

1 PRODUCTION OF AN ALCOHOLIC BEVERAGE FROM RICE (*Oryza sativa*)
2 USING NIGERIAN PALM WINE YEASTS

3
4 **Abstract**

5 Rice wine is an alcoholic beverage of cereal produced as a result of fermentation of rice by selected
6 yeasts isolated from different substrates. In this study the effects of starter cultures on physico-
7 chemical and sensory properties of rice wine were investigated. The yeasts isolated belong to six
8 genera and these are *Saccharomyces*, *Kluveromyces*, *Schizosaccharomyces*, *Candida*, *Rhodotorula*
9 and *Geotrichum*. The species isolated included *S. cerevisiae*, *S. uvarum*, *S. chevallieri*, *Klu.*
10 *africanus*, *Schizosaccharomyces japonicus*, *S. pombe*, *C. castelli*, *C. intermedia*, *R. graminis* and
11 *G. candidium*. The wort produced from rice had fermentable sugars and nitrogen sources for yeast
12 metabolism and alcoholic fermentation. The wort fermented with *Saccharomyces cerevisiae* had
13 the highest ethanol concentration of 3.90% compared to 2.16% and 3.7% recorded for *Klu.*
14 *africanus* and *S. uvarum*, respectively. The rice wine produced with *S. cerevisiae* also received the
15 highest scores in all the parameters evaluated by the taste panelist. Therefore, it could be concluded
16 that rice wine of acceptable quality could be produced by using *S. cerevisiae* isolated from palm
17 wine.

18
19 **Introduction**

20 Rice is the seed of the monocot plant *Oryza sativa* of the grass family gramineae (Kadiri *et al.*,
21 2014). Rice is an increasingly important crop in Nigeria. It is relatively easy to produce and it is
22 grown for sale and for home consumption. In some areas there is a long tradition of rice growing
23 but for many, it is considered a luxury food for special occasions only. Since rice has become more
24 available, it is now part of the everyday diet of many people in Nigeria. There are different types
25 of rice grown in Nigeria, some of these are local varieties while others have been introduced into
26 the country (Shittu *et al.*, 2019). Fermentation is the oldest transformation method used to preserve
27 and enhance **flavor**, **aroman** and nutritive values of food (Kishneth and Charles, 2013). In this
28 regard, rice fermentation for the production of alcoholic beverage is an ancient process practiced
29 in most Asian countries. Rice wine is widely consumed during social and cultural events and is
30 part of offering for a good harvest, traditional medicine and postnatal recovery (Chiang *et al.*,
31 2006). The Chinese Huangiiu, Indian Sonti, Japanses sake and Korean Yakju are a few examples

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32 of fermented rice wines (Steinkraus, 2002). In rice wine production, starch (sugar) from rice is
33 converted into alcohol via fermentation by yeast, fungus and lactic acid bacteria (LAB)
34 (Chuenchomrat *et al.*, 2008). Palm wine is the fermented sap of various palm trees especially
35 palmyra, silver date palm and coconut palms. Palm wine is an alcoholic beverage produced from
36 the sap of various palm tree species and usually consumed in parts of Africa, Asia and South
37 America (Nwakanma *et al.*, 2015). In Africa, the sap is most often taken from mild date palms
38 such as *Phoenix sylvesteris* (the palmyra) and *Caryotaurens* from oil palms such as *Elaeis*
39 *guineensis* or from raphia, kithul or nipa palms. The liquid collected is a cloudy whitish beverage
40 with a sweet alcoholic taste of flavours varying from sweet unfermented to sour fermented and
41 vinegary (Chandrasekhar *et al.*, 2021). The microorganisms in the overnight palm wine have the
42 effect on the palmwine and this effect is due to the increase in their population as well as their
43 activities (Ogbulle *et al.*, 2007). Upon fermentation by the natural microbial flora, the sugar level
44 decreases rapidly as it is converted to alcohol and other products (Obire, 2005). Palm wine contain
45 good amount of microorganisms. The types and numbers of organisms encountered vary widely
46 even from tree to tree (Nwakanma *et al.*, 2015). From the microbial analysis of palm wine, it
47 includes both yeast and bacteria. The present government in Nigeria is encouraging large scale
48 production of rice and in the next few years surplus quantity of rice will be produced. Therefore,
49 there is need to develop alternative method of industrial utilization of rice apart from the traditional
50 utilization as food. The present study was carried out to produce wine from rice using local yeasts
51 isolated from palm wine.

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53

54 **Materials and methods**

55 **Sample collection**

56 **Indigenous rice** which is the raw material used was collected from the Nigerian Cereal Research
57 Institute (NCRI) Apata, Ibadan metropolis. The sample was collected in a clean polythene bag and
58 taken to the laboratory. Also, palm wine was obtained from different sources at Mammy Market
59 in Barracks, Ibadan. The samples were collected in sterile containers and were taken to the
60 laboratory.

