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Physiochemical And Proximate Analysis Of Wine Produced From Fermented Rice Grains Hydrolysed With Dilute Hydrocloric Acid

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Abstract: ***This study evaluated the physiochemical and proximate composition of rice wine using three local rice varieties cultivated in Nigeria (Ilah, Ekpoma and Abakaliki rice). Rice samples were steeped for 2hrs, cooked to gelatinize the starch and hydrolyzed using 1.1% dilute hydrochloric acid. The hydrolysate was neutralized using 1.0M sodium hydroxide to pH 7.0. The hydrolysate was filtered and the filtrate was anaerobically fermented using Saccharomyces cerevisiae at room temperature. Rice samples not hydrolysed with acid but subjected to all other experimental conditions served as control. The acid hydrolyzed samples recorded higher physiochemical values when compared than the control samples across the three rice varieties. The specific gravity ranged between 1.000 ± 1.5 – 1.055 ± 0.1, alcohol content 0.01 ± 0.0% – 6.99 ± 2.5%, pH 5.8 ± 1.1 - 7.3 ± 0.3, reducing sugar 0.00 ± 0.0% – 40.51 ± 5.1% and total sugar 0.02 ± 0.0 % – 51.43 ± 4.6 % respectively. pH and specific gravity decreased as fermentation progressed while alcohol content, reducing and total sugar increased respectively. Protein content ranged between 0.14% – 1.49%, moisture 77.5% – 98.6%, carbohydrate 1.6% - 48.4%, ash 0.20% - 0.90% and fats 0.00% - 0.03%. Among the rice varieties utilized in the study, Ekpoma rice was best for the production of rice wine because it scored the highest physicochemical parameters. The physiochemical properties of the rice wine produced using acid hydrolysis reported in this study compares favourably with those from rice wine produced using enzyme hydrolysis reported in other studies.***

Keywords: ***Acid hydrolysis, Starch, Glucose, Anaerobic fermentation, Saccharomyces cerevisiae, Rice wine***

I. INTRODUCTION

Rice is the seed of the monocot plant *Oryza sativa* of the grass family Gramineae. Rice is grown virtually in all the agro-ecological zones in Nigeria (Kadiri et al., 2014). There are many varieties of rice grown in Nigeria; some of these are local varieties while others have been introduced into the country (Shittu et al., 2019)

Rice is a staple food and can be used for the production of alcoholic beverage. The well-known fermented beverages are rice wine, rice beer, and rice vinegar. Wine is a fermented beverage of fruits juice or a solution containing simple sugars.

Starch is the major constituent of rice and makes up to 90% of rice in dry weight. Starch is a polymer of glucose and contains amylase and amylopectin as building blocks. To release the free glucose, these building blocks have to be broken by hydrolysis. Starch may be hydrolyzed by enzymes, inorganic and organic acids. Acid hydrolysis is the chemical breakdown of starch into glucose via addition of water in the presence of an acid. Acid hydrolysis is a simple and cheap method of starch hydrolysis (Ramprakash and Muthukumur, 2014). Rice wine is the alcoholic beverage produced after hydrolysis of rice starch into glucose by action of microbes, enzymes or acids and fermentation of the hydrolysate by microorganisms such as yeast (Dziedzie and Kearsley, 2012). Starter cultures used in the rice wine fermentation comprise mixed cultures containing fungi and bacteria with dehulled rice (*Oryza sativa L.*), glutinous rice (*Oryza sativa var. glutinosa*), and purple glutinous rice as the substrate (Dung et al., 2015).

Global interest in cereal-based fermented products is increasing due to low fat/cholesterol, high minerals, dietary

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fibers, and photochemical content (Blandino et al., 2003). Fermentation enriches the rice, supplements it with different essential amino acids, vitamins, minerals, prebiotics, probiotic organisms, and degrades anti-nutrients (phytic acid, tannins, and polyphenols). Thus, its nutrition, energy contents, and therapeutic potentialities are increased (Mohan et al., 2014). Rice wine is consumed in different parts of the World. Rice wine is called sake in Japan, cheongju in Korea, Indian Sonti, and shaosingjiu in China. (Steinkraus, 2002).

The quality of wines can be influenced by the type of rice fermented and different physiochemical attributes: acidity, specific gravity, reducing sugar, density, pH, alcohol content, etc (Vairappan and Kishneth, 2013; Kadiri *et al.*, 2014).

