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ORIGINAL PAPER

Wine produced from date palm (*Phoenix dactylifera* L.) fruits using *Saccharomyces cerevisiae* X01 isolated from Nigerian locally fermented beverages

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Abstract

The study focused on the production of wine from date palm fruits (*Phoenix dactylifera* L.) using a strain of yeast isolated from selected Nigerian locally fermented beverages ('pito', 'brukutu' and palm wine). Seven (7) distinct yeasts were isolated and identified using cultural, microscopy and biochemical tests (temperature tolerance, flocculation, ethanol tolerance, H_2S production and killer toxin production and the ability to assimilate and ferment sugars. The yeast isolates were screened using refractometric and spectrophotometric methods to select the isolate with the best ability for wine production. This isolate was molecularly characterized, grown in 1 L of potatoes dextrose broth, freeze dried and used for wine production. The population of yeast, bacteria and the physicochemical analysis of the must were monitored during fermentation. Populations of bacteria in the wine were assessed by standard pour plate count. The proximate content and physicochemical properties of the produced wine before and after ageing, as well as the sensorial quality of the produced wine was determined. All the yeast isolates possessed the ability to flocculate, tolerate ethanol concentration of between 5 and 20% and temperature range of 30–45 °C, produced low concentration of H_2S and does not produce killer toxins. A palm wine isolate, identified as *Saccharomyces cerevisiae* X01 was selected as the best isolate with the most ability for wine production. The total yeast count increases as the period of fermentation progressed while the total viable bacterial count reduced as the fermentation period progressed. There was a significant difference ($P < 0.05$) in the physicochemical properties of the must during fermentation. At the end of the fermentation, the produced wine had 5.22% and 4.86% ethanol content for *S. cerevisiae* QA23 and *S. cerevisiae* X01 respectively. There was no significant difference ($P > 0.05$) between the proximate and physicochemical compositions of the produced wine before and after ageing using *Saccharomyces cerevisiae* X01 when compared to the control *S. cerevisiae* QA23. This study revealed that the Nigerian locally sourced *S. cerevisiae* X01 can be used as an alternative substrate for industrial scale production of wine with a mild alcoholic content.

Keywords Pito · Brukutu · Palmwine · Refractometry · Spectrophotometry · *Saccharomyces cerevisiae*

Introduction

Yeasts are industrially important microorganisms because they possess dynamic activities and as such, have been used for diverse industrial applications such as the production

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of many important products like beer, wine, bread, biofuels, and biopharmaceuticals (Martínez et al. 2012; Johnson 2013). The most industrially exploited yeast by man is *Saccharomyces cerevisiae,* which is also known as brewers or bakers' yeast. *S. cerevisiae* has more aerobic growth habit, maximum yielding capability and stability during storage and it is usually derived from special selection of naturally occurring yeast strains with short generation time (Hong and Nielsen 2012). Since nineteenth century, *S. cerevisiae* has been used for the production of different kinds of products during fermentation, this yeast strain might have mutated due to the constant industrial use and might have silently lost some of its key attributes.

Isolation and development of suitable yeast for use in production processes of economic importance are crucial in biotechnology. The type or nature of yeasts with fermentative activity isolated from selected local fermented alcoholic beverages from Minna, Niger State, Nigeria may possess some peculiar characteristics. Information on physiological studies of such indigenous yeasts is not available; this information is required for domesticating such yeasts for wine production. Therefore, these indigenous strains must be isolated and subjected to rigorous studies before they can be reliably used for this purposes, as starter cultures in wine production. Further studies on these indigenous yeasts isolated from locally produced alcoholic beverages may give some good qualities and properties which will improve products in the wine industry. The findings of this study would provide a basis for comparing the isolated wild yeasts from selected locally fermented alcoholic beverages with those described in earlier reports and would also help to confer wild yeasts with different industrial useful properties.

One of the oldest cultivated plants that have been used as food for over 6000 years is the date palm (*Phoenix dactylifera* L.) (Johnson 2012). In the Middle East, North Africa and some Asian countries, date palm serves as an important food crop and is considered to be one of the most important fruit tree particularly, because the fruit contributes to the economic and social life within these regions and also makes up as vital component of their diet (Nwaokobia et al. 2018). Date palm trees are often referred to as the "tree of life" because of the high nutritional value of the fruit, great yields and its long life (Bankefa and Oyedeji 2015). Date palm fruits are commonly known as "Dabino" in the Northern part of Nigeria. It is a monocotyledonous woody perennial fruit species belonging to the Arecaceae family with over 3000 species and 200 genera (Garba and Galadima 2012).

Date fruits are said to contain simple sugars like fructose, sucrose and glucose thus making them one of the major sources of carbohydrate. It also contains dietary fibre and some important minerals like; iron, potassium, selenium, calcium, copper, cobalt magnesium, fluorine, manganese, phosphorus, potassium, sodium, boron, sulphur, zinc and vitamins like vitamin C, B_1 (thiamine), B_2 (riboflavin), A, and niacin (nicotinic acid) while the fat and protein contents are considerably low with the protein in dates containing 23 types of amino acids (Guizani et al. 2010).

The word ''wine'' was coined from the Greek word ''oines'' which means wine and the study of wine is referred to as ''oenology''. The process of wine making starts with the harvest of grapes from the vine, followed by separation of the juice from the grape before fermentation process is being carried out and it is concluded with the variety of storage and ageing steps (Awe and Nnadoze 2015). Wines are categorized using the following characteristics, fruit variety, region of origin, colour, name of the wine maker or viticulturalist, or its production technique, but the three basic groups of wines that are most easily distinguishable for the consumer are; table wines, sparkling wines and fortified wines. For over thousands of years, wine has been produced and enjoyed by many people, from peasants to kings (Awe and Nnadoze 2015).

Apples, berries, grapes and several other fruits have been used for the production of wine through fermentation process. These fruits possess some natural chemical balance that allows for a complete fermentation without the addition of sugar, acid, enzymes or other nutrients (Johnson 2012). The wine produced from these fruits then serve as a rich source of vitamins, essential amino acids, minerals and fatty acid, although research is still on going to discover more fruits with these characteristics that are effective for wine production (Awe 2011).

Wine fermentation is popularly carried out by yeast, which are single-celled microorganisms and the mostly commonly used is the *Saccharomyces cerevisiae,* which is commonly isolated from sugary foods and fruits (Olowonibi 2017). *S. cerevisiae* has the ability to carry out fermentative and oxidative reactions, thus, breaking down the sugar found in juice extracted from the fruits, producing alcohol and carbon dioxide under anaerobic conditions. It can also grow rapidly on sugar and produce high yields of biomass under aerobic conditions (Olowonibi 2017).

