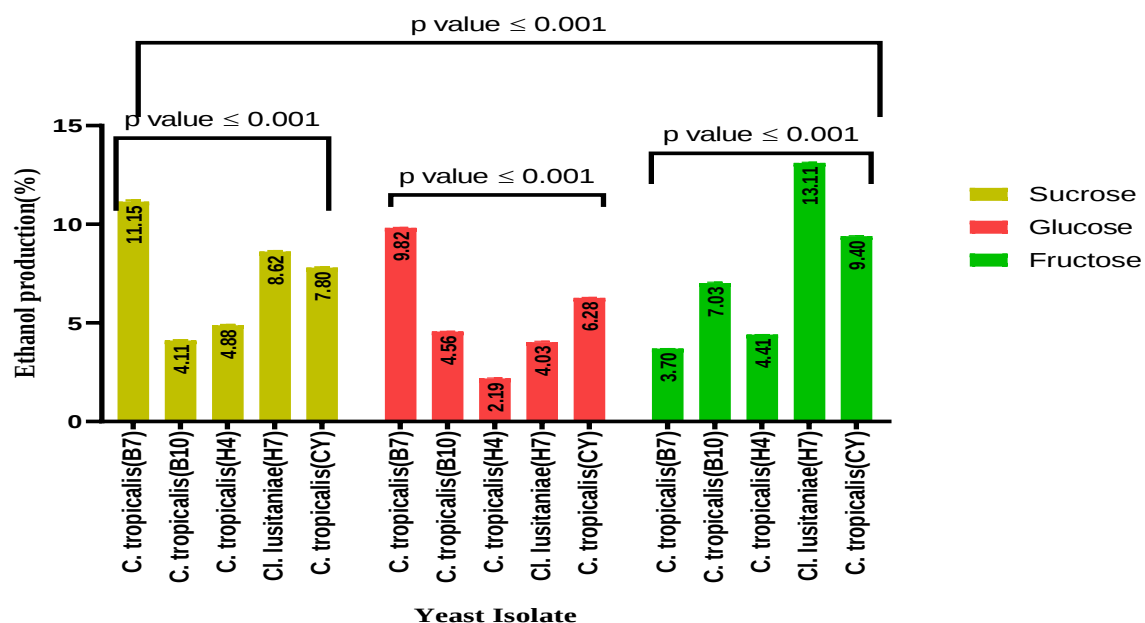
| Isolates                            | Sucrose | Glucose | Fructose |
|-------------------------------------|---------|---------|----------|
| <i>C. tropicalis</i> <sup>B10</sup> | +       | +       | +        |
| <i>C. tropicalis</i> <sup>B7</sup>  | +       | +       | +        |
| <i>C. tropicalis</i> <sup>H4</sup>  | +       | +       | +        |
| <i>Cl. lusitaniae</i> <sup>H7</sup> | +       | +       | +        |
| <i>C. tropicalis</i> <sup>CY</sup>  | +       | +       | +        |

+ signifies the ability to ferment sugar. – signifies a lack of ability to ferment sugar.

### Alcohol production ability

The alcohol production ability of yeast isolates from sucrose, glucose, and fructose is illustrated in Figure 1. The concentration (%) of alcohol produced by the yeast isolates vary with the type of sugar fermented to alcohol. The percentage of alcohol produced from the sucrose fermentation ranges from 4.11 – 11.50 (%) and individual yeast isolates recorded the following: B7(11.50%) > H7(8.62%) > CY (7.80%) > H4(4.88%) > B10 (4.11%), yeast isolates B7(11.50%) and H7(8.62%) produced more alcohol than the commercial wine yeast, CY (7.80%). The alcohol produced from glucose fermentation ranges from 2.19 – 9.82 (%), while individual yeast isolates recorded the

