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CHARACTERIZATION OF INDIGENOUS YEASTS SPECIES ISOLATED FROM FRUITS FOR PINEAPPLE WINE PRODUCTION

Fadahunsi Ilesanmi Festus[1]✉, Akoja Abiodun David[1], Ozabor Temilade Praise[1]

[1]Department of Microbiology University of Ibadan, Ibadan Nigeria
✉Sanmifadahunsi@yahoo.com

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ABSTRACT

This study was designed to characterize yeast species isolated from fruits and use as starter cultures in pineapple wine production. Forty yeast isolates were obtained from fermenting pineapple, watermelon and cashew juices using culture-dependent method and screened for pathogenicity properties. Eleven of the yeast isolates were non-pathogenic and were investigated for their abilities to produce invertase, tolerate ethanol, sugars, grow at different temperatures and pH by spectrophotometric method. Identification of the yeast isolates was carried out using API (ID 32C) kit. The result obtained showed that Isolate PIN32 *(Saccharomyces cerevisiae* 4) had the highest invertase activity of 40.04±0.5 Umol/min followed by 30.17±0.1 Umol/min produced by WAM8 (*Saccharomyces cerevisiae* 1). The highest tolerance to ethanol was demonstrated by isolate PIN32 (*Saccharomyces cerevisiae* 4) and WAM8 (*Saccharomyces cerevisiae1*) with a growth of 1.31±0.3 and 1.26±0.2 respectively. Optimum glucose tolerance was observed in WAM8 (*Saccharomyces cerevisiae1*), while PIN32 (*Saccharomyces cerevisiae* 4) demonstrated the highest growth in 20% sucrose. Similarly PIN32 (*Saccharomyces cerevisiae* 4) and WAM8 (*Saccharomyces cerevisiae 1*) recorded the highest growth of 1.55 at pH 6. All isolates exhibited optimum growths at 30^0 C with PIN32 recording the highest growth. The isolates were identified as *Saccharomyces cerevisiae, Pichia farinosa, Saccharomyces kluyveri, Kloeckera japonica, Pichia ohmeri, Debaromyces polymorphus, Candida kefyr.* The result showed that PIN32 and WAM 8 could be selected as potential starter cultures for pineapple wine production based on the empirical findings in this work.

1. Introduction

Yeasts are eukaryotic microorganisms and they are widely encountered in ecological niches such as fruits, grains, soil and fermented food but they are mostly isolated from citrus juices and sugar cane (Tamang,2016) They are important in many complex ecosystems, as frequent early colonizers of nutrient rich substrates (Djelal et al.2017). *Saccharomyces cerevisiae* (baker's and brewer's yeast) is the most studied species because it utilizes hexose sugar to produce CO_2, ethanol, and variety of secondary metabolites such as esters, aldehyde and amino acids that contribute to the development of flavour and aroma of fermented foods (Pretotoritus, 2000). Unlike bacteria, yeasts are osmophilic and can grow in media of low water activities and acid pH. (Deak, 2006).) These biotechnological properties exhibited by saccharomyces cerevisiae have made them suitable in the production of wine of good quality and consumers acceptability(Turker, 2014).However Yeast metabolism and physiology are thus strongly dependent on sugar and oxygen Unlike bacteria, yeasts are osmophilic and can grow in media of low water activities and acid pH.(Dickinson and Kruckeberg, 2006)..Its enhanced applications in

alcoholic fermentation, bread-making, single cell protein, vitamin production, synthesis of recombinant proteins, and biological control is well documented (Akabanda et al.,. 2013).Its high tolerance to sugar ,ethanol , high temperature are the biotechnological properties leading to its selection for alcoholic fermentation (Islam et.al.,2015)

