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

Abstract

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

Introduction

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

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

Materials and methods

Sample collection

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

Isolation and identification of yeasts

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

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

Starter culture preparation

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

Malting of rice grains

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

Wort preparation

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

mash dropped to 60^{o}C and was maintained at 60^{o}C in the water bath. Filtration was very poor so the mash was centrifuged at 1300xg. The sediment was mixed with water and centrifuged at 1,300kg. The supernatant from the second extract was used to adjust the first extract. The wort was divided into three equal portions (300ml) and was sterilized at 100^{o}C for 30 min in an autoclave.

Determination of reducing sugar of the wort

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

Determination of specific gravity

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

Density	=	Weight / volume
Specific gravity	=	Density of wort / Density of water

Determination of pH of wort

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

Determination of titratable acidity (TA) of wort

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

Fermentation of the wort

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

Analysis of the rice wine

pH determination

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

Alcohol content

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

Determination of titratable acidity (TA)

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

Microbial analysis (Yeast count)

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

Sensory evaluation

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

Statistical analysis

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

Results

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

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

The results of the yeast count are presented in table 6. The results revealed that viable cell count increased with incubation period and ranged between 3.80 and 4.71 $\log_{10}$cfu/ml at the end of fermentation. The organoleptic evaluation is presented in table 7. Sample prepared with *S. cerevisiae* as starter culture had the highest scores for all the attributes considered. The results from this study indicated that acceptable alcoholic beverage could be produced from rice by using *Saccharomyces cerevisiae* isolated from palm wine

Table 1: Percentage occurrence of yeasts isolated from palm wine

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

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

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

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

Discussion

The yeasts isolated in this study are *Saccharomyces cerevisiae, Candida intermedia, Candida castelli, Rhodotorulagraminis, Kluveromyces 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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