Commented [SA6]: samples were...

Commented [SA7]: How many samples? How much? How often? Describe the reasons for obtaining the palm wine from different source.

61 **Isolation and identification of yeasts**

62 The samples of palm wine were serially diluted in sterile distilled water and appropriate dilutions
63 was plated on Potato Dextrose Agar (PDA) supplemented with 3ug/ml of streptomycin. The plates

64 were incubated at 27°C for 48 hours under aerobic condition (Nwakanma *et al.*, 2015). The yeasts
65 that developed were sub-cultured on PDA and purified by repeated streaking. The pure yeast
66 isolates were then characterized by physiological and biochemical tests which include sugar
67 fermentation tests, acid production from glucose, nitrate assimilation and urease test. The sugars
68 tested include glucose, fructose, mannitol, sucrose, melibiose, lactose, arabinose, galactose,
69 raffinose and maltose. The yeasts were then identified according to the scheme of Barnett *et al.*
70 (2000).

71

72 Starter culture preparation

73 Pure cultures of yeast stocked on potato dextrose agar slants were made in sterile wort. Loopful of
74 yeast from potato dextrose agar slants were inoculated into 10ml wort in cotton-plugged test tubes.
75 The tubes were incubated for 12 hours after which 2ml of each were transferred to a fresh 10ml
76 wort and incubated further for 12 hours under static conditions.

77

78 Malting of rice grains

79 Rice grains were manually cleaned to remove stones, sticks and other extraneous matters. The
80 grains were steeped for 24 hours to raise the moisture level and activate the metabolic process of
81 dormant grains. After the steeping, the grains were kept in air tight sack and the white tips of the
82 rootless emerged. The grains were then spread on a tray to a depth of approximately 1cm and
83 incubated at 30°C for 96 hours. Light watering was carried out at 12 hours interval. The grains
84 were turned to ensure uniform aeration and temperature. The grains were then allowed to
85 germinate for 4 days, the germination process was stopped by drying the grains in the oven at 50°C
86 for 24 hours and the moisture content was brought to about 4%. The shoot less and root less grains
87 were packed in air tight polythene bags until they are needed for use.

88

89 Wort preparation

90 Malted grains were ground in a hammer mill to pass through a sieve of pore 1.0mm. The
91 grist(500g) was weighted into 2 liters of water at 50°C causing the temperature to drop to 48°C. It
92 was stirred after 30 min and left to settle for another 30 min. A 500ml sample of the supernatant
93 was decanted and held. The rest of the mash was gently boiled with stirring for 10 min cooled to
94 65°C and the supernatant added and mixed thoroughly. The temperature of the mash dropped to

Commented [SA8]: what temperature? What is the reference method?

Commented [SA9]: What temperature?

Commented [SA10]: Do you mean until the white tips emerged? Please correct. At what temperature/

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Commented [SA12]: which supernatant? If you mean the initially decanted supernatant, re-write and make that clear.

95 60°C and was maintained at 60°C in the water bath. Filtration was very poor so the mash was
96 centrifuged at 1300xg. The sediment was mixed with water and centrifuged at 1,300g. The
97 supernatant from the second extract was used to adjust the first extract. The wort was divided into
98 three equal portions (300ml) and was sterilized at 100°C for 30 min in an autoclave.
99

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100 Determination of reducing sugar of the wort

101 The reducing sugar was determined by (DNSA), 3, 5-dinitrosalicylic acid method according to the
102 procedure by Shrestha (2002).
103

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104 Determination of specific gravity

105 Fifteen ml of wort was taken and transferred into the bottle of known weight. The bottle and the
106 wort were then weighed. The density was determined by dividing the weight of the sample by the
107 volume of the sample. The specific gravity was then determined by dividing the density of the
108 wort by the density of the water as stated below:

Commented [SA15]: What is the reference method? Why didn't you use a hydrometer to measure specific gravity? Usually, s. gravity is measured at specific temperature!

$$\begin{aligned} \text{Density} &= \text{Weight / volume} \\ \text{Specific gravity} &= \text{Density of wort / Density of water} \end{aligned}$$

113 Determination of pH of wort

114 The pH of the liquid wort was determined using a previously calibrated pH meter
115

116 Determination of titratable acidity (TA) of wort

117 Acidity of the wort was measured by titrating 10ml of the sample with 0.1M NaOH and indicated
118 as the volume of 0.1M NaOH(ml) needed for neutralization (Teramoto *et al.*, 1998).
119

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120 Fermentation of the wort