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Wine is a fermented beverage prepared from fresh fruit juices by normal alcoholic fermentation (Okafor,2007). In wine production ,yeast ferments the hexose sugar present in the substrate a process named alcoholic fermentation to ethanol, carbon dioxide and other secondary metabolites.(Robinson,2006) The quality of wine depends largely on the yeast strain used (Idise, 2012).Wines produced from grape (Vitis species) are called the true wines while wines from other fruits are referred to as fruit wines and they are named using the name of the fruit used for their production e.g., orange wine, banana wine, cherry wine and pineapple wine (Lea et al., 2003). It is reported that wine and other alcoholic drinks are used in entertaining guests during conferences, rallies, marriage, christening, and burial ceremonies.. This has made it an integral part of the Nigerian society and many people believe strongly in its ability to prevent cardiovascular disease because of its high content of polyphenol such resveratrol, anthocyanin and catechins (Snopek et al.,2018). Most of the common fruits being utilized for wine and juice making are cashew, pineapple, mango and orange. Fruits supplement the quality of our diet by providing essential ingredients like vitamins, minerals, carbohydrates. They are seasonal products and are available in large quantity during their harvesting period but a higher percentage is wasted due to lack of available storage facilities and underutilization. This wastage can be arrested by processing and conversion of these fruits into other useful valued products which will make them available all year round. Such possible products are wines, concentrate and confectionaries (Bolarin et al., 2016).

Pineapple (Ananas comosus)belongs to the Bromeliaceae family and is planted in different regions of Nigeria either for export or for the local consumption. It can be eaten fresh or process into fruit juices or concentrates for future use. Nutritionally it contains 81.2-86.2% moisture, 13-19% total solids of which sucrose, glucose, and fructose are the main components with 2-3% fiber, a high level of vitamin C and calcium (Sun et al.,2016). It is also reported to possess proteolytic activity due to the presence of bromelin couple with low amount of lipids and nitrogenous compounds (Shetty et al.,2019)The pineapple varieties commonly grown in Africa have sufficient sugar levels and favorable pH (4.5-6.5) for fermentation to occur (Idise, 2012). Thus, through fermentation the highly perishable pineapple fruit could be converted into a highly nutritious wine which can be made available all year round (Keller, 2010). In Nigeria, 50% of the pineapple harvested annually is wasted due to lack of adequate storage facility. It is therefore suggested that government and private investors should look inwards as to developing wineries that will exploit optimally the utilization of this fruit, considering the fact that a huge amount of foreign exchange and employment will be derived from the exportation of wine (Akubo, et al., 2003).

In this present work, attempt was made to characterize indigenous yeast species isolated from fruits to select potential starters for pineapple wine production with the aim of developing wine with long shelf life, desirable sensory attributes coupled with improved nutritional quality and consumer's acceptability.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Samples of pineapple, water melon and cashew nut were collected from Military Cantonment Oshodi in Lagos and Bodija market in Ibadan, Nigeria in sterile ethylene bags and

transported to the Food and Applied Microbiology laboratory, Department of Microbiology University of Ibadan, Nigeria.

2.2. Methods

2.2.1. Treatment of sample and isolation procedure

The barks of the pineapple, water melon and cashew fruits were removed and cut into pieces using a sharp knife. They were allowed to ferment naturally for three days and their juices were extracted using a juice extractor machine. The juices were serially diluted with one ml from dilutions 10^{-4} and 10^{-5} was inoculated into sterile Petri dishes containing malt extract agar and incubated at 30°C for three days. The Petri dishes were observed for microbial growth and representative colonies sub-cultured repeatedly to obtain pure cultures which were maintained on malt extract agar slant at 4°C.

2.2.2. Pathogenicity Test

*2.2.2.1.Gelatin Liquefaction***:**

The method described by dele-Cruz and Torres (2012) was used. One ml from a 24h old culture of the yeast cells suspension was inoculated into sterile gelatin medium in 50mL Erlenmeyer flasks containing 10% malt extract and incubated at 30^0C for 7 days. The temperature was reduced to observe liquefaction. At lower temperature, liquefaction of gelatin indicates positive reaction while un-liquefied gelatin indicates negative reaction.

2.2.2.2. Hemolysis test

This was carried out by streaking a colony from a 24h old culture of the yeast cells on blood agar plates and incubated for 24-72h at 30^0C.The plates were observed for alpha, beta or gamma hemolytic reaction (Akinjogunla *et.al.* 2014).

2.2.2.3. DNase test

The method described by Akinjogunla *et. al.*. (2014). was adopted by picking a colony from a 24h old culture of yeast cells and streaked on DNase agar plates containing methyl green indicator and incubated at 37^0C for 72h. The plate was observed for green color fades surrounded by a colorless zone.