The aim of this study was to produce wine from date palm (*Phoenix dactylifera* L.) fruits using S*accharomyces cerevisiae* X01 isolated from Nigerian locally fermented beverages.

Materials and methods

Collection of samples (alcoholic beverage and date palm fruit samples)

Three Nigerian locally produced fermented alcoholic beverages ('pito', 'burukutu' and palm wine) were bought in triplicate from Maitunbi, Minna, Niger State and was

transported to the Federal University of Technology Bosso, campus laboratory using ice-packed box so as to maintain its temperature.

Fresh date palm fruits and dried date palm fruits were obtained from Engr. A.A. Kure market Minna, Niger State, Nigeria. They were sorted and washed to remove dirt, before air drying at room temperature (28 °C ± 2).

Media preparation

Potatoes dextrose agar (PDA), potatoes dextrose broth, yeast peptone glucose broth (YPGB) and nutrient agar were prepared according to manufacturer's instruction and was sterilized using autoclave at 121 °C for 15 min. The media was allowed to cool before adding 250 mg of antibiotic (chloramphenicol) to 250 mL of PDA to prevent bacterial growth.

Isolation of yeast from locally produced alcoholic beverage samples

The locally produced alcoholic beverage samples ('pito', 'burukutu' and palm wine) were serially diluted and 0.1 mL of the diluted samples was taken from 10^{-7} dilution factor test tubes and plated on 20 mL of molten PDA in a 15 × 100 mm Petri dish. This was incubated at 30 °C for 72 h. Distinct colonies of isolates were subcultured to obtain pure isolates. Purity of isolates was checked by streaking all isolates on PDA and the pure cultures was maintained on PDA slant at 4 °C for short term storage (Castillo-Castillo et al. 2016).

Identification and characterization of yeast isolates

The following tests were carried out to identify and characterise the yeast isolates: microscopy and sugar fermentation tests (Barnett et al. 1990), temperature tolerance test (Valdineia et al. 2008), flocculation test (Valdineia et al. 2008), ethanol tolerance test (Olowonibi 2017), H_2S production test (Olowonibi 2017) and killer toxin production test (Olowonibi 2017). The isolates were also tested for their ability to assimilate and to ferment 1% of the following sugars: glucose, lactose, sucrose, arabinose, fructose, mannitol, sorbitol, rhamnose, xylose and maltose using methods of Olowonibi (2017).

Cultural, morphology and microscopy examination of yeast isolates

Each distinct isolate's colony elevation, colour, texture, shape and other distinct and unique features was observed macroscopically while for the microscopy observation; thin smear was prepared by emulsifying a loopful of each isolate on a clean slide with a drop of normal saline. The film was spread to make a thin film and then air dried after which it was Gram stained and observed with a light microscope (Amscope B490B-3M, UK) under 10X and 40X objective lenses (Castillo-Castillo et al. 2016).

Screening for wine production ability

The fermentative ability of yeast isolate was tested using YPGB. Ten (10) millilitre of YPG broth was dispensed in test tubes and autoclaved at 121 °C for 15 min. The broth was allowed to cool before inoculating each test tube with 5 µL of different suspension of yeast isolate that was standardized using 0.5 McFarland standard (Zapata and Ramirez-Arcos, 2015). The test tubes were incubated at 35 °C ± 2 and 200 rpm for 5 days in an incubator shaker (PEC ZHP-100, USA). This test was carried out in duplicate. A control was set up without inoculation of isolate. The absorbance reading was taken at 630 nm wavelengths using a spectrophotometer (UV–VIS 752, China) and the sugar utilization rate was measured using a refractometer (Lasany LI-R501, India) (Gidado et al. 2016).

Molecular characterization of yeast with most ability for wine production

The yeast cell with the most ability for wine production was molecularly characterized to reveal the identity of the isolate by DNA extraction, polymerase chain reaction, gel electrophoresis and sequence analysis (Valdineia et al. 2008).

Production of yeast cell biomass

The yeast cell with the most ability for wine production, subsequently identified as *Saccharomyces cerevisiae* X01 was grown in 1 L of potatoes dextrose broth and placed in an incubator shaker (PEC ZHP-100, USA) at 30 °C and 200 rpm for 6 days. After incubation, the medium was aseptically poured into sterile Petri dishes and freeze dried using lyophilizer (LGJ18, China). Ten gram (10 g) of powered yeast biomass was obtained. The sample was first pre dried in the drying tray and lowered into the freezing compartment, it was frozen to − 50 °C for 2 h. The sample holder was removed from the freezing compartment to the drying compartment and dried at 20 pa with starting temperature of − 50 °C and ambient temperature (28 °C ± 2) indicating completion of drying process (Balogu and Towobola 2017).

Preparation of date fruit wine

The knife, grinder, bowl, muslin cloth and stirring rod used for wine production were sterilized with sodium metabisulphate solution. The date palm fruit was sliced using a sterile sharp knife to remove the seed and was macerated using a

clean sterilized grinding engine to produce a small granular texture of the date palm fruit so as to increase the surface area of the fruit for the activity of yeast for fermentation. Two litres of water was sterilised at 121 °C for 15 min and was allowed to cool to about 45 °C before it was used to mix 481.5 g of the crushed date palm fruit and it was evenly mixed using a sterilized stirrer, the fruit pulp also known as the "must" produced was poured into sterilized fermentor and was sterilized with standardized sodium metabisulphate to remove microbial contaminants 24 h before the addition of yeast This process was duplicated for control (Awe and Nnadoze 2015).

Fermentation process

Two gram (2 g) of *Saccharomyces cerevisiae* X01 biomass was added to the must in one of the fermenting jar by sprinkling it over the surface of the wine and then stirred while the other fermenting jar was sprinkled with 2 g of Lavin wine yeast; *Saccharomyces cerevisiae* QA23 as control. The inoculated must was covered with sterile muslin cloth and incubated at room temperature (28 °C ± 2). It was aerated daily by stirring twice to encourage yeast multiplication. Aerobic fermentation was terminated after 6 days and the must was sieved to remove the shaft and debris of the crushed fruits. For the anaerobic fermentation phase, the filtrate obtained after sieving the must was transferred into a separate anaerobic fermentation jar. Sodium metabisulphate solution was added to each filtrate to supply sulphur dioxide, then air traps, filled with sterile water was fixed to each fermenting jar and was incubated at room temperature (28 °C ± 2). The anaerobic fermentation was terminated after four weeks; the wine was then stored for 1 week to allow the yeast to flocculate. The resulting wine was aged for 1 month. The aged wine was pasteurized at 60 °C for 15 min to stop fermentation. The clarified wine was decanted into sterile bottles and corked (Awe and Nnadoze 2015). All processes of the wine production were carefully carried out under aseptic condition using proper aseptic techniques.