121 Concentrated yeast cells in 10ml of 0.9% NaCl were made in sterile worts. Two ml of each strain
122 (*Kluyveromyces africanus*, *Saccharomyces cerevisiae* and *S. uvarum*) were aseptically transferred
123 to a fresh 10ml of wort in separate test tubes and incubated for 12 hours. Five ml each of the yeast
124 cultures were then used to inoculate 200ml of fresh wort in 300ml cotton-plugged Erlenmeyer
125 flask. Fermentation was then carried out at 30°C under static conditions, and samples were
126 withdrawn at 24 hours intervals for analysis. After three days of fermentation, organoleptic
127 assessment was carried out on the samples of rice wine prepared with different starter cultures.
128

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129 **Analysis of the rice wine**

130 **pH determination**

131 pH of the samples was recorded by using pH meter standard solutions of pH 4.0 and 7.0 were used
132 as reference to calibrate.

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134 **Alcohol content**

135 Rice wine sample (100ml) was poured in to the 100ml of volumetric flask. The samples were
136 distilled until 25ml of distillate is collected. The volume of distillate was restored to 100ml with
137 distilled water. Then the specific gravity was measured by using hydrometer and recorded and
138 corresponding alcohol percentage volume (Henock *et al.*, 2014).

140 **Determination of titratable acidity (TA)**

141 Sodium hydroxide (0.1M NaOH) was prepared, poured into burette attached to a retort stand. Ten
142 ml of each sample of rice beer was pipetted into a conical flask, and then two drops of
143 phenolphthalein indicator was added and then titrated. Development of pink coloration was
144 observed as the end point. The titre volume of each homogenate was multiplied by 0.09 to give
145 the percentage TA as lactic acid (Adesokan *et al.*, 2010).

147 **Microbial analysis (Yeast count)**

148 Each sample of rice beer was thoroughly mixed together and then serially diluted in sterile distilled
149 water. Appropriate dilutions of the rice wine samples were then plated on potato dextrose agar
150 supplemented with streptomycin (3µg/ml). Enumeration was carried out at 24 hours interval for
151 three days.

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153 **Sensory evaluation**

154 Sensory evaluation of rice wine samples was estimated by ten consumer sensory panelists. Sensory
155 parameters which include flavor, taste, aroma appearance and overall acceptability of rice wine
156 were evaluated using a Hedonic scale of 1 to 5 where 5 being "excellent" and 1 "poor" (Henock
157 *et al.*, 2014).

159 **Statistical analysis**

160 All data obtained from sensory evaluation were subjected to statistical analysis by using Analysis
161 of Variance and DUNCAN Multiple Range Test. Significance of variations in the analyzed data
162 was tested at 95% confident limit.

163 Results

164 A total of 50 yeasts were isolated from seven different samples of palm wine analysed. The yeast
165 isolates belong to six genera and these are *Saccharomyces*, *Kluveromyces*, *Schizosaccharomyces*,
166 *Candida*, *Rhodotorula*, and *Geotrichium* (Table 1). *S. cerevisiae* had the highest frequency of
167 occurrence of 36% while *Schizosaccharomyces japonicus* and *Schizosaccharomyces pombe* had
168 2% each.

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170 The results of wort analysis are presented in table 2. The results showed that the wort had reducing
171 sugar, soluble nitrogen, specific gravity, pH and acidity of 6.55mg/ml, 0.35%, 1.062, 5.88 and
172 0.27% respectively. The wort was fermented with selected starter culture based on ethanol
173 production. The results of the characteristics of the fermented wort are presented in table 3-5. The
174 results showed that pH of the fermented wort decreased steadily with corresponding increase in
175 titratable acidity. The pH ranged between 3.09 and 4.68 after 72 hours of fermentation. The
176 titratable acidity ranged between 0.313 and 0.477% at the end of fermentation. Also, the reducing
177 sugar and the soluble nitrogen ranged between 3.07 and 4.26 mg/ml and 0.09 and 0.24%
178 respectively. Moreover, ethanol concentration ranged between 2.16 and 3.90% (v/v).

Commented [SA22]: Should have included Standard deviation or S. error to judge the accuracy of the reported results.