2.3. Identification procedure

Identification of the isolates was carried out by considering their macroscopic and microscopic characteristics (Tika *et al*.(2017) and by employing the API (ID 32C) test kit.

2.4. Determination of invertase production.

Determination of invertase production was carried out by inoculating 1 ml from a 24h old culture of yeast cells suspension into sucrose (4% w/v, 2 ml) in 10ml solution of acetate buffer(pH 5) and incubated for 5 min at 30°C. The amount of reducing sugar released was determined by dinitro-salicylic acid method Tika *et al*.(2017).The amount of enzyme which liberate 1 µ mole reducing sugar per minute was defined as one unit of Invertase activity.

2.5. Determination of ethanol tolerance

This was carried out by inoculating 0.1 ml from a 24h old culture of yeast cells suspension into 10ml sterile malt extract broth containing different concentrations of ethanol (0, 5, 10, 15 and 20%) The tubes were incubated at 30°C for 48 h. and the initial optical densities were read from an UV spectrophotometer (Pharamacia LK13Ultraspec II) set at 560nm. The tubes were transferred into a gyratory shaker incubator set at 150rpm at 30^0C for 24 h and the final optical density was read.

2.6. Determination of sugar tolerance

The modified method described by Shankar *et. al*.(2013) was used by inoculating 0.1 ml from a 24h old culture of the yeast cells suspension into sterile malt extract broth in several 100ml Erlenmeyer flasks containing 10%, 20, 3% and 40%) glucose concentrations and incubation was carried outat 30^0C for 72h. Growth was determined using an UV visible spectrophotometer (Pharamacia LK13Ultraspec II) set at 560nm.The experiment was repeated by replacing glucose with sucrose.

2.7. Determination of growth at different pH

1 ml from a 24h old culture of the yeast cells suspension was into several sterile malt extract broth in 100ml Erlenmeyer flasks with pH

adjusted to 2, 4, 6 and 8 and incubated at 30^0C for 72h h and growth was determined using an UV visible spectrophotometer (Pharamacia LK13Ultraspec II) set at 560nm.

2.8. Determination of growth at different temperatures

One ml from a 24h old culture of the yeast cells suspension was inoculated into sterile malt extract broth in several 100ml Erlenmeyer flasks with pH adjusted to 6. Incubation was carried out at different temperatures (20, 25, 30, 35, 40 and 45) for 72 h and growth was determined by using an UV visible spectrophotometer (Pharamacia LK13Ultraspec II) set at 560nm.

All experiments reported in this study were carried out in triplicates

Statistical analysis

Descriptive statistical method in statistical package for social science (SPSS version17) was used for data analysis to determine means and standard errors.

3. Results and discussions

The screening for pathogenic properties of the yeast isolates is shown in Table 1. It was observed that the eleven isolates tested were negative to gelatin liquefaction, heamolysis and DNase production.

Table 1. Pathogenicity test of yeast isolates

Isolate Code	Gelatin Liquefaction	Haemolytic Test	Dnase Test
WAM 8	_	_	_
WAM 11	_	_	_
WAM 20	_	_	_
WAM 25	_	_	_
PIN 10	_	_	_
PIN 40	_	_	_
PIN 32	_	_	_
PIN 12	_	_	_
CAS 15	_	_	_
CAS 36	_	_	_
CAS 3	_	_	_

Key - WAM-8:Saccharomyces cerevisiae 1,WAM-11:Pichia farinosa,WAM-20: Saccharomyces cerevisiae2,WAM-25:Kloeckera japonica; PIN-10:Saccharomyces kluyveri, PIN–40: Saccharomyces cerevisiae3,PIN-32: Saccharomyces cerevisiae 4, PIN-12:Saccharomyces cerevisiae 5 CAS-15: Pichia ohmeri;CAS-36:Debaryomyces polymorphus;CAS-3: Candida kefyr.

The result of Invertase production by the yeast isolates is presented in Table 2.