Physicochemical analysis of must before and after aerobic fermentation and during anaerobic fermentation

An aliquot quantity of the must was taken aseptically before and after aerobic fermentation and on a weekly basis during anaerobic fermentation to monitor the physicochemical content. The pH was measured with a pH meter (Jenway 3510, UK). The titratable acidity was determined using wine maker's acid kit. The specific gravity, percentage alcohol and H_2S content of the samples was determined using triple scale hydrometer (Alla 5451, France). The conductivity of the wine samples was determined using conductivity metre (Wincom DDS-307, China).

Enumeration of yeast and total viable bacteria during fermentation

The population of yeast in the fermenting must during aerobic and anaerobic phases was monitored by microscopic counting using haemocytometer. Colony were expressed as cells/mL. Bacterial populations in the wine were assessed by standard pour plate method using nutrient agar. Tenfold serial dilution of the wine was made and 0.1 mL of desired dilution plated. Colony were counted and expressed as colony forming unit per millilitre (cfu/mL).

Determination of physiochemical properties, alcohol content and proximate content of produced wine

The proximate composition and physiochemical analysis of the wine produced was determined before and after ageing using the method described by Association of Official Analytical Chemists, AOAC (2000). These were moisture, crude lipids, crude fibre, crude protein, ash and carbohydrate contents.

The pH of the wine produced was measured with a pH meter (Jenway 3510, UK). The titratable acidity was determined using wine maker's acid kit. The specific gravity, percentage alcohol and H_2S content of the samples was determined using triple scale hydrometer (Alla 5451, France) for beer and wine. The conductivity of the wine samples was determined using conductivity metre (Wincom DDS-307, China). The 0.1 mL of the wine produced was plated on nutrient agar and PDA plates to check for microbial growth.

Sensory evaluation of produced wine

The sensorial quality of the date wine produced was evaluated for overall impressions in a trial with 10 trained or consuming panellists using ten points hedonic scale (from grade 1: extremely disliked to grade 10: extremely liked) and compared with the control date fruit wine (Balogu and Towobola, 2017).

Statistical analysis of data

The data obtained from this study was analysed using one-way ANOVA and Duncan multivariable post-hoc test for comparison between control and wine produced. *P* values less than 0.05 was considered statistically significant.

Results

Biochemical characteristic of yeast isolates

Seven distinct colonies of *S. cerevisiae* were isolated from the fermented alcoholic beverage ('pito', 'burukutu' and palm wine) samples, 3 distinct colonies isolated from palm wine were designated as PW_1, PW_2 and PW_3, two distinct colonies isolated from 'pito' and 'burukutu' each were designated as PT_1, PT_2, and BKT_1, BKT_2 respectively. The yeast isolates' colour ranges from creamy to white, the shape ranges from circular to punctiform and irregular with either flat or raised elevation. The yeast isolates all have oval shape when viewed under the microscope.

All the yeast isolates were able to ferment and assimilate glucose, fructose and sucrose while other sugar like lactose, maltose, sorbitol, mannitol, xylose and rhamnose were not utilized. All the yeast isolates possessed the ability to tolerate ethanol at lower concentrations. There was an intensive growth response at 5% ethanol concentration, moderate growth response at 10% and 15% concentrations and at 20% concentration there was a low growth response for isolates PT_1, PT_2, BKT_1 and BKT_2 while for isolate PW_1, PW_2 and PW_3, there was no growth response at the same concentration. For temperature tolerance ability, there was an intensive growth response at 30 °C and 37 °C for all yeast isolates and at 45 °C there was a low response for isolate PW_1, PW_2 and PW_3 while for isolate PT_1, PT_2, BKT_1 and BKT_2, there was no growth response at the same temperature. All the isolates have the ability to flocculate and do not produce killer toxin while only isolate PW_1 does not produce H_2S (Table 1).

Wine production ability of yeast isolates

The refractometry method showed that PW_3 gave the lowest refractive index value (1.3250) when compared with the control (1.3410) and this revealed that the sugar in the medium has been utilized by PW_3 (Fig. 1a). The spectrophotometry method also showed that PW_3 had the highest absorbance rate (1.286) at 630 nm (Fig. 1b), therefore this revealed that PW_3 is the best isolate with wine production ability (Supplementary File, Plate 1).

Molecular identity of the yeast isolate with most ability for wine production

The gel electrophoresis micrograph of amplified product of PW_3 had 600 base pairs (Supplementary File, Plate 2) and the sequenced results of the ITS region of yeast isolate revealed that PW_3 is 99% identical to *Saccharomyces*

Table 1 Biochemical characteristics of yeast isolates

Isolates	Ethanol tolerance test				Temperature tolerance test			Flocculation	H_2S production	Killer toxin production	Probable yeast
	5%	10%	15%	20%	30 °C	37 °C	45 °C				
PW_1	+++	++	++	–	+++	+++	+	++	–	–	*S. cerevisiae*
PW_2	+++	++	++	–	+++	+++	+	++	+	–	*S. cerevisiae*
PW_3	+++	++	++	–	+++	+++	++	+++	+	–	*S. cerevisiae*
PT_1	+++	++	++	+	+++	+++	–	+	+	–	*S. cerevisiae*
PT_2	+++	++	++	+	+++	+++	–	++	+	–	*S. cerevisiae*
BKT_1	+++	++	++	+	+++	+++	–	+	+	–	*S. cerevisiae*
BKT_2	+++	++	++	+	+++	+++	–	+++	+	–	*S. cerevisiae*

Keys: +++ = intensive response, ++ = moderate response, + = low response, – = no response