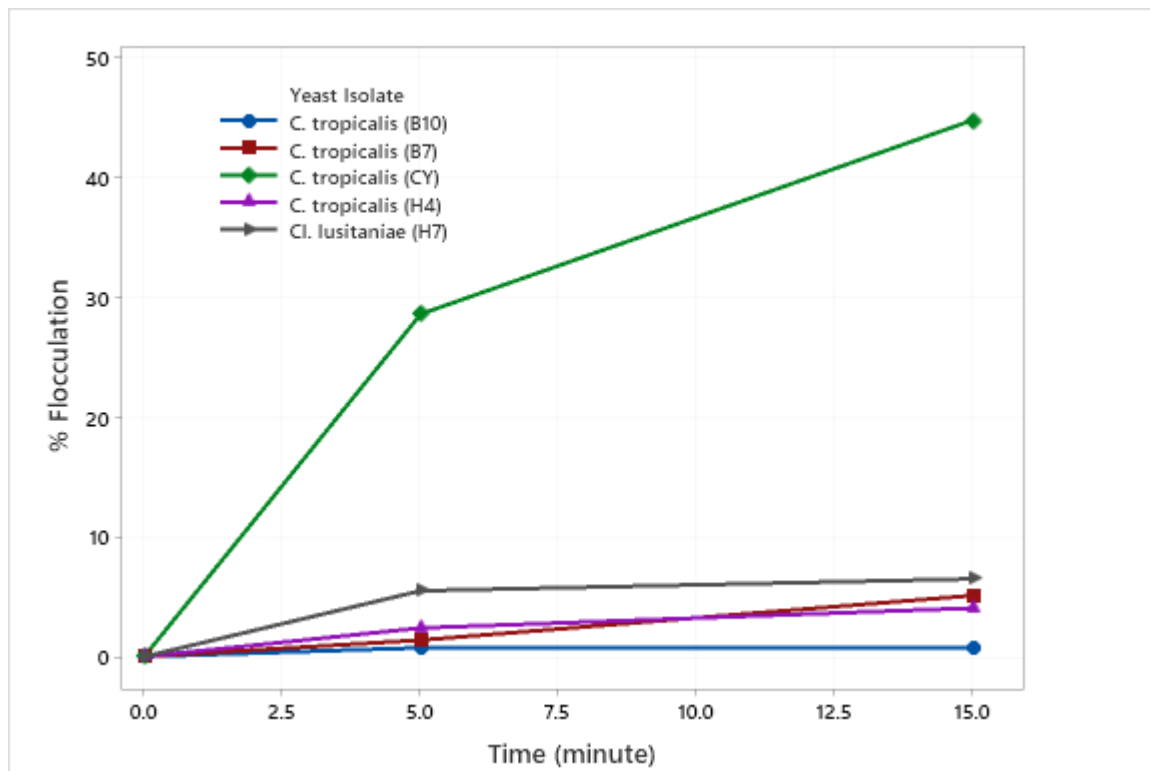
following: B7(9.82%) > CY (6.28%) > B10(4.56%) > H7(4.03%) > H4(2.19%), only B7(9.82%) produced more alcohol than the commercial wine yeast, CY (6.28%). Alcohol produced from fructose fermentation ranges from 3.70 to 13.11 (%), and individual yeast isolates recorded the following: H7(13.11%) > CY(9.40%) > B10(7.03%) > H4(4.41%) > B7(3.70%), only H7(13.11%) produced more alcohol than the commercial wine yeast, CY (9.40%). Statistically, there is a significant difference in the concentration of alcohol produced between yeast isolates from sucrose, glucose, and fructose at the P value < 0.001, and the three different sugars fermented by yeast isolates, show a significant difference in the percentage of alcohol produced by yeast isolates from sucrose, glucose, and fructose, respectively, at the P value < 0.001.



**Figure 1. Alcohol production ability of yeast isolates using glucose, fructose, and sucrose.**

### Flocculation ability

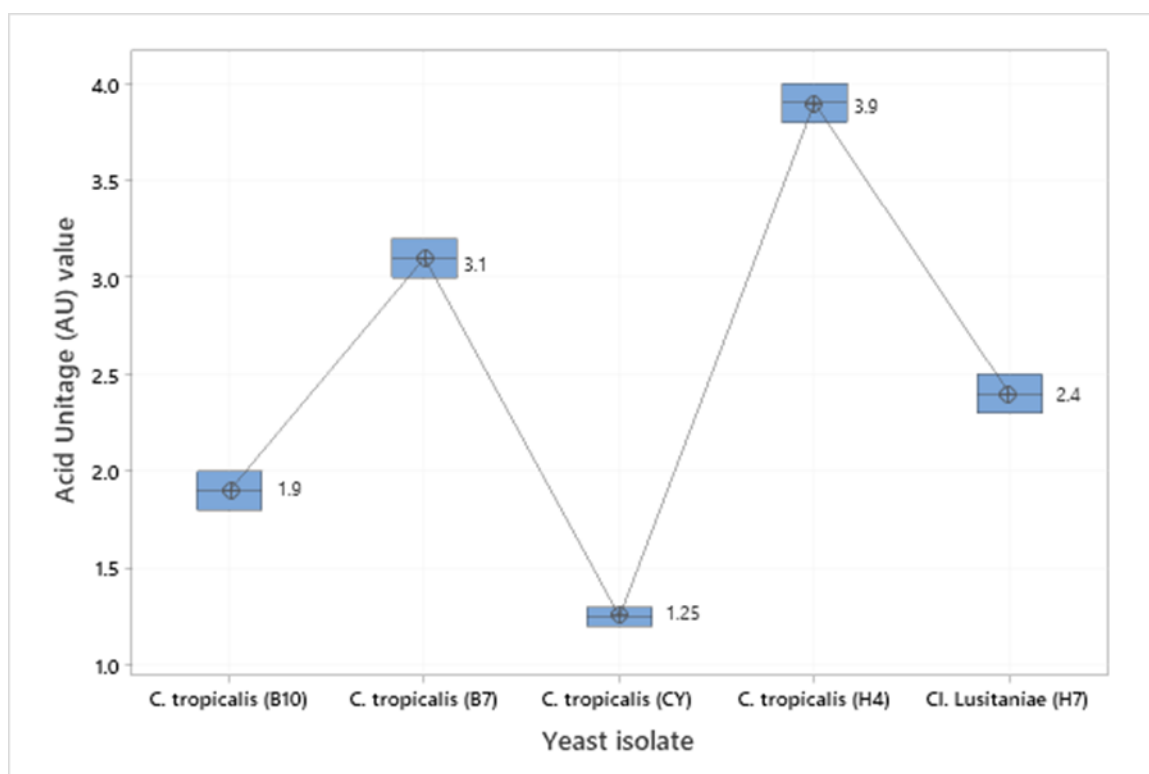
The flocculation ability of yeast isolates is graphically presented in Figure 2. Yeast isolate CY demonstrated increasing flocculation of 28.55 and 44.75 (%) at 5 and 15 (minutes) respectively, while the wild yeast isolates B10, B7, H4, and H7 had percentage flocculation < 10 % at 5 and 15 (minutes) respectively. Statistically, there is a significant difference in flocculation and time between yeast isolates at P-value < 0.001.



