Original Research Article

Technological characteristics of wild non-Saccharomyces sourced from bananafruit and honey

Comment [A1]: banana fruit

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 (Clavisporalusitaniae), 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

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The *Saccharomycescerevisiae*yeasthas been themost widely accepted microorganism industrially (Dorota, 2008; Nandy and Srivastava, 2018), due to its ability to complete sugar fermentation, high alcoholyield,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).

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Review and harmonization of the microbialtaxonomy employed in food fermentation (Bourdichon *et al*., 2012), have led to the adoptionofyeast 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)asstandard(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), thusrevealing 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,isunderutilized, such asthe potential benefits of non-Saccharomyces yeasts in wine production that unavoidably have a different list of desired properties (Mateo and Maicas, 2016). The characteristics ofyeasts without Saccharomyces (Candida, Kloeckera, Hanseniaspora, Zygosaccharomyces, Schizosaccharomyces, Torulaspora, Brettanomyces, Saccharomycodes, Pichia and Williopsis genera) in vinificationare receiving increasing attention from wine microbiologists in countries producing Old and New World wines(Jolly *et al*., 2016)andindigenous 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 possessgood 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), forthe 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).

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Flocculation is a natural, reversible active assemblage of cells into flocs. Aggregation of microorganismsis common among bacteria, filamentous fungi, algae, and yeasts.Dominant flocculation genes (FLO genes) FL01, FL05, and FL08 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 flocculentis 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-encodingflocculin 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_2S) 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$H_2S$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_2S 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_2S 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). 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 evaluatethetechnological characteristic of non-Saccharomyces wild bananas sourced from the wild.

Materials and Method

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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 ℃. After incubation, an aliquot (0.1ml) of the broth was transferred to prepared yeast extract peptone dextrose agar plates(YEPDA) supplemented with chloramphenicol and evenlyspread using a sterile bent glass rod. Plates were also incubated at 30 ℃ 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.

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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, thoroughlymixed 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, gentlymixed 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 towashed pellet and then centrifuged at 14000 at 4°C for 2 minutes. The pellet was air-dried after thesupernatant 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 MgCl2, 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 PRISMTM 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.

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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 tubesterilized 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 alcoholproduction

Yeast isolates were screened for alcoholproductioncapacity according to the method of Ambadas (2011) and Reddy *et al*. (2009) modified usingpeptone water containing sucrose, glucose, and fructose (20 % (w/v)) respectively as substrate. The bottles were sterilized and rapidly cooled to room temperatureand 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) ×131.25. Ogu (2011). …….. Equation 1.

where:

OG = original gravity of the sample.

FG = Final Gravity of the sample.

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For specific gravity correction, sample temperature below 20 ºC 0.0002 is subtracted per degree ºC and temperature above 20 ºC 0.0002 is added per degree ºC (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) $CaCl_2$, while the initial OD600 nmculture for each was determined. After agitationon 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 OD600nm determined using spectrophotometric method (Nayyar *et al*., 2014). Flocculation ability (F) was determined by the following equation: F = (1-B/A) × 100%. Where A is the absorbance at 600 nm (OD600) immediately before the cells were shaken in flocculation buffer and B is the absorbance at 600 nm (OD600) 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 Uzah*et al*. (2020). After a 3 - 5 days incubation period at 28 – 30 ° C of Czapek-Doxagar medium, supplemented with 0.5 g of Ca_2CO_3 and bromocresol green asindicator, 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_2S) 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 ℃. The qualitative measure for H_2S 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

Yeastisolates isolated from banana fruit and wild honey were subjected to sugar fermentation to determine their fermenting ability of fructose, glucoseandsucrosedue 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. andyeast isolates identified as B10 (*Candida tropicalis*), B7 (*Candida tropicalis*), H4 (*Candida tropicalis*), H7 (*Clavisporalusitaniae*), 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
C. tropicalis^{B10}	+	+	+
C. tropicalis^{B7}	+	+	+
C. tropicalis^{H4}	+	+	+
Cl. lusitaniae^{H7}	+	+	+
C. tropicalis^{CY}	+	+	+

+ 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 toalcohol. The percentage of alcohol produced from the sucrose fermentation ranges from 4.11 – 11.50 (%) and individual yeast isolates recorded thefollowing: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%) producedmorealcohol than the commercial wine yeast, CY (6.28%). Alcoholproducedfrom 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 morealcohol than the commercial wine yeast, CY (9.40%). Statistically, there is a significant difference in the concentration of alcoholproducedbetween yeast isolates from sucrose, glucose, and fructose at the P value <0.001, and the three different sugarsfermentedby 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.