Local production of rice in Nigeria has improved and researchers are investigating the potential of using rice as a raw material for the production of other consumables. Non-food application of rice has in recent times become an area of interest by industrialists, researchers and biotechnologists. Research to validate the use of rice for wine production is imperative. Also there is no scientific study utilizing acid hydrolysis of rice starch for wine production. Hence this study was aimed at evaluating the physicochemical properties of rice wine produced from three selected Nigerian local rice varieties hydrolyzed with mineral acid (HCl)

II. MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANTS

Rice samples cultivated in Ilah in Delta State, Ekpoma in Edo State and Abakaliki in Ebonyi State, all in Nigeria were collected and identified by different taxonomists, prominent of which are Prof. E.I. Aigbokhan and Prof. H. Akinibosun both of the Department of Plant Biology and Biotechnology, University of Benin, Benin-City. Samples were obtained and transported immediately to the laboratory for experimentation and analysis.

Rice starch gelatinization: One kilogram (1.0 Kg) of each variety of rice (Ilah, Ekpoma and Abakaliki) was weighed and washed 2 to 3 times with water to remove rice bran and husks or until the water became clear. Rice was steeped in water for 2 hours and thereafter cooked (rice grain: water = 2:1) at 100^{o}c for 60 minutes and cooled at room temperature (28~30°C) (Palaniveloo and Vairappan, 2013).

ACID HYDROLYSIS OF RICE STARCH

The cooled cooked rice samples were divided into two equal parts. One part was treated with 1.1% concentration of dilute HCl (rice: acid = 1:3) in a water bath at 50^{0}c for 90 minutes with steady agitation (Henry, 2009). The media was cooled and neutralized with 0.1M sodium hydroxide until pH was 7.0 and thereafter filtered through Whatman No1 filter paper into various fermenters (Cory and Bruce, 1997). The other part of the gelatinized rice sample (control) were treated with the equivalent volume of sterilized distilled water and subjected to same experimental conditions.

Inoculation of Rice with Starter Cultures: Five hundred milliliter (500ml) of each filtered hydrolysate and control samples were introduced into different fermentation jars. Five millilitre (5 ml) of the inoculum (wine yeast: *Saccharomyces cerevisiae* cells diluted to the turbidity of 0.5 McFarland standard) was aseptically transferred into each of the fermenters (Ansah, 2011)

Fermentation

The rice liquor and inoculum mixture were left to ferment anaerobically for 120 hours (5 days) at room temperature. Temperature, Reducing sugar, specific gravity, alcohol content, pH etc were monitored during the fermentation process every 24 hour. (Palaniveloo and Vairappan, 2013). After fermentation, rice wine was filtered using a muslin cloth. The wine was stored under refrigeration (< 4°C).

PHYSIOCHEMICAL ANALYSIS OF RICE WINE

Colour determination of Rice Wine: About 50 ml of rice liquor from each treatment and replicate were dispensed into clean coded glass cups and placed on serving plates. Panel of judges consisting of students of the University of Benin, Benin- City, were asked to give the colour by visual observation.

Determination of the pH: The pH was measured using a Hanna HI98129 hand held pH meter (Hanna Instruments, Woonsocket, RI). Approximately 25 ml of wine were placed into a 50 ml beaker. The probe was inserted into the liquid and gently stirred until a stable pH reading was displayed. All experiments were carried out in triplicates and their mean values were calculated and recorded (Veith, 2007)

Determination of Specific: Specific gravity was measured using a brix hydrometer. 50ml of must was measured into a 100ml measuring cylinder. A clean brix hydrometer was dipped into it and the specific gravity was measured and recorded (Veith, 2007).

Determination of Reducing Sugar: The wine was filtered through whatman No 1 filter paper. The supernatant was used for determination of quantity of reducing sugar. The quantity of reducing sugar produced was determined by the method of Miller (1959) as described by Itelima et al., (2013) using the 3,5- dinitrosalicilic acid method **.** 1.0ml of 3,5- dinitrosalicilic acid was added to 1.0ml of filtrate in a test tube. The mixture was boiled for 5 minutes in a boiling water bath and 10.0ml of distilled water was added to the mixture. A blank containing 1.0ml of distilled water and 1.0ml of DNSA was prepared. Optical density of the samples was measured against the blank using a Spectrophotometer (JENWAY: 6400, UK), set at 540nm. The concentration of reducing sugar was obtained from a standard glucose curve at concentrations ranging from 0.5 to 2.0g/l of glucose

Determination of Alcohol from Rice Wine: Alcohol content in the fermented wine was determined using the specific gravity method using a brix hydrometer. 50ml of filtrate was measured into a 100ml measuring cylinder. A clean brix hydrometer was dipped into it and the specific gravity of the hydrolysate was measured and recorded at room temperature. The alcohol content was read using data from the specific gravity table.