**Figure 2. Flocculation proportion of yeast cells with time.**

### Organic acid production

The ability of microorganisms to produce organic acid is measured in Acid Unitage (AU) as presented in figure 3. The organic acid produced by the yeast isolates ranges from 1.25 – 3.90 (AU), while the individual yeast isolates B10, B7, CY, H4 and H7 recorded the followings:  $1.90 \pm 0.41$ ,  $3.10 \pm 0.41$ ,  $1.25 \pm 0.07$ ,  $3.90 \pm 0.41$  and  $2.40 \pm 0.41$  (AU) respectively. H4 (3.90 AU) and CY (1.25 AU) had the highest and least organic acid production, statistically, there is a significant difference in organic acid production among the yeast isolates at  $P\text{-value} < 0.001$ ; but there is no significant difference in the acid unitage of B10 ( $1.90 \pm 0.41$ ) and H7 ( $2.40 \pm 0.41$ ).



**Figure 3. Acid unitage values of yeast isolate on Czapek-Dox agar.**

Hydrogen sulphide production is an undesired but inevitable metabolite expressed by yeast isolates during fermentation. A qualitative method of screening yeast isolates for  $H_2S$  carryout on Lead Acetate Agar (LAA) and Bismuth Glycine Glucose Yeast extract (BIGGY) agar is shown in Table 2. Hydrogen sulphide production was measured base on colour and colour intensity: White colony, no hydrogen sulphide production; light brown colony, low hydrogen sulphide production; brown colony, medium hydrogen sulphide production; and black colony, high hydrogen sulphide production. On LAA and BIGGY agar yeast isolate, B10 had a brown colony. Yeast isolates B7, CY, and H7 produced similar light brown colonies in the medium, while H4 showed variation in colour intensity between LAA and BIGGY agar.

**Table 2. Production of hydrogen sulfide of yeast isolates on lead acetate agar and bismuth glycine glucose yeast extract (BIGGY) agar**

| Isolates                            | Lead Acetate Agar | BIGGY agar |
|-------------------------------------|-------------------|------------|
| <i>C. tropicalis</i> <sup>B10</sup> | ++                | ++         |
| <i>C. tropicalis</i> <sup>B7</sup>  | +                 | +          |
| <i>C. tropicalis</i> <sup>H4</sup>  | +                 | ++         |
| <i>Cl. lusitaniae</i> <sup>H7</sup> | +                 | +          |
| <i>C. tropicalis</i> <sup>CY</sup>  | +                 | +          |

**keys: - white colonies; + light brown; ++ brown; +++ dark brown/black**

## Discussion

The present study was aimed at evaluating the technological characteristic of wild non-Saccharomyces sourced from banana fruit and wild honey in southern, Nigeria in order to select appropriate autochthonous starter cultures for the production of typical safe regional wine and traditional fermented food

### Sugar Fermentation Capacity

The yeast isolates B10 (*Candida tropicalis*), B7 (*Candida tropicalis*), H4 (*Candida tropicalis*), H7 (*Clavisporalutitanae*), and CY (*Candida tropicalis*) fermented sucrose, glucose, and fructose. My finding is consistent with the report of Lee *et al.* (2011) and Tao *et al.* (2011) that wild yeast isolates fermented of sugar, such as glucose, fructose, and sucrose. Non-Saccharomyces have been implicated in fermentation sugar (Panagiotis *et al.*, 2013), and *Candida* spp isolated from palm wine was able to ferment sugar (Olowonibi, 2017), which can play a major role in the production of fermented beverages (Di Paola *et al.*, 2020). The fermentation of glucose, fructose, and sucrose implies the possession of the hexose transporter gene (hxt 1- hxt 7) (Boles and the Hollenberg, 1997; Lazar *et al.*, 2017), the periplasmic invertase and sucrose H<sup>+</sup> symporter AGT1 gene are associated with yeast isolates (Batista *et al.*, 2004).