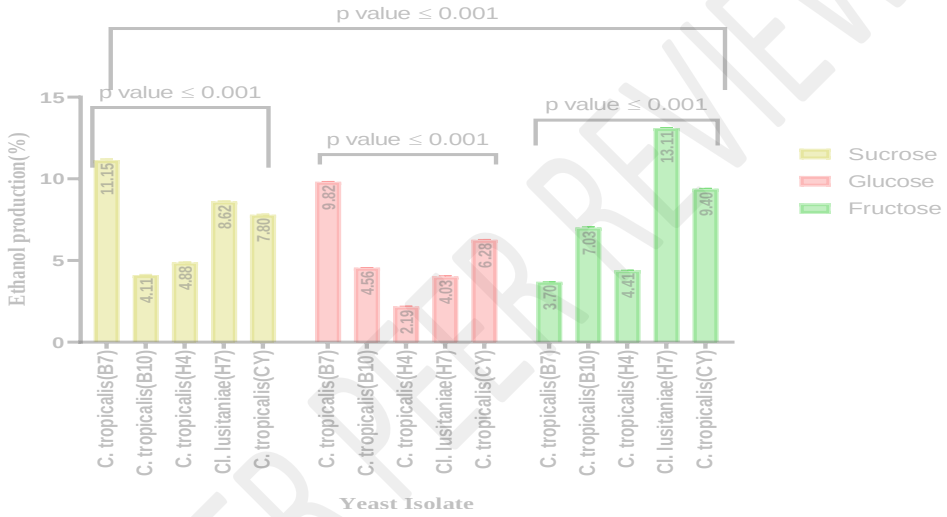

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Figure 1. Alcohol production ability of yeast isolates using glucose, fructose, and sucrose.

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.

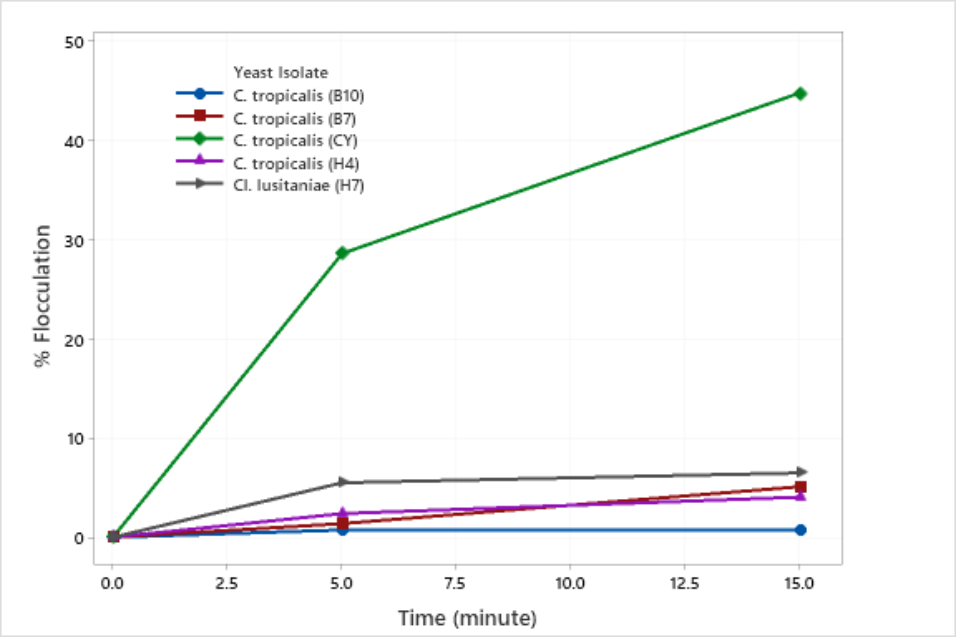

Figure 2. Flocculation proportion of yeast cells with time.