All the yeast isolates produced varying amounts of invertase with Isolate PIN32(*Saccharomyces cerevisiae* 4) showing the highest invertase activity of 40.04±0.5Umol/min followed by an activity of 30.17±0.1Umol/min produced by WAM8 (*Saccharomyces cerevisiae* 1) and the least activity of 13.87±0.2Umol/min was recorded in CAS36 (*Debaromyces polymorphus*).

The observed result in the screening of yeast for pathogenicity properties is in conformity with the findings of Eze *et al.*(2011). He previously reported that yeast isolates are not gelatinase producers and the absence of gelatinase, haemolysis and dnase production by these micro-organisms accord them acceptability in the food industry as starter cultures.

Table 2. Invertase production by the yeast isolates

Isolate number	Invertase activity Umol/min
WAM 8	30.17±0.1
WAM 11	18.85±0.1
WAM 20	25.64±0.2
WAM 25	20.51±0.4
PIN 10	28.82±0.3
PIN 40	26.28±0.
PIN 32	40.04±0.5
PIN12	14.66±0.9
CAS 15	20.03±0.7
CAS36	13.87±0.2
CAS3	18.27±0.2

Production of invertase by a wide range of microorganisms such as *Saccharomyces cerevisiae* and *S.carlsbergensis* had been earlier reported (Sivakumar et al.; 2013) Mahesh et al.(2012) reported that *S. cerevisiae* possesses a great ability to secrete invertase while Talekar *et al.* (2010) confirmed that this enzyme is highly significant in the cleavage of α-1,4 glycosidic linkage between α-D-glucose and β-D-fructose molecules of sucrose by hydrolysis and releases monosaccharide. In addition, Guimaraes *et al*. (2007) described the general use of invertase in the production of confectionary with liquid or soft center invert syrup, calf feed preparation, pharmaceutical, food and fermentation of cane molasses into ethanol. The tolerance of the yeasts isolates to different concentration of ethanol is shown in Table 3. The result showed that all the yeasts isolates tolerated the different concentrations (5% -15%) of ethanol used producing different levels of growth with the highest recorded at lower concentration of ethanol. The highest tolerance to ethanol was demonstrated by Isolate PIN32 (*Saccharomyces cerevisiae* 4) by producing a growth of 1.31±0.3 (optical density) followed by a growth of 1.26±0.2 recorded by WAM8 (*Saccharomyces cerevisiae1*) while the least growth of 0.72±0.3 was seen in CAS3 (*Candida kefyr*) at 15% ethanol.

Table 3. Tolerance of yeast isolates to different ethanol concentrations

Isolate code	5%v/v	10%v/v	15%v/v	20%v/v
WAM8	1.74±0.5	1.54±0.5	1.26±0.2	0.00±0.0
WAM11	1.37±0.9	1.15±0.2	1.00±0.3	0.00±0.0
WAM20	1.61±0.1	1.37±0.0	1.21±0.6	0.00±0.0
WAM25	1.44±0.0	1.18±0.9	1.02±0.4	0.00±0.0
PIN10	1.25±0.3	1.14±0.8	0.90±0.0	0.00±0.0
PIN40	1.54±0.3	1.37±0.2	1.11±0.1	0.00±0.0
PIN32	1.83±0.1	1.58±0.3	1.31±0.3	0.00±0.0
PIN12	1.47±0.8	1.04±0.7	0.76±0.0	0.00±0.0
CAS15	1.44 ±0.2	1.05±0.6	0.80±0.0	0.00±0.0
CAS36	1.33±0.6	1.06±0.4	0.81±0.1	0.00±0.0
CAS3	1.37±0.0	1.05±0.1	0.72±0.2	0.00±0.0