### Alcohol production

The non-Saccharomyces yeast isolates B10 (*Candida tropicalis*), B7 (*Candida tropicalis*), H4 (*Candida tropicalis*), H7 (*Clavisporalutitanae*), and CY (*Candida tropicalis*) are fermenter yeasts but possess alcohol production ability (Brooks, 2008). My finding agrees with the report of Matsushika *et al.* (2009) and Kang and Lee (2015) that yeasts are known for their common characteristic of alcohol production from sugars. Sucrose metabolism requires the action of sucrose synthases or invertases (Santaniello *et al.*, 2014; Zayed *et al.*, 2014) and multiple genes for the transportation of hexose, Hxt(1-17) (Batista *et al.*, 2004; Boles and Hollenberg, 1997) are associated with *Candida tropicalis* (Chattopadhyay *et al.*, 2020). More than 95% of the ethanol produced is monosaccharide or disaccharides, with low yields of alcohol due to the formation of end products such as acetate, butyrate and CO<sub>2</sub> (Jessen and Orlygsson, 2012; Verstrepen *et al.*, 2003).

The noticeable difference in the concentration (%) of alcohol produced from the fermentation of fructose, glucose, and sucrose by the respective non-Saccharomyces yeast isolates, could be due to strain variation among the non-Saccharomyces species. My observation was in accordance with a report that better alcohol yield depends on the selection of microorganisms (Zayed *et al.*, 2014). Although, my findings disagree with the report of greater yields of ethanol using glucose (0.37 gg<sup>-1</sup>) than fructose (0.32 gg<sup>-1</sup>) (Díaz-Nava *et al.*, 2017).

### Flocculation ability

Flocculation of yeast is an essential oenological property expressed at the end of sugar fermentation for yeast cells recovery and wine clarification. Commercial wine yeast (CY) had superior flocculating ability compared to wild yeast isolates. Flocculation of a yeast strain is absolutely dependent upon the presence of calcium on the cell surface (Mill, 1964). Cell-cell interactions have been proposed to be facilitated by specific recognition and adhesion factors (Kamada and Murata, 1984). The flocculation genes FLO1, FLO5, and FLO8 have been proposed as structural genes that encode proteins (lectins) involved in flocculation (Stewart, 2018; Straver *et al.*, 1993). In another study it was revealed that flocculation is carried out by FLO-genes, FLO1, FLO5, FLO9, FLO10, and FLO11, located at telomeres and regulated by Flo8 and Mss11 (Bernardi *et al.*, 2018) and the degree of flocculation induced by these genes expression seem to differ (Rossouw *et al.*, 2015)

### **Organic acid production**

The yeast isolates expressed the ability to produce organic acid during fermentation which was measured in acid unitage. My result is in agreement with the report that non-conventional yeast, *Yarrowialipolytic* have been identified as organic acids producers (Darvishi *et al.*, 2009), also in a study conducted by Klinket *et al.* (2009), *Candida albican* was implicated as organic acid producer. Organic acid production was regulated by mutations in genes such as GTR1, GTR2, LIP5, LSM1, PHO85, PLM2, RTG1, RTG2, and UBP3, and genes related to succinate dehydrogenase such as EMI5, SDH1, SDH2, SDH4, TCM62, and YDR379C-A (Yoshida and Yokoyama, 2012). The organic acid metabolisms of *Candida zemplinina* and *Candida stellata* are different from each other and from that of the *Saccharomyces* species (Chidi *et al.*, 2015, 2018; Magyar *et al.*, 2014).

### **Hydrogen sulphide production**

Hydrogen sulphide (H<sub>2</sub>S) production is a product of amino acid decarboxylation by microorganisms during fermentation production, that brings about off-flavour in an alcoholic beverage. The results obtained revealed that the yeast isolates are hydrogen sulphide producers, but the amount of hydrogen sulphide produced by the wild yeast isolates is within the limit when compared with the commercial wine yeast. My observation is in line with the report that H<sub>2</sub>S is a compulsory intermediate in the assimilation of sulphur catalyzed by sulphite reductase in yeast through the sulphate reduction sequence and wine yeast (*Saccharomyces cerevisiae*) plays a vital role in volatile sulphur compounds production in wine (Cordente *et al.*, 2009; C. Huang *et al.*, 2014). The growth of brown colony on BIGGY medium signifying hydrogen sulphide production was also reported by Linderholmet *et al.* (2010) and the MET2 and SKP2 genes have been identified as regulators of mild hydrogen sulphide metabolism in wine yeasts (Noble *et al.*, 2015).

### **Conclusions**

Non-Saccharomyces could be the hope of the wine microbiologist to ease the challenges in the wine industry. When the technological properties of wild yeast isolated from bananas and honey are

compared to commercial wine yeast, wild yeasts are potential wine yeast waiting for deployment in the wine industry. As they could ferment sucrose, fructose, glucose, and carry out alcohol fermentation with the sugars. Produce moderate organic acid and a low amount of hydrogen sulphide, but, the wild yeasts are inferior to commercial wine yeast inability to flocculate.