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 recordedthefollowings: 1.90±0.41, 3.10±0.41, 1.25±0.07, 3.90±0.41 and 2.40±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-value < 0.001;but there is no significant difference in the acid unitage of B10 (1.90±0.41) and H7 (2.40±0.41).

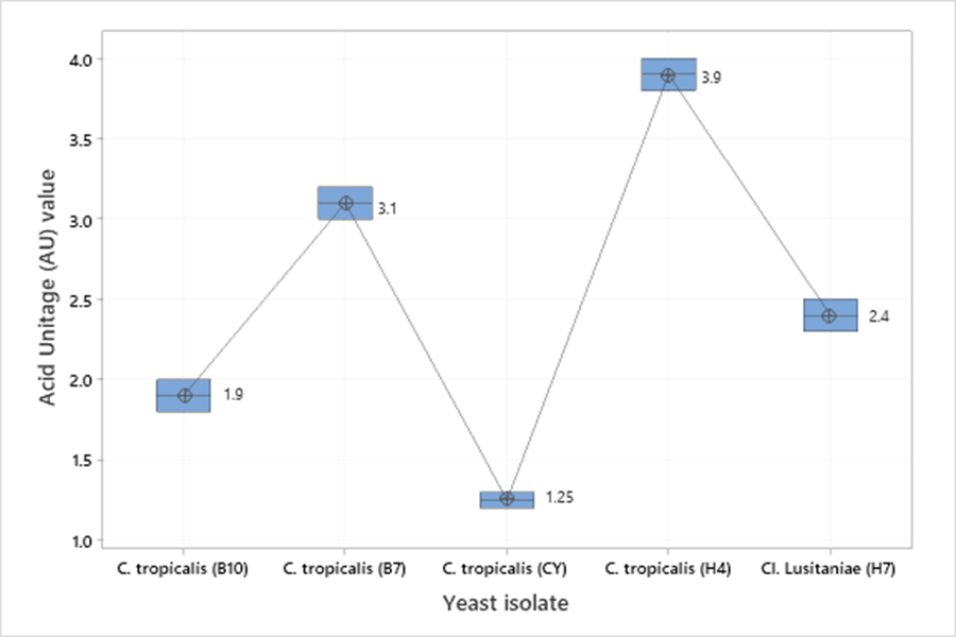

Figure 3. Acid unitage values of yeast isolate on Czapek-Dox agar.

Hydrogen sulphide production is an undesired but inevitablemetaboliteexpressed 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 sulphideproduction; 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 coloniesin 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
C. tropicalis^{B10}	++	++
C. tropicalis^{B7}	+	+
C. tropicalis^{H4}	+	++
Cl. lusitaniae^{H7}	+	+
C. tropicalis^{CY}	+	+

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 wildhoney in southern, Nigeria in order to select appropriate autochthonous starter cultures fortheproductionof typical safe regional wine and traditional fermented food

Sugar Fermentation Capacity

The yeast isolates B10 (Candida tropicalis), B7 (Candida tropicalis), H4 (Candida tropicalis), H7 (Clavisporalusitaniae), 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 periplasmicinvertase and sucrose H^+symporterAGT1 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 (Clavisporalusitaniae), 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 ofsucrosesynthases or invertases(Santaniello *et al*., 2014; Zabed *et al*., 2014)and multiplegenesforthe transportation of hexose,Hxt(1-17)(Batista *et al*., 2004; Boles and Hollenberg, 1997) are associated with *Candidatropicalis*(Chattopadhyay *et al*., 2020) More than 95% of the ethanol produced ismonosaccharideor disaccharides, with low yields of alcohol duetothe formation of end products such as acetate, butyrate and CO_2 (Jessen and Orlygsson, 2012; Verstrepen *et al*., 2003).

Thenoticeable 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 ontheselectionofmicroorganisms(Zabed *et al*., 2014). Although, my findings disagree with the report of greater yields of ethanol using glucose (0.37 gg^{-1}) than fructose (0.32 gg^{-1})(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 comparedto 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 Klinke*et al*. (2009),*Candida albican* was implicated as organic acid producer. Organic acidproduction 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_2S) 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_2S is a compulsory intermediate in the assimilation of sulphur catalyze by sulphitereductase 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 Linderholm*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.

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