The ethanol tolerance of yeast isolates seen in this present work is observed to be species dependent. The yeast species were able to grow in different concentrations of ethanol due to the

ability of their cell wall to withstand osmotic stress (plasmolysis) (Gomar-Alba et al.2015). This observation had earlier been reported by Dash *et al*. (2015); Alloysius *et al*. (2015). However, Osho (2005) reported that the alcoholic tolerance for most brewing yeast strains was within 9-10% ethanol concentration. Studies have shown that ethanol tolerant yeasts are also sugar-tolerant (Balakmar and Arasatnam,, 2012;Techaparin *et al.,*.2017) and the combination of the two properties are important in the selection of yeast species for industrial application especially in fermentative production of ethanol, wine, alcoholic beverages and baking products (Moneke *et al*., 2008;Patruscus *et al*.,2009). Ethanol tolerance is a crucial characteristic of microorganisms involved in the production of alcohol because the process will be inhibited if the fermenting microorganism could not tolerate the alcohol produced (Thammasttirong *et al.,*2013). (Thammasttirong *et al*.,.2013) Albergaria and Arneborg (2016);Alonso del-real *et al*.(2017) explained that the ability of yeast spp. to survive in high alcoholic wine is an indication of their high ethanol tolerance and this characteristics is used in the selection of yeast spp. for industrial ethanol production (Chandasena *et al*., 2006; Patruscus *et al.,*.2009).

Table 4 shows the result of tolerance of yeast isolates to different glucose concentration. All the isolates had maximum growth at 20% glucose but at higher concentration growth decreased. In addition, the highest glucose tolerance was observed in WAM8 (*Saccharomyces cerevisiae1*) recording a growth of 2.46±0.1 followed by PIN32 (*Saccharomyces cerevisiae 4*) with a growth of 2.34±0.1 and the least was observed in WAM25 (*Kloeckera japonica*) with a growth of 2.04±03 in 20% glucose.

Table 4. Tolerance of yeasts isolates to different glucose concentration

Isolates	10%	20%	30%	40%
WAM8	2.11±0.8	2.46±0.1	2.25±0.5	2.16±0.1
WAM11	2.07±0.6	2.20±0.0	2.10±0.6	2.05±0.0
WAM20	2.07±0.6	2.06±0.3	2.00±0.0	1.90±0.5
WAM25	2.01±0.6	2.04±0.3	1.99±0.1	1.83±0.9
PIN10	2.03±0.0	2.06±0.2	1.91±0.2	1.80±0.3
PIN40	2.01±0.1	2.09±0.5	1.81±0.1	1.72±0.4
PIN32	2.09±0.1	2.34±0.1	2..20±0.8	2.10±0.4
PIN12	2.01±0.2	2.18±0.7	2.10±0.0	1.88±0.2
CAS15	2.07±0.5	2.20±0.0	2.00±0.9	1.90±0.6
CAS36	2.06±0.7	2.18±0.6	2.01±0.3	1.70±0.7
CAS3	2.02±0.3	2.07±0.8	1.99±0.1	1.72±0.0

The result of tolerance of the yeast isolates to different sucrose concentration is presented in Table 5. It was observed that PIN32 (*Saccharomyces cerevisiae 4*) recorded the highest growth of 2.39±0.1 followed by a growth of 2.30±0.3 produced by WAM8 (*Saccharomyces cerevisiae1*) while the least growth of 2.10±0.0recorded by WAM11 (*Pichia farinosa)* in 20% sucrose.

Table 5. Tolerance of yeasts isolates to different sucrose concentration

Isolate code	10%	20%	30%	40%
WAM8	2.20±0.3	2.30±0.3	1.83±0.1	1.01±0.7
WAM11	2.02±0.6	2.10±0.2	1.43±0.0	0.89±0.5
WAM20	2.07±0.2	2.14±0.0	1.50±0.6	0..66±0.7

WAM25	2..08±0.7	2.16±0.9	1.53±0.2	0.64±0.6
PIN10	2.10±0.0	2.16±0.4	1.50±0.5	0.59±0.1
PIN40	2.05±0.1	2.11±0.0	1.45±0.1	0.50±0.4
PIN32	217±0.8	2.39±0.1	1.89±0.0	1.18±0.5
PIN12	2.11±0.6	2.28±0.7	1.80±0.2	1.00±0.8
CAS15	2.06±0.6	2.18±0.5	1.60±0.1	0.85±0.3
CAS36	2.08±0.2	2.20±0.1	1.64±0.9	0.93±0.7
CAS3	2.10±0.1	2.16±0.0	1.55±0.3	0.79±0.1