## References

- Alabere, A., Ogbonna, D. N., and Williams, J. O. (2020). Screening of yeast cells for the production of wine from banana and pineapple substrates. *Journal of Advances in Microbiology*, 20(7), 38–55. <https://doi.org/10.9734/jamb/2020/v20i730264>
- Ali, M. S., and Latif, Z. (2016). Molecular characterization of yeast strains isolated from different sources by restriction fragment length polymorphism. *Pakistan Journal of Botany*, 48(1), 363–370.
- Ambroset, C., Petit, M., Brion, C., Sanchez, I., Delobel, P., Guérin, C., Chiapello, H., Nicolas, P., Bigey, F., Dequin, S., and Blondin, B. (2011). Deciphering the molecular basis of wine yeast fermentation traits using a combined genetic and genomic approach. *G3: Genes, Genomes, Genetics*, 1(4), 263–281. <https://doi.org/10.1534/g3.111.000422>
- Batista, A. S., Miletti, L. C., and Stambuk, B. U. (2004). Sucrose fermentation by *Saccharomyces cerevisiae* lacking hexose transport. *Journal of Molecular Microbiology and Biotechnology*, 8(1), 26–33. <https://doi.org/10.1159/000082078>
- Bayly, J., Douglas, L., Pretorius, I., Bauer, F., and Dranginis, A. (2005). Characteristics of Flo11-dependent flocculation in. *FEMS Yeast Research*, 5(12), 1151–1156. <https://doi.org/10.1016/j.femsyr.2005.05.004>
- Bely, M., Stoeckle, P., Masneuf-Pomarède, I., and Dubourdieu, D. (2008). Impact of mixed *Torulaspora delbrueckii*-*Saccharomyces cerevisiae* culture on high-sugar fermentation. *International Journal of Food Microbiology*, 122(3), 312–320. <https://doi.org/10.1016/j.ijfoodmicro.2007.12.023>
- Bernardi, B., Kayacan, Y., and Wendland, J. (2018). Expansion of a Telomeric FLO/ALS-Like Sequence Gene Family in *Saccharomycopsis fermentans*. *Frontiers in Genetics*, 9, 536. <https://doi.org/10.3389/fgene.2018.00536>
- Boles, E., and Hollenberg, C. P. (1997). The molecular genetics of hexose transport in yeasts. *FEMS Microbiology Reviews*, 21(1), 85–111. <https://doi.org/10.1111/j.1574-6976.1997.tb00346.x>
- Bourdichon, F., Casaregola, S., Farrokhi, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I. B., Prajapati, J. B., Seto, Y., Schure, E. T., Boven, A. V., Vankerckhoven, V., Zgoda, A., Tuijelaars, S., and Hansen, E. B. (2012). Food fermentations: Microorganisms with technological beneficial use. *International Journal of Food Microbiology*, 154, 87–97.
- Brice, C., Cubillos, F. A., Dequin, S., Camarasa, C., and Martínez, C. (2018). Adaptability of the *Saccharomyces cerevisiae* yeasts to wine fermentation conditions relies on their strong ability to consume nitrogen. *PLoS ONE*, 13(2), 1–20. <https://doi.org/10.1371/journal.pone.0192383>
- Brooks, A. A. (2008). Ethanol production potential of local yeast strains isolated from ripe banana peels. *African Journal of Biotechnology*, 7(20), 3749–3752.
- Chattopadhyay, A., Singh, R., Das, A. K., and Maiti, M. K. (2020). Characterization of two sugar transporters responsible for efficient xylose uptake in an oleaginous yeast *Candida tropicalis*