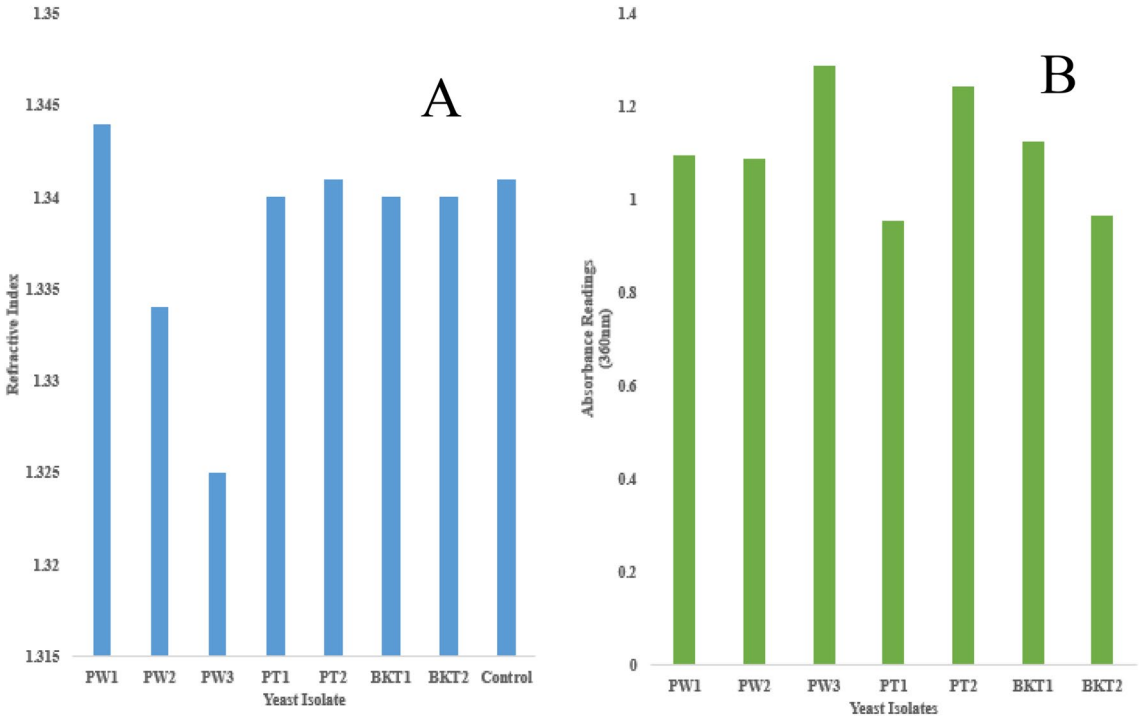

Fig. 1 Refractometry (**a**) and spectrophotometric (**b**) screening for wine production ability of yeast isolates

cerevisiae X01 accession number MT478139. Since it is a palm wine isolate, the phylogenetic tree of *Saccharomyces cerevisiae* strain X01 (Fig. 2) shows that it is related to other yeasts like *Saccharomyces chevalieri* Accession No. AF005714, *Saccharomyces ellipsoideus* Accession No. AF005716.1, *Saccharomyces cerevisiae* QA23 Accession No. 764098, *Saccharomyces cerevisiae* CBS 1171 Accession NR_111007, *Kazachstania africana* CBS:2655 Accession KY103621, *Kazachstania bulderi* strain P7FP8 Accession MT645410, *Kluyveromyces aestuarii* UCDFST:61- Accession MH595310, *Kluyveromyces lactis* CBS 683 Accession NR_166044, *Pichia kluyveri* strain SM12UFAM Accession MN268784, *Pichia kudriavzevii* TTG-81- Accession No. MT321167.

Microbial count during fermentation

The microbiological analysis of the must, which include enumeration of total viable bacterial count and monitoring of yeast growth during aerobic and anaerobic fermentation is presented in Fig. 3. The highest total viable bacterial count was recorded on the first day of aerobic fermentation (Fig. 3a) and as the period of fermentation progressed, there was a decline in the total viable count. The lowest was recorded at week 4 of anaerobic fermentation (Fig. 3b). The total yeast count increases as the period of fermentation progressed depicting a normal batch culture growth curve. Highest total yeast count was recorded on day 3 of the aerobic fermentation (Fig. 3c) while on the 3rd and 4th week of anaerobic fermentation (Fig. 3d), there was no growth.

Physicochemical analysis of must before and after aerobic fermentation and during anaerobic fermentation

The means of the physicochemical analysis before and after aerobic and anaerobic fermentation of the must prepared using *S. cerevisiae* X01 and *S. cerevisiae* QA23 are presented in Tables 2 and 3. After the aerobic fermentation, the pH, specific gravity, conductivity and sugar values of the must for both isolates reduced while the titratable acidity and ethanol content must be increased. This trend was also observed in the physicochemical properties of the must for both organisms during the anaerobic fermentation.

Proximate content of the wine produced before and after clarification and ageing

The means of the proximate component of the wine before and after ageing and clarification are presented in Table 4. There was no significant difference ($P > 0.05$) between the moisture, carbohydrate and crude protein of wine produced by *S. cerevisiae* X01 and *S. cerevisiae* QA23 (control) but there was a significant difference ($P < 0.05$) in their ash content.

Sequencing results of The ITS region of PW_3

Sample is 99% identical to *Saccharomyces cerevisiae* strain X01

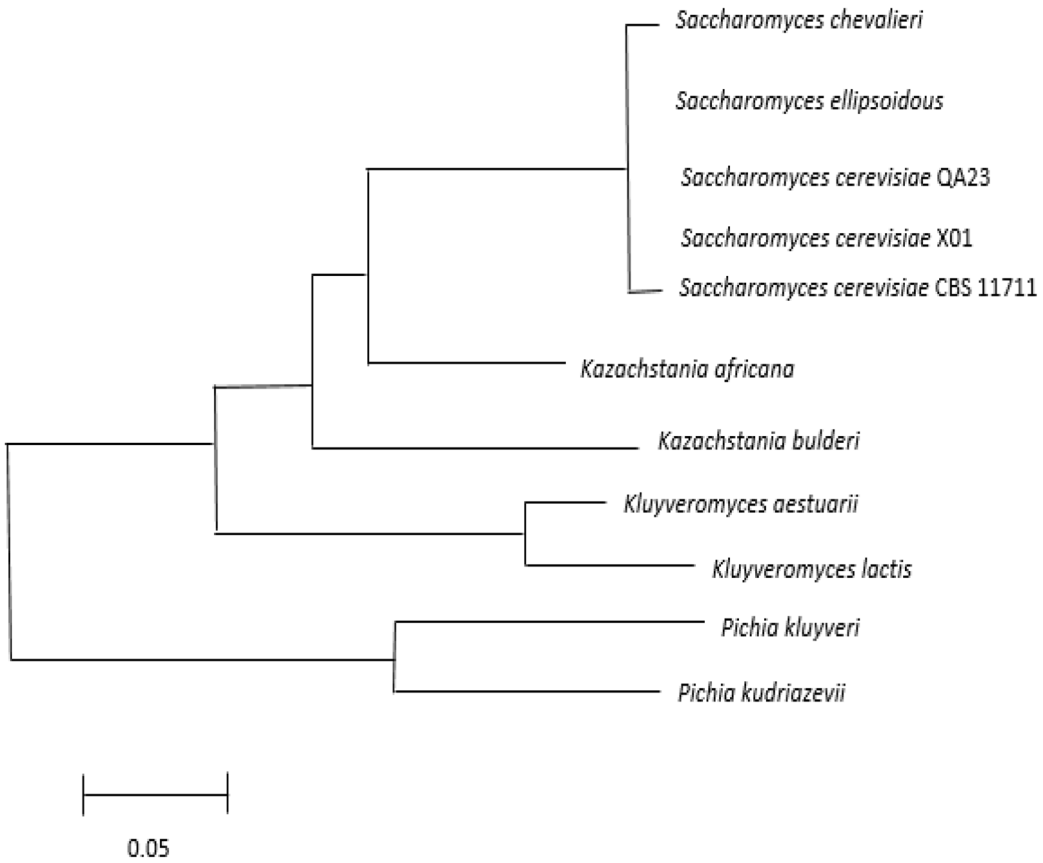

Fig. 2 Phylogenetic tree of *Saccharomyces cerevisiae* strain X01

Physicochemical content of the wine produced before and after clarification and ageing