All previously reported observations on sugar tolerance are in agreement with the findings in this work. The ability of the yeast isolate to tolerate sugar concentration is due to the possession of osmotic stress resistant cells caused by dehydration (Homann,2003; Gomar-Alba *et al.*,.2015) They are frequently isolated from high-sugary substrates such as fruits, honey, and jam. (Patruscus *et al.*,.2009; Alakeji *et al.* (2015). Ogunremi *et al.* (2015) and Sulieman *et al*. (2015) confirmed their ability to cause food spoilage. Examples of some sugar tolerant yeast spp are *Candida bombicola; Tolulaspora delbrueckii; Zygosaccharomyces bailli; Zygosaccharomyces rouxii* and it has been reported that they grow in medium containing 40-70% (w/w) sugar .Sugar tolerant yeasts are osmophillic in nature, desirable and are good candidates for production of poly-alcohols thus showing potential for industrial application (Deak, 2006; Turker, 2014) Their sugar-tolerant attribute is exploited in the processing of functional foods (health drinks, enzyme drinks, and fermented vegetable extract) which possess various health-regulating functions. Their immense contribution to the unique functions of these novel foods is partly due to the possession of antibacterial activity (Boirivant and Stober,2007)

The result of growth of yeast isolates at different pH is shown in Table 6. It was observed that all the yeast species grew within the pH range of 2 to 8 showing different levels of growth with optimum growth at pH 6 after which no growth was observed. PIN32 (*Saccharomyces cerevisiae 4*) and WAM8 (*Saccharomyces cerevisiae 1*) recorded the highest growth of 1.55 followed by WAM 20 (*Saccharomyces cerevisiae* 2) producing a growth of 1.47±0.3 and least (0.94±0.1) was recorded by CAS36 (*Debaromyces polymorphus*). Minimum growths were recorded at extreme pH (2 and 8) and all the yeast isolates showed no growth at pH 8. The growth pattern demonstrated by yeast spp. with pH variation had previously been reported by Narendramata and Power (2005) and Deak,(2006). Alakeji *et al.*(2015) reported that mould and yeasts could tolerate range pH between 2-8 and their ability to tolerate low pH (acidic pH) serves as a strategy to eliminate spoilage microbes and create a conducive environment for growth of desirable microorganisms (Boirivant and Stober,2007).

Table 6. Tolerance of yeasts isolate to different pH

Isolate code	**2**	**4**	**6**	**8**
WAM8	0.36±0.2	1.12±0.1	1.55±0.5	0.00±0.0
WAM11	0.34±0.1	1.27±0..6	1.29±0.1	0.00±0.0
WAM20	0.47±0,1	1.23±0.2	1.47±0.3	0.00±0.0
WAM25	0.18±0.0	1.01±0.0	1.18±0.2	0.03±0.0
PIN10	0.34±0.0	1.11±0.3	1.32±0.0	0.00±0.0
PIN40	0.22±0.1	0.94±0.0	1.25±0.7	0.00±0.0
PIN32	0.21±0.0	1.27±0.1	1.55±0.1	0.00±0.0
PIN12	0.26±0.3	1.09±0.5	1.28±0.6	0.00±0.0

CAS15	0.21±0.2	0.71±0.2	0.94±0.4	0.00±0.0
CAS36	0.19±0.1	1.04±0.3	1.31±0.0	0.09±0.0
CAS3	0.17±0.0	1.13±0.7	1.40±0.2	0.00±0.0

Table 7 represents the growth of the yeast isolates. at different temperatures The optimum temperature for growth of all the yeast isolates was 30^{o}C but a sharp decline in growth was observed as the temperature reached 40^{o}C and no growth was recorded at 45^{0}C.The isolates grew optimally within a temperature range of 30-35^{0}C with PIN32 (*Saccharomyces cerevisiae Saccharomyces*4) producing the highest growth of 2.50±0.6 followed by WAM8 (*Saccharomyces cerevisiae* 1) with a growth of 2.45±0.1 and the least 2.15±0.3 was recorded by CAS 36 (*Debaromyces polymorphus*).The growth pattern of yeast isolates obtained at different temperatures in this study is in consonance with the finding of Caspeta *et.al.*(2016) and Taluhder *et al*.(2016) that reported the activity of yeasts within a temperature range of 20-30^{0}C The inability of the yeast species to grow at extreme temperature of 45^{0}C is due to the high stress associated with this temperature (Sathees *et al.*, 2011;Choudhary,2016). The growth of yeast spp. within a temperature range of 20^{0}C-40^{0}C confirmed the vast difference in their thermo stability and maximum growth at 30^{0}C makes the yeast species suitable in fermentation process as this temperature corresponds with the temperature of fermentation which usually takes place within a temperature range of 20-30^{o}C (Keller, 2010).