- Chidi, B. S., Bauer, F. F., and Rossouw, D. (2018). Organic Acid Metabolism and the Impact of Fermentation Practices on Wine Acidity: A Review. *South African Journal of Enology and Viticulture*, 39(2). <https://doi.org/10.21548/39-2-3172>
- Chidi, B. S., Rossouw, D., Buica, A. S., and Bauer, F. F. (2015). Determining the Impact of Industrial Wine Yeast Strains on Organic Acid Production Under White and Red Wine-like Fermentation Conditions. *South African Journal of Enology and Viticulture*, 36(3), 316–327. <https://doi.org/10.21548/36-3-965>
- Cocolin, L., Bisson, L. F., and Mills, D. A. (2000). Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbiology Letters*, 189, 81–87.
- Cordente, A. G., Heinrich, A., Pretorius, I. S., and Swiegers, J. H. (2009). Isolation of sulfite reductase variants of a commercial wine yeast with significantly reduced hydrogen sulfide production. *FEMS Yeast Research*, 9(3), 446–459. <https://doi.org/10.1111/j.1567-1364.2009.00489.x>
- Darvishi, F., Nahvi, I., Zarkesh-Esfahani, H., and Momenbeik, F. (2009). Effect of Plant Oils upon Lipase and Citric Acid Production in *Yarrowia lipolytica* Yeast. *Journal of Biomedicine and Biotechnology*, 2009, 1–7. <https://doi.org/10.1155/2009/562943>
- Di Paola, M., Meriggi, N., and Cavalieri, D. (2020). Applications of Wild Isolates of *Saccharomyces* Yeast for Industrial Fermentation: The Gut of Social Insects as Niche for Yeast Hybrids' Production. *Frontiers in Microbiology*, 11(578425), 1–8.
- Díaz-Nava, L. E., Montes-Garcia, N., Domínguez, J. M., and Aguilar-Uscanga, M. G. (2017). Effect of carbon sources on the growth and ethanol production of native yeast *Pichia kudriavzevii* ITV-S42 isolated from sweet sorghum juice. *Bioprocess and Biosystems Engineering*, 40(7), 1069–1077. <https://doi.org/10.1007/s00449-017-1769-z>
- Domingues, L., Vicente, A. A., Lima, N., and Teixeira, J. A. (2000). Applications of yeast flocculation in biotechnological processes. *Biotechnology and Bioprocess Engineering*, 5(4), 288–305. <https://doi.org/10.1007/BF02942185>
- Dorota, K. (2008). Physiology and metabolism of Crabtree-negative yeast *Debaryomyces occidentalis*. *Food Chemistry and Biotechnology*, 72, 36–46.
- Ebabhi, A. M., Adekunle, A. A., Okunowo, W. O., and Osuntoki, A. A. (2013). Isolation and characterization of yeast strains from local food crops. *Journal of Yeast and Fungal Research*, 4(4), 38–43.
- Hine, C., Harputlugil, E., Zhang, Y., Ruckenstein, C., Lee, B. C., Brace, L., Longchamp, A., Treviño-Villarreal, J. H., Mejia, P., Ozaki, C. K., Wang, R., Gladyshev, V. N., Madeo, F., Mair, W. B., and Mitchell, J. R. (2015). Endogenous Hydrogen Sulfide Production Is Essential for Dietary Restriction Benefits. *Cell*, 160(1–2), 132–144. <https://doi.org/10.1016/j.cell.2014.11.048>
- Hong, Y. A., and Park, H. D. (2013). Role of non-*Saccharomyces* yeasts in Korean wines produced from Campbell Early grapes: Potential use of *Hanseniaspora uvarum* as a starter culture. *Food Microbiology*, 34(1), 207–214. <https://doi.org/10.1016/j.fm.2012.12.011>
- Huang, C., Roncoroni, M., and Gardner, R. C. (2014). MET2 affects production of hydrogen sulfide during wine fermentation. *Applied Microbiology and Biotechnology*, 98(16), 7125–7135. <https://doi.org/10.1007/s00253-014-5789-1>
- Huang, C.-W., Walker, M. E., Fedrizzi, B., Roncoroni, M., Gardner, R. C., and Jiranek, V. (2016). The yeast TUM1 affects production of hydrogen sulfide from cysteine treatment during fermentation. *FEMS Yeast Research*, 16(8), fow100. <https://doi.org/10.1093/femsyr/fow100>

- Jessen, J. E., and Orlygsson, J. (2012). Production of ethanol from sugars and lignocellulosic biomass by *Thermoanaerobacter j1* isolated from a hot spring in Iceland. *Journal of Biomedicine and Biotechnology*, 2012, 1–7. <https://doi.org/10.1155/2012/186982>
- Jolly, N. P., Augustyn, O. P. H., and Pretorius, I. S. (2016). The Role and Use of Non-Saccharomyces Yeasts in Wine Production. *South African Journal of Enology and Viticulture*, 27(1), 15–39. <https://doi.org/10.21548/27-1-1475>
- Kamada, K., and Murata, M. (1984). On the Mechanism of Brewer's Yeast Flocculation. *Agricultural and Biological Chemistry*, 48(10), 2423–2433. <https://doi.org/10.1080/00021369.1984.10866535>
- Kang, A., and Lee, T. S. (2015). Converting Sugars to Biofuels: Ethanol and Beyond. *Bioengineering*, 2(4), 184–203. <https://doi.org/10.3390/bioengineering2040184>
- Klinke, T., Kneist, S., de Soet, J. J., Kuhlisch, E., Mauersberger, S., Förster, A., and Klimm, W. (2009). Acid Production by Oral Strains of *Candida albicans* and *Lactobacilli*. *Caries Research*, 43(2), 83–91. <https://doi.org/10.1159/000204911>
- Knop, M. (2011). Yeast cell morphology and sexual reproduction – A short overview and some considerations. *Comptes Rendus Biologies*, 334(8–9), 599–606. <https://doi.org/10.1016/j.crv.2011.05.007>
- Lazar, Z., Neuvéglise, C., Rossignol, T., Devillers, H., Morin, N., Robak, M., Nicaud, J.-M., and Crutz-Le Coq, A.-M. (2017). Characterization of hexose transporters in *Yarrowia lipolytica* reveals new groups of Sugar Porters involved in yeast growth. *Fungal Genetics and Biology: FG and B*, 100, 1–12. <https://doi.org/10.1016/j.fgb.2017.01.001>
- Lee, Y.-J., Choi, Y.-R., Lee, S.-Y., Park, J.-T., Shim, J.-H., Park, K.-H., and Kim, J.-W. (2011). Screening Wild Yeast Strains for Alcohol Fermentation from Various Fruits. *Mycobiology*, 39(1), 33–39.
- Li, Y., Zhang, Y., Liu, M., Qin, Y., and Liu, Y. (2019). *Saccharomyces cerevisiae* isolates with extreme hydrogen sulfide production showed different oxidative stress resistances responses during wine fermentation by RNA sequencing analysis. *Food Microbiology*, 79, 147–155. <https://doi.org/10.1016/j.fm.2018.10.021>
- Linderholm, A., Dietzel, K., Hirst, M., and Bisson, L. F. (2010). Identification of MET10-932 and characterization as an allele reducing hydrogen sulfide formation in wine strains of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 76(23), 7699–7707. <https://doi.org/10.1128/AEM.01666-10>
- Magyar, I., Nyitrai-Sárdy, D., Leskó, A., Pomázi, A., and Kállay, M. (2014). Anaerobic organic acid metabolism of *Candida zemplinina* in comparison with *Saccharomyces* wine yeasts. *International Journal of Food Microbiology*, 178, 1–6. <https://doi.org/10.1016/j.ijfoodmicro.2014.03.002>
- Malfeito-Ferreira, M. (2011). Yeasts and wine off-flavours: A technological perspective. *Annals of Microbiology*, 61(1), 95–102. <https://doi.org/10.1007/s13213-010-0098-0>
- Mateo, J. J., and Maicas, S. (2016). Application of non-saccharomyces yeasts to wine-making process. *Fermentation*, 2(14), 1–13.
- Matsushika, A., Inoue, H., Kodaki, T., and Sawayama, S. (2009). Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: Current state and perspectives. *Applied Microbiology and Biotechnology*, 84(1), 37–53. <https://doi.org/10.1007/s00253-009-2101-x>
- Mill, P. J. (1964). The Nature of the Interactions between Flocculent Cells in the Flocculation of *Saccharomyces cerevisiae*. *Journal of General Microbiology*, 35(1), 61–68. <https://doi.org/10.1099/00221287-35-1-61>