The means of the physicochemical component of the wine before and after ageing and clarification are presented in Table 5. There was a significant difference ($P < 0.05$) in their titratable acidity and conductivity. There was no significant difference ($P > 0.05$) between the pH, specific gravity, H_2S and ethanol contents of wine produced by *S. cerevisiae* X01 and *S. cerevisiae* QA23 (control), except the specific gravity after ageing and clarification that was significantly different.

Sensory evaluation of produced wine

The sensory evaluation of the wine produced using *Saccharomyces cerevisiae* X01 and Lavin wine yeast *Saccharomyces cerevisiae* QA23 as control from date palm fruit are presented in Fig. 4. The means of the sensory parameters shows that there was no significant difference ($P > 0.05$) on the panellists' perception on colour clarity and sourness of the date wine while for parameters such as aroma, sweetness, and general acceptability the date wine produced using Lavin wine yeast *Saccharomyces cerevisiae* X01 was preferred over the wine produced using *Saccharomyces cerevisiae* QA23 with significant difference ($P < 0.05$) in the means of these sensory parameters.

Sequencing results of the ITS region of PW_3

Sample is 99% identical to *Saccharomyces cerevisiae* strain X01.

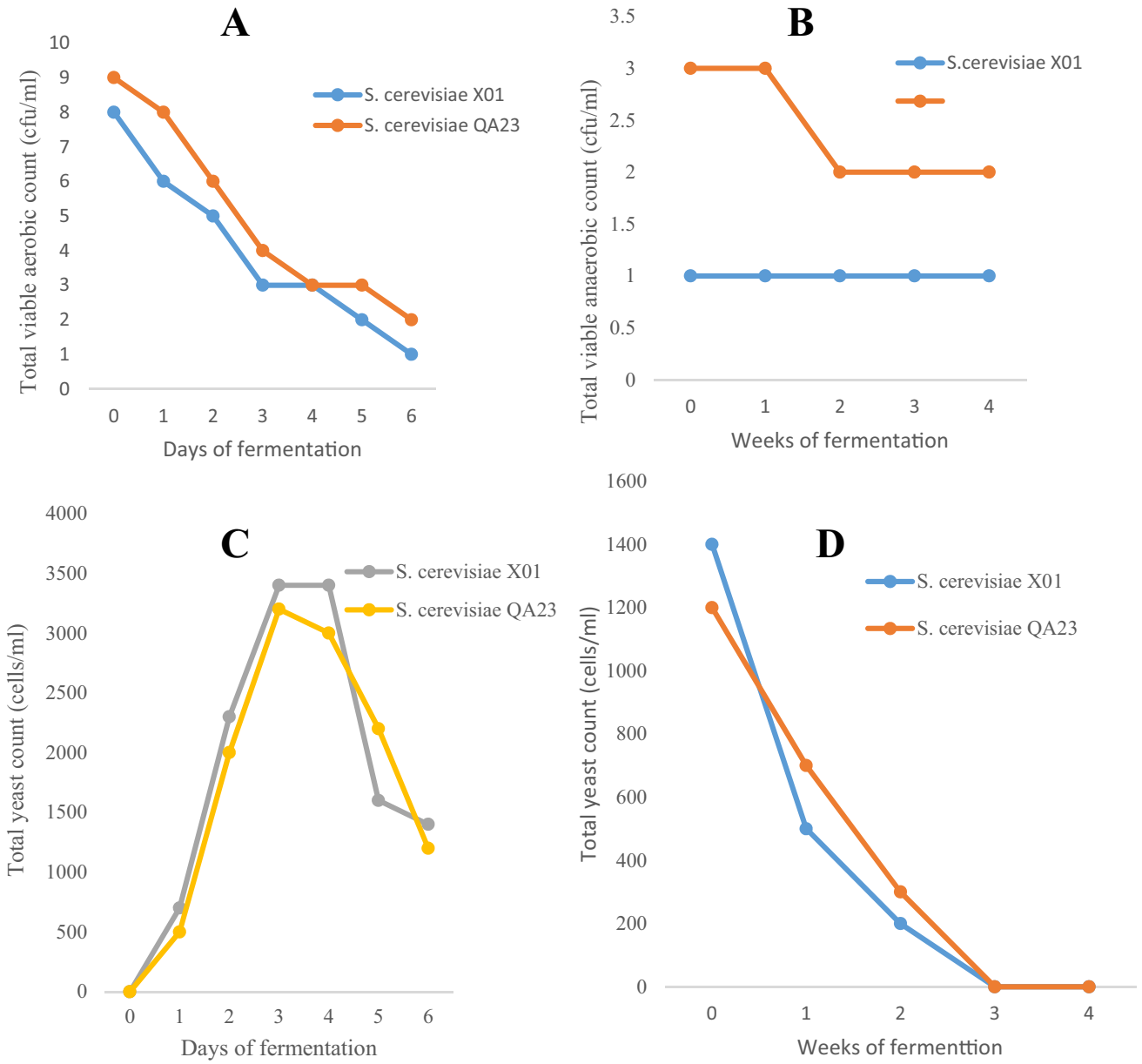

Fig. 3 Microbial count during fermentation. **a** Total viable count during aerobic fermentation, **b** total viable count during anaerobic fermentation, **c** total yeast count during aerobic fermentation, **d** total yeast count during anaerobic fermentation

Table 2 Physicochemical analysis of must before and after aerobic fermentation

Parameters	*S. cerevisiae* X01		*S. cerevisiae* QA23	
	Before	After	Before	After
pH	5.02 ± 0.02^{a}	3.20 ± 0.20^{b}	5.00 ± 0.50^{a}	3.00 ± 0.20^{b}
Titratable acid	3.93 ± 0.60^{b}	5.50 ± 0.25^{a}	3.54 ± 0.40^{b}	6.12 ± 0.12^{a}
Conductivity	7934 ± 4.00^{a}	6542 ± 1.00^{b}	6948 ± 3.00^{a}	5997 ± 2.00^{b}
Specific gravity	1.75 ± 0.30^{a}	1.25 ± 0.02^{b}	1.83 ± 0.00^{a}	1.30 ± 0.10^{b}
Percentage sugar	15.00 ± 2.00^{a}	0.02 ± 0.00^{b}	15.00 ± 1.00^{a}	0.01 ± 0.00^{b}
Ethanol	0.00 ± 0.00^{b}	2.46 ± 0.01^{a}	0.00 ± 0.00^{b}	2.75 ± 0.20^{a}