179 The results of the yeast count are presented in table 6. The results revealed that viable cell count
180 increased with incubation period and ranged between 3.80 and 4.71 log₁₀cfu/ml at the end of
181 fermentation. The organoleptic evaluation is presented in table 7. Sample prepared with *S.*
182 *cerevisiae* as starter culture had the highest scores for all the attributes considered. The results from
183 this study indicated that acceptable alcoholic beverage could be produced from rice by using
184 *Saccharomyces cerevisiae* isolated from palm wine

Table 1: Percentage occurrence of yeasts isolated from palm wine

Isolates	Frequency of occurrence	Percentage occurrence
<i>Sacharomyces cerevisiae</i>	18	36
<i>Sacharomyces chevallieri</i>	4	8
<i>Sacharomyces uvarum</i>	3	6
<i>Schizosacharomyces</i> <i>japanicus</i>	1	2
<i>Schizosacharomyces pombe</i>	1	2
<i>Candida intermedia</i>	2	4
<i>Candida castelli</i>	5	10
<i>Rhodotorula graminis</i>	2	4
<i>Kluveromyces africanus</i>	12	24
<i>Geotrichum candidum</i>	2	4

Table 2: Characteristic of wort produced from rice

Reducing Sugar(mg/ml)	6.55
Soluble nitrogen (%)	0.35
Specific gravity	1.062
pH	5.88
Acidity (%)	0.27

UNDER PEER REVIEW

Table 3: Characteristics of wort fermented with *Klu.africanus*

Fermentation time (Hr)	0 (Hrs)	24(Hrs)	48(Hrs)	72 (Hrs)
Ethanol (% v/v)	Nd	2.03	2.09	2.16
Acidity (%)	0.27	0.297	0.311	0.313
pH	5.88	5.80	5.01	4.68
Reducing sugar (mg/ml)	6.55	6.26	5.32	4.26
Soluble nitrogen (%)	0.35	0.32	0.27	0.24

Table 4: Characteristics of wort fermented with *S. uvarum*

Fermentation time	0 (Hr)	24(Hrs)	48(Hrs)	72 (Hrs)
Ethanol (% v/v)	Nd	3.24	3.53	3.70
Acidity (%)	0.27	0.324	0.423	0.459
pH	5.88	5.68	4.33	3.14
Reducing sugar (mg/ml)	6.55	5.94	5.14	3.22
Soluble nitrogen (%)	0.35	0.30	0.21	0.13

UNDER PEER REVIEW

Table 5: Characteristics of wort fermented with *S. cerevisiae*

Fermentation time (Hours)	0	24	48	72
Ethanol (% v/v)	Nd	3.40	3.70	3.90
Acidity (%)	0.27	0.325	0.441	0.477
pH	5.88	5.63	4.30	3.09
Reducing Sugar (mg/ml)	6.55	5.89	5.08	3.07
Soluble nitrogen (%)	0.35	0.22	0.14	0.09

UNDER PEER REVIEW

Table 6: Viable count (yeast) (\log_{10} cfu/ml)

Sample	0 (Hr)	24(Hrs)	48(Hrs)	72 (Hrs)
A	ND	nd	3.76	3.80
B	2.40	2.57	4.59	4.66
C	2.45	2.61	4.63	4.71

Key: Sample A-Wort fermented with *Klu. africanus*; Sample B-wort fermented with *S. uvarum* and Sample C-wort fermented with *S. cerevisiae*.

nd means not detected

ND means not determined

Table 7: Organoleptic attributes of rice beer as determined by a sensory panel

Sample	Appearance	Taste	Flavour	Aroma	Overall acceptability
A	2.6	2.1	2.3	2.6	2.4
B	3.6	3.4	3.5	3.2	3.4
C	3.8	3.6	3.7	3.4	3.6

Key: The sample codes are as stated in table 6

UNDER PEER REVIEW

Discussion

The yeasts isolated in this study are *Saccharomyces cerevisiae*, *Candida intermedia*, *Candida castelli*, *Rhodotorulagraminis*, *Kluyveromyces africanus*, *Schizosaccharomyces pombe*, *S. uvarum* and *S. chevallieri*. Similar yeasts have been reported from palm wine by other workers (Adesokan *et al.* 2021, Yabaya *et al.* 2016 and Nwakanma *et al.* 2015). The wort produced from the rice indicated the presence of enough fermentable medium for the yeast isolates to be able to produce alcoholic beverage. During fermentation the yeast consumed the fermentable sugar which resulted in decrease in pH and a corresponding increase in titratable acidity. Decrease in pH, increase in acidity and production of ethanol must have resulted in inhibition of pathogenic and spoilage organisms.

The pH, acidity (%) and alcohol (%) reported during fermentation of rice for wine production vary from one author to another (Pakuwal and Manandhar, 2020; Bhuyan *et al.*, 2014 and Henock *et al.*, 2014). The reasons for this could be the use of different variety of rice and methods of wine production in different locality. The yeast population was increasing throughout the period of fermentation. This could be as a result of their ability to multiply rapidly in the presence of increasing concentration of ethanol. The total count of *Saccharomyces cerevisiae* was the highest at the end of wort fermentation. The wine produced by this yeast also received the highest scores for appearance, taste, **flavor**, aroma and overall acceptability. Therefore, it could be concluded from this study that an acceptable wine could be produced from rice using *S. cerevisiae* isolated from Nigerian palm wine.

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