- Mills, D. A., Phister, T., Neeley, E., and Johannsen, E. (2008). Wine fermentation. In L. Cocolin and D. Ercolini (Eds.), *Molecular techniques in the microbial ecology of fermented foods* (pp. 162–192). Springer.
- Morata, A. (2019). Enological Repercussions of Non-Saccharomyces Species in Wine Biotechnology. *Fermentation*, 5(3), 72. <https://doi.org/10.3390/fermentation5030072>
- Nandy, S. K., and Srivastava, R. K. (2018). A review on sustainable yeast biotechnological processes and applications. *Microbiological Research*, 207, 83–90.
- Nayyar, A., Walker, G., Canetta, E., Wardrop, F., and Adya, A. K. (2014). *Cell surface properties and flocculation behaviour of industrial strains of Saccharomyces cerevisiae*. 2, 64–72.
- Noble, J., Sanchez, I., and Blondin, B. (2015). Identification of new *Saccharomyces cerevisiae* variants of the MET2 and SKP2 genes controlling the sulfur assimilation pathway and the production of undesirable sulfur compounds during alcoholic fermentation. *Microbial Cell Factories*, 14(1), 68. <https://doi.org/10.1186/s12934-015-0245-1>
- Nogami, S., Ohya, Y., and Yvert, G. (2007). Genetic complexity and quantitative trait loci mapping of yeast morphological traits. *PLoS Genetics*, 3(2), 0305–0318. <https://doi.org/10.1371/journal.pgen.0030031>
- Olowonibi, O. O. (2017). Isolation and characterization of palm wine strains of *Saccharomyces cerevisiae* potentially useful as bakery yeasts. *European Journal of Experimental Biology*, 7(2), 1–13.
- Panagiotis, T., Kanellis, A., Logothetis, S., and Nerantzis, E. (2013). Use of non-Saccharomyces *Torulaspora delbrueckii* yeast strains in winemaking and brewing. *Matica Srpska Journal of Natural Sciences*, 124, 415–426.
- Rainier, S., and Pretorius, L.S. (2000). Selection and improvement of wine yeasts. *Annals of Microbiology*, 50, 15–31.
- Rossouw, D., Bagheri, B., Setati, M. E., and Bauer, F. F. (2015). Co-Flocculation of Yeast Species, a New Mechanism to Govern Population Dynamics in Microbial Ecosystems. *PloS One*, 10(8), 1–17. <https://doi.org/10.1371/journal.pone.0136249>
- Santaniello, A., Loreti, E., Gonzali, S., Novi, G., and Perata, P. (2014). A reassessment of the role of sucrose synthase in the hypoxic sucrose-ethanol transition in *Arabidopsis*. *Plant, Cell and Environment*, 37(10), 2294–2302. <https://doi.org/10.1111/pce.12363>
- Sorrentino, A., Boscaino, F., Cozzolino, R., Volpe, M. G., Elena, I., Guerriero, S., Picariello, T., and La-Cara, F. (2013). Characterization of Free Volatile Compounds in Fiano Wine Produced by Different Selected Autochthonous Yeasts. *Chemical Engineering Transactions*, 32, 1837–1842.
- Stewart, G. (2018). Yeast Flocculation—Sedimentation and Flotation. *Fermentation*, 4(2), 28. <https://doi.org/10.3390/fermentation4020028>
- Straver, M. H., Kijne, J. W., and Smit, G. (1993). Cause and control of flocculation in yeast. *Trends in Biotechnology*, 11(6), 228–232. [https://doi.org/10.1016/0167-7799\(93\)90133-T](https://doi.org/10.1016/0167-7799(93)90133-T)
- Tao, N., Gao, Y., and Liu, Y. (2011). Isolation and characterization of a *Pichia anomala* strain: A promising candidate for bioethanol production. *Brazilian Journal of Microbiology*, 42, 668–675.
- Teramoto, Y., Sato, R., and Ueda, S. (2005). Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. *African Journal of Biotechnology*, 4(2), 160–163.