Values are means of triplicate values. Means with dissimilar letter (s) in column differ significantly according to the Duncan multiple range test

Table 3 Physicochemical analysis of the must during anaerobic fermentation

	Parameters	Week 0	Week 1	Week 2	Week 3	Week 4
S. cerevisiae X01	pH	3.20 ± 0.20^{a}	3.30 ± 0.15^{a}	3.40 ± 0.30^{a}	3.42 ± 0.04^{a}	3.43 ± 0.02^{a}
	Titratable acid	5.50 ± 0.20^{b}	5.42 ± 0.10^{b}	5.00 ± 0.16^{a}	4.93 ± 0.04^{a}	4.70 ± 0.10^{a}
	Specific gravity	1.245 ± 0.00^{e}	1.106 ± 0.00^{c}	1.119 ± 0.00^{d}	1.083 ± 0.00^{b}	0.998 ± 0.00^{a}
	% Ethanol content	2.36 ± 0.02^{a}	2.97 ± 0.02^{b}	3.46 ± 0.03^{c}	4.21 ± 0.02^{d}	4.86 ± 0.01^{e}
	% Sugar	0.02 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Conductivity	6542 ± 3.00^{e}	6023 ± 4.50^{d}	5542 ± 7.00^{c}	5033 ± 3.00^{b}	4536 ± 2.00^{a}
S. cerevisiae QA23	pH	3.00 ± 0.10^{a}	3.15 ± 0.02^{b}	3.19 ± 0.04^{bc}	3.20 ± 0.02^{bc}	3.26 ± 0.02^{c}
	Titratable acid	6.12 ± 0.04^{d}	5.91 ± 0.01^{a}	5.62 ± 0.03^{c}	5.52 ± 0.03^{a}	5.05 ± 0.04^{a}
	Specific gravity	1.296 ± 0.003^{e}	1.128 ± 0.004^{d}	1.096 ± 0.004^{c}	1.032 ± 0.002^{b}	1.002 ± 0.003^{a}
	% Ethanol content	2.94 ± 0.02^{a}	3.31 ± 0.01^{b}	3.98 ± 0.03^{c}	5.16 ± 0.03^{d}	5.22 ± 0.04^{d}
	% Sugar	0.01 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	Conductivity	5997 ± 7.00^{e}	5523 ± 7.00^{d}	5146 ± 2.00^{c}	5009 ± 9.00^{b}	4510 ± 21.00^{a}

Values are means of triplicate values. Means with dissimilar letter (s) in column differ significantly according to the Duncan multiple range test

Table 4 Proximate composition of wine produced by before and after ageing and clarification

Parameter	Wine produced by *S. cerevisiae* X01 (%)		Wine produced by *S. cerevisiae* QA23 (control) (%)	
	Before	After	Before	After
Moisture content	97.56^{ab}	97.72^{a}	97.54^{b}	97.77^{a}
Ash	0.03^{b}	0.04^{ab}	0.07^{a}	0.05^{a}
Crude protein	0.43^{b}	0.46^{ab}	0.43^{b}	0.48^{a}
Crude fibre	0.00	0.00	0.00	0.00
Carbohydrate	1.90^{a}	1.72^{ab}	1.89^{a}	1.63^{b}

Mean values in the same row with the same superscripts are not significantly different ($P > 0.05$) from each other

Table 5 Physicochemical content of Wine produced by *S. cerevisiae* X01 before and after ageing and clarification

Parameter	Wine produced by *S. cerevisiae* X01		Wine produced by *S. cerevisiae* QA23(control)	
	Before	After	Before	After
pH	3.43^{a}	2.90^{ab}	3.26^{a}	2.62^{b}
Conductivity	4536^{a}	3177^{c}	4510^{b}	3093^{d}
Titratable acidity (g/100 mL)	4.70^{d}	5.55^{b}	5.05^{c}	5.85^{a}
Specific gravity	0.998^{ab}	0.993^{b}	1.002^{a}	0.999^{a}
H_2S content (mg/l)	0.02^{a}	0.006^{b}	0.04^{a}	0.006^{b}
Ethanol Content (%)	4.86^{c}	5.55^{b}	5.22^{bc}	7.15^{a}

Mean values in the same row with the same superscripts are not significantly different ($P > 0.05$) from each other

Discussion

The yeast cell (PW_3) with the most ability for wine production, was subsequently identified as *Saccharomyces cerevisiae* X01. This strain of *S. cerevisiae* was isolated from the palm wine samples. The findings in this study revealed that PW_3 is 99% identical to *Saccharomyces cerevisiae* strain X01. The phylogenetic tree of *Saccharomyces cerevisiae* strain X01 showed that it is related to *Saccharomyces cerevisiae* QA23, which was the commercial typed yeast strain that was used as control for the production of wine as well as the following yeasts: *Saccharomyces chevalieri, Saccharomyces ellipsoideus, Kazachstania Africana, Kazachstania bulderi, Kluyveromyces aestuarii, Kluyveromyces lactis, Pichia kluyveri,* and *Pichia kudriavzevii.*

The phylogenetic similarities between *Saccharomyces cerevisiae* X01 and Lavin wine yeast *S. cerevisiae* QA23, explains the reason why there was no significant difference ($P > 0.05$) between the proximate and physicochemical composition of the date wine produced using *S. cerevisiae* X01 and Lavin wine yeast *Saccharomyces cerevisiae* QA23 as control, before and after clarification and ageing of the date wine. The proximate and physicochemical composition of the wine produced was not in line with that of Awe and Nnadoze (2015) and Mohammed et al. (2018). The cause of this variation could be attributed to the type of date used, the climate condition, soil of where the date was grown and the time of the year. Therefore, from the findings of this research it could be deduced that a strain of *Saccharomyces cerevisiae* isolated from palm wine and used for wine production, had attributes similar to the commercial typed wine yeast strain.