Table 7. Tolerance of yeasts isolate to different temperatures

Isolate code	**20^{0}C**	**25^{0}C**	**30^{0}C**	**35^{0}C**	**40^{0}C**	**45^{0}C**
WAM 8	1.05±0.4	1.60±0.6	2.45±0.1	2.34±0.2	0.50±0.1	0.00±0.0
WAM 11	1.17±0.9	1.55±0.3	2.29±0.6	2.19±0.3	0.41±0.2	0.00±0.0
WAM 20	1.21±0.5	1.59±0.5	2.21±0.8	2.13±0.2	0.33±0.0	0.00±0.0
WAM 25	1.12±0.4	1.64±0.1	2.34±0.0	2.20±0.1	0.36±0.1	0.00±0.0
PIN 10	1.38±0.2	1.73±0.3	2.16±0.1	2.08±0.0	0.46±0.1	0.00±0.0
PIN 40	1.24±0.2	1.47±0.0	2.18±0.2	2.05±0.5	0.46±0.3	0.00±0.0
PIN 32	1.21±0.5	1.66±0.2	2.50±0.6	2.32±0.2	0.78±0.2	0.00±0.0
PIN 12	1.16±0.1	1.59±0.5	2.11±0.1	2.04±0.3	0.14±0.1	0.00±0.0
CAS 15	0.79±0.4	1.46±0.7	2.16±0.2	2.11±0.3	0.06±0.2	0.00±0.0
CAS 36	1.14±0.0	1.58±0.2	2.15±0.3	2.10±0.8	0.09±0.0	0.00±0.0
CAS 3	1.29±0.3	1.62±0.0	2.19±0.1	2..08±0.2	0.09±0.1	0.00±0.0

Table 8 shows the result of the identification process of the eleven yeast isolates. They were identified as *Saccharomyces cerevesiae* (5)*, Saccharomyces kluyveri* (1)*,* Pichia *farinose* (1)*, Kloeckera japonica*(1)*, Pichia ohmeri* (1) *Debaryomyces polymorphus*(1), *Candida tropicalis* (1)and *Candida kefyr.*(1).

Table 8. Identification result of the Isolates

Isolate code	Source	Number of isolate	Identity
WAM 8	Water melon	1	*Saccharomyces cerevisiae* 1
WAM 11	Water melon	1	*Pichia farinose*
WAM 20	Water melon	1	*Saccharomyces cerevisiae* 2
WAM 25	Water melon	1	*Kloeckera japonica*
PIN 10	Pineapple juice	1	*Saccharomyces kluyverii*
PIN 40	Pineapple juice	1	*Saccharomyces cerevisiae* 3
PIN 32	Pineapple juice	1	*Saccharomyces cerevisiae* 4
PIN 12	Pineapple juice	1	*Pichia ohmeri*
CAS 15	Cashew juice	1	*Saccharomyces cerevisiae* 5
CAS 36	Cashew juice	1	*Debaromyces polymorphus*
CAS 3	Cashew juice	1	*Candida kefyr*

Isolation and identification of yeast from fruits, fermented foods and beverages had earlier been reported by Maragatham and Panneerselvam (2011), Somdatta et. al. (2011) and Zerihun (2016). The involvement of yeasts in different types of indigenous fermented foods and fruits has been documented (Ogunremi et al.,2015). Their existence in fermented foods and fruits is due to their sugar loving characteristics and their fermentative ability (Djelal et al.,2017)

4. Conclusions

Considering the result obtained in this study isolates PIN32 (Saccharomyces cerevisiae 4) and WAM 8 (Saccharomyces cerevisiae 1) may be selected as potential starter cultures for the production of pineapple wine.

5. References

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