- Úbeda, J., Maldonado Gil, M., Chiva, R., Guillamón, J. M., and Briones, A. (2014). Biodiversity of non-Saccharomyces yeasts in distilleries of the La Mancha region (Spain). *FEMS Yeast Research*, 14(4), 663–673. <https://doi.org/10.1111/1567-1364.12152>
- Ugliano, M., Kolouchova, R., and Henschke, P. A. (2011). Occurrence of hydrogen sulfide in wine and in fermentation: Influence of yeast strain and supplementation of yeast available nitrogen. *Journal of Industrial Microbiology and Biotechnology*, 38(3), 423–429. <https://doi.org/10.1007/s10295-010-0786-6>
- Uzah, G. A., Akani, N. P., and Odu, N. N. (2020). Screening of Aspergillus and Candida Species with Utmost Potential to Synthesize Citric Acid. *Journal of Advances in Microbiology*, 20(4), 10–18. <https://doi.org/10.9734/jamb/2020/v20i430232>
- Van Mulders, S. E., Ghequire, M., Daenen, L., Verbelen, P. J., Verstrepen, K. J., and Delvaux, F. R. (2010). Flocculation gene variability in industrial brewer's yeast strains. *Applied Microbiology and Biotechnology*, 88(6), 1321–1331. <https://doi.org/10.1007/s00253-010-2843-5>
- Varize, C. de S., Maria Christofoleti-Furlan, R., Muynarsk, E. de S. M., Pereira, G. V. de M., Dantas Lopes, L., and Carlos Basso, L. (2019). Biotechnological Applications of Nonconventional Yeasts. In *Yeasts in Biotechnology* (pp. 1–26). intechopen.
- Verstrepen, K. J., Derdelinckx, G., Verachtert, H., and Delvaux, F. R. (2003). Yeast flocculation: What brewers should know. *Applied Microbiology and Biotechnology*, 61(3), 197–205. <https://doi.org/10.1007/s00253-002-1200-8>
- Viana, N. C., Portugal, C., and Helena, S. (2017). Morphophysiological and molecular characterization of wild yeast isolates from industrial ethanol process. 11(37), 1422–1430. <https://doi.org/10.5897/AJMR2017.8691>
- Vicente, J., Calderón, F., Santos, A., Marquina, D., and Benito, S. (2021). High Potential of Pichia kluyveri and Other Pichia Species in Wine Technology. *International Journal of Molecular Sciences*, 22, 1196. <https://doi.org/10.3390/ijms22031196>
- Whiting, G. C. (1976). Organic acid metabolism of yeasts during fermentation of alcoholic beverages—a review. *Journal of the Institute of Brewing*, 82(2), 84–92. <https://doi.org/10.1002/j.2050-0416.1976.tb03731.x>
- Winter, G., Cordente, A. G., and Curtin, C. (2014). Formation of Hydrogen Sulfide from Cysteine in Saccharomyces cerevisiae BY4742: Genome Wide Screen Reveals a Central Role of the Vacuole. *PLoS ONE*, 9(12), e113869. <https://doi.org/10.1371/journal.pone.0113869>
- Yoshida, S., and Yokoyama, A. (2012). Identification and characterization of genes related to the production of organic acids in yeast. *Journal of Bioscience and Bioengineering*, 113(5), 556–561. <https://doi.org/10.1016/j.jbiosc.2011.12.017>
- Zabed, H., Faruq, G., Sahu, J., Azirun, M., Hashim, R., and Nasrulhaq Boyce, A. (2014). Bioethanol Production from Fermentable Sugar Juice. *The Scientific World Journal*, 2014, 1–11. <https://doi.org/10.1155/2014/957102>
- Zara, G., and Nardi, T. (2021). Yeast Metabolism and Its Exploitation in Emerging Winemaking Trends: From Sulfite Tolerance to Sulfite Reduction. *Fermentation*, 7(2), 57. <https://doi.org/10.3390/fermentation7020057>
- Khan, Rijwan, Nipun Tyagi, and Nikita Chauhan. "Safety of Food and Food Warehouse Using VIBHISHAN." *Journal of Food Quality* 2021 (2021).
- Sahni, Varsha, Sandeep Srivastava, and Rijwan Khan. "Modelling Techniques to Improve the Quality of Food Using Artificial Intelligence." *Journal of Food Quality* 2021 (2021).

UNDER PEER REVIEW