Yeasts were isolated from the fermented products study (namely pito', 'burukutu' and palm wine) used in this. This is similar to reports by other authors, for example Danmadami

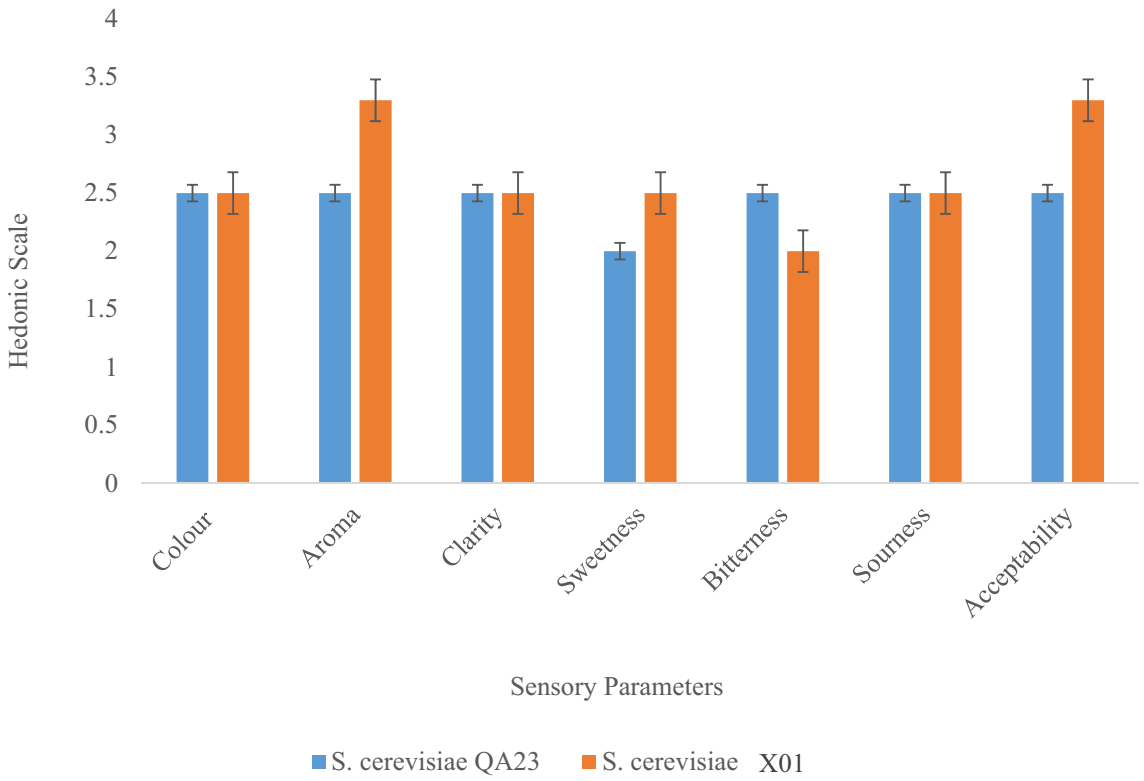

Fig. 4 Sensory evaluation of the date fruit wine produced

et al. (2017) and Ukponobong et al. (2018) reported the presence of yeast in fermented products. Thus, yeasts are said to be one of the major microorganisms that carry out ethanoic fermentation and they originate from several sources during the process of production. The isolates fermented and assimilated glucose and fructose, which are monosaccharides and sucrose, which is a disaccharide obtained from glucose and fructose. This could be attributed to the metabolic activities of the yeast during fermentation. Other sugars like lactose, maltose, sorbitol, mannitol, xylose and rhamnose were not utilized. This may imply that the yeast isolates do not possess enzymes responsible for the utilization of these sugars (Balarabe et al. 2017).

All the yeast isolates possessed the ability to tolerate ethanol at lower concentrations. There was an intensive growth response at 5% ethanol concentration, moderate growth response at 10% and 15% concentrations while at 20% concentration there was a low growth response for 'pito' and 'burukutu' isolates while for palm wine isolate there was no growth response at the same concentration. This may imply that the yeasts isolates were able to withstand industrial stress and this could be a huge advantage in their application for industrial processes. The reason for low or no growth response at higher ethanol concentration could be attributed to toxicity of high alcohol concentration to the yeasts cells by inhibiting the cell growth due to the destruction of the cell membrane (Tsegaye et al. 2018). The yeast isolates were able to thrive at temperature range of 30–37 °C. This was due to an intensive growth response at this temperature range. However, at 45 °C there was a low response for all palm wine isolates and there was no growth response for 'pito' and 'burukutu' isolates at the same temperature. This suggest that the yeast isolates could not tolerate excess heat generated from the fermentation process and may not perform a wide temperature range fermentation. The seven yeast isolates have the ability to flocculate and they do not produce killer toxin while for H_2S production only isolate PW_1 did not produce H_2S although the response for the H_2S production was very low. There were variations in the report of some of the biochemical tests when compared with the findings of Balarabe et al. (2017) and Ukponobong et al. (2018) that recorded yeast isolate that had the ability to tolerate ethanol concentration of 10–15% and temperature range of between 25 and 37 °C while Tsegaye et al. (2018) recorded ethanol and temperature tolerance of between 2–13% and 25–37 °C, respectively. This could be attributed to genetic variations among the isolates.

The refractometric method revealed that PW_3 gave the lowest refractive index value (1.3250) when compared with the control (1.3410). This implies that the sugar in the

medium has been utilized by PW_3 and gave an insight that *S. cerevisiae* X01 possessed the ability to be used for wine fermentation effectively within a short period of time. However, the spectrophotometric method showed that PW_3 has the highest absorbance rate (1.286) at 630 nm, therefore this revealed that in spite of the ability of PW_3 to utilize sugar in a fermentation medium, the growth is not intensive, thereby making cell removal for reuse after fermentation easier since it also has the ability to flocculate, tolerate a wide range of industrial stress (temperature and high concentration of ethanol) and does not produce killer toxins.

The means of physiochemical analysis of the must before and after aerobic fermentation of the must prepared using *S. cerevisiae* X01 and Lavin wine yeast *S. cerevisiae* QA23 as inoculum revealed that their pH reduced after day 6 of aerobic fermentation with *S. cerevisiae* QA23 having the lowest pH. This then explained the reason why it has higher titratable acidity (Adeleke and Abiodun 2010). There was a significant difference ($P < 0.05$) in the conductivity, specific gravity, sugar and ethanol content of the must. The reduction in specific gravity, conductivity and sugar shows that the yeast were able to metabolise the sugar in the must, which serve as a substrate to produce organic acids and alcohol in the process of fermentation. This reason further explains why there is an increase in the production of ethanol in the must. This same trend was observed in the physicochemical analysis of the must during anaerobic phase of fermentation. The aerobic and anaerobic physicochemical analysis of the must was slightly different from the report of Awe and Nnadoze (2015). This could be attributed to the variety of date used, the climate condition, soil of where the date was grown and location where the research was carried out.

The microbiological analysis of the must, which include enumeration of total viable bacterial count and monitoring of the growth of the yeast carrying out the aerobic and anaerobic fermentation showed that as the period of aerobic and anaerobic fermentation increase, there was a general decline in the total number of viable cell. This could be as a result of nutrient depletion and production of organic acids and alcohol into the must, thereby making the must unconducive for the growth of other unwanted microorganisms (Awe 2011). As the period of fermentation increases, initially there was an initial exponential increase in the total yeast count, which signifies the log phase of growth especially during the aerobic and early anaerobic fermentation period. This could be attributed to the high availability of nutrients in the must. This was followed by a short stationary phase before moving to the death phase where there was a rapid decline in the total yeast count due to nutrient depletion and production of inhibitory metabolite into the must. This result is slightly different from that of Awe and Nnadoze (2015) and could also be attributed to the type of date used, the climate condition and soil factor and location where the research was carried out.

The means of the sensory parameters shows that there was no significant difference ($P > 0.05$) in the panellists' perception on colour clarity and sourness of the date wine while for parameters such as aroma, sweetness, and general acceptability the date wine produced using *S. cerevisiae* X01 was preferred over the wine produced using Lavin wine yeast *S. cerevisiae* QA23 with significant difference in the means of these sensory parameters. This implies that the date wine produced using *S. cerevisiae* X01 was preferred and enjoyed by the panellists.

Conclusion

Saccharomyces cerevisiae X01 isolated from palm wine, can be used as an alternative substrate for industrial scale wine production with mild alcoholic content. This can augment the not-readily available and expensive commercial wine yeast (*Saccharomyces cerevisiae* QA23). This study also revealed that date palm fruit is a suitable substrate for the production of wine instead of disposing them off due to poor storage facilities.

References

Adeleke RO, Abiodun OA (2010) Physicochemical properties of commercial local beverages in Osun State Nigeria. Pak J Nutr 9(9):853–855

AOAC (2000) Official methods of analysis of the AOAC, 18th edn. Author, Washington

Awe S (2011) Production and microbiology of pawpaw (*Carica papaya*) wine. Curr Res J Biol Sci 3(5):443–447

Awe S, Nnadoze SN (2015) Production and microbiological assessment of date palm (*Phoenix dactylifera* L.) fruit wine. Br Microbiol Res J 8(3):480–488

Balarabe MM, Mohammed SSD, Orukotan AA (2017) Screening of fermentative potency of yeast isolates from indigenous sources for dough leavening. Int J Microbiol Biotechnol 2(1):12–17

Balogu TV, Towobola O (2017) Production and quality analysis of wine from honey and coconut milk blend using *Saccharomyces cerevisiae*. Fermentation 3(2):16

Bankefa EO, Oyedeji OM (2015) Synergistic fermentative nutritional quality *of Lactobacillus delbrueckii* and *Bacillus pumilus* on date fruits (*Phoenix dactylifera*). Afr J Food Sci 9(5):307–313

Barnett JA, Payne RW, Yarrow D (1990) Yeast: characteristics and identification, 2nd edn. Cambridge University Press, Cambridge

Castillo-Castillo Y, Ruiz-Barrera O, Burrola-Barraza ME, Marrero-Rodriguez Y, Salinas-Chavira J, Angulo-Montoya C, Corral-Luna A, Arzola-Alvarez C, Itza-Ortiz M, Camarillo J (2016) Isolation and characterization of yeasts from fermented apple bagasse as additives for ruminant feeding. Braz J Microbiol 47:889–895

Danmadami RN, Yabaya A, Yahaya O, Abraham OJ, Bobai M, Orukotan AA (2017) The efficiency of *Saccahromyces cerevisiae* strain isolated from palm wine in the production of 'burukutu'. Int J Res 5(11):70–85

Garba MD, Galadima A (2012) Anti-diarrhoea and phytochemical evaluation of *Phoniex dactylifera* L. extracts. Elixir Appl Chem 49:9808–9812

Gidado RSM, Olatiilu OA, Etuk-Udo GA, Onyenekwe PC, Isu RN, Habu J (2016) Isolation and characterization of yeast inhabiting alcohol processing environment in Bayelsa state, Nigeria. Adv Appl Sci 1(3):78–85

Guizani N, Al-Saidi GS, Rahman MS, Bornaz S, Al-Alawi AA (2010) State diagram of dates: glass transition, freezing curves and maximal-freeze- concentration condition. J Food Eng 99:92–97

Hong KK, Nielsen J (2012) Metabolic engineering of *Saccharomyces cerevisiae*: a key cell factory platform for future biorefineries. Cell Mol Life Sci 69:2671–2690

Johnson DV (2012) Enhancement of date palm as a source of multiple products: examples from other industrialized palms. Emir J Food Agric 24(5):408–414

Johnson EA (2013) Biotechnology of non-*Saccharomyces* yeasts: the *Ascomycetes*. Appl Microbiol Biotechnol 97:503–517

Martínez JL, Liu L, Petranıvic D, Nielsen J (2012) Pharmaceutical protein production by yeast: towards production of human blood proteins by microbial fermentation. Curr Opin Biotechnol 23:965–971

Mohammed SSD, Yohanna B, Wartu JR, Abubakar NL, Bello S (2018) Wine produced from fermentation of honey slurry and dates palm fruit juice blend using *Saccharomyces cerevisiae* isolated from palm wine. Int J Biol 10(3):53–61

Nwaokobia KR, Ogboru O, Idibie CA (2018) Investigating the proximate, ultimate and chemical composition of four cultivars of date seed, *Phoenix dactylifera L.* World News Nat Sci 18(2):52–61

Olowonibi OO (2017) Isolation and characterization of palm wine strains of *Saccharomyces cerevisiae* potentially useful as bakery yeasts. Eur J Exp Biol 7(11):1–13

Tsegaye Z, Tefera G, Gizaw B, Abatenh E (2018) Characterization of yeast species isolated from local fruits used for bakery industrial application. J Appl Microbiol Res 1(1):17–26

Ukponobong EA, Otobong DA, Nsikak U, Stephen CK, Nseobong GA (2018) Isolation and screening of yeast isolates indigenous palm wine for ethanol production. Philipp J Sci 147(3):411–417

Valdineia AO, Maristela AV, Luciano GF, Ieso-de-Miranda C, Maurıcio XC, Dorit S, Henrique A, Margarida C, Juliana-de-Oliveira S, Leandro DA, Paulo HA, Rogelio LB (2008) Biochemical and molecular characterization of *Saccharomyces cerevisiae* strains obtained from sugar-cane juice fermentations and their impact in cachaca production. Appl Environ Microbiol 74(3):693–701

Zapata A, Ramirez-Arcos S (2015) A comparative study of McFarland turbidity standards and the densimat photometer to determine bacterial cell density. Curr Microbiol. https://doi.org/10.1007/s00284-015-0801-2