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Determination of Total Sugar: The soluble sugar content of the sample was determined using the method described by James, (1995). In the process, 20ml homogenate of the samples was dissolved in 250 ml of distilled water and continuously agitated for 3hours using a shaker, followed by filtration. Four milliliter of the filtrate was pipetted into three test tubes while 4ml of distilled water was introduced into a fourth test tube as blank and 4 ml of glucose into a fifth test tube as a standard. To each of the test tube was added 2 ml each of Fehling's solutions (A and B). It was boiled gently in a water bath for 3 minutes. After which 10 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. Each tube was labeled and placed in a test tube rack and allowed to stand for 12 minute before transfer to spectrophotometer and absorbance read at 630 nm against the blank. After which the total available sugar as percentage reducing sugar was calculated as shown:

$$\text{Total sugar (\%)} = \frac{25\ A_1 \times 100}{W \times A_2}$$

Where: W = weight of sample
25 = Constant
A_1 = Absorbance of diluted sample
A_2 = Absorbance of diluted standard

PROXIMATE ANALYSIS OF RICE WINE

The Association of official Analytical Chemistry methods was used in the determination of the percentage moisture, protein, ash, fat, fibre and carbohydrate contents of the wine samples (AOAC, 2012)

STATISTICAL ANALYSIS

Data obtained were subjected to descriptive statistics (mean and standard deviation of mean) as well as inferential (Turkey's multiple comparison test) analysis using Graph pad prism (UK) soft ware version 6 (Berthold and Hand, 2003).

III. RESULTS AND DISCUSSIONS

In this study, rice grains samples were steeped in water and cooked to gelatinize the starch to make it more susceptible to hydrolysis by the hydrochloric acid. The hydrolysate was neutralized into salt and water using sodium hydroxide to pH 7.0 in order to support the growth and activities of the yeast: *Saccharomyces cerevisiae.* The fermentation was carried out anaerobically in order for the yeast to convert the sugars into alcohol, and at room temperature because it is best for yeast fermentation.

The results of the physicochemical properties of rice wine from the three different varieties are presented in table 1.0.

The acid hydrolysis favoured majority of the rice wine physicochemical parameters when compared with the untreated control samples across the three rice varieties (Ilah, Ekpoma and Abakaliki). This is because the acid acts as a catalyst, speeding up the hydrolysis and yielding more glucose molecules that are fermented by the yeast which in turn gave higher alcohol and other physiochemical properties of the wine. The presence of reducing sugar and alcohol in the control samples despite not been treated with the acid, is as a result of water molecules hydrolyzing the rice starch. When starch molecules react with water, they break down into smaller sugar molecules however at a slower rate which in turn resulted in the lower physiochemical properties of the control samples.

The colour of the fermenting rice liquour varied slightly from cream to brown in all samples all through the experimental period.

Specific gravity measures the density liquids. Brewers utilize this measurement to indicate the amount of sugar in solution and to determine the rate of fermentation. The acid hydrolyzed samples had higher specific gravity than the control samples in all the rice varieties. The specific gravity recorded in this study ranged between 1.000 ± 1.5 – 1.055 ± 0.1 and decreased with fermentation time. This decrease in specific gravity is as a result of the fermentative activities of the wine yeast resulting in production of alcohol and Co_2 gas

Alcohol content was higher in the acid hydrolyzed samples than the control all through the experiment. Alcohol content ranged between 0.01 ± 0.0% – 6.99 ± 2.5% and increased as the fermentation progresses. The alcoholic content recorded in this study compares with those reported by Chim et al., (2015), (Alc 5.6 – 7.0%) using microbial enzymes for rice starch hydrolysis. The presence of alcohol is a positive indicator of a successful fermentation. Alcohol is produced from the conversion of sugar by microbes (Dung et al., 2007). Alcohol content in wine is inversely proportional to the sugar content and with sufficient sugar for microbes; alcohol will continue to be produced (Pramanik and Rao, 2005).

The pH of the wine was measured to evaluate the rate of fermentation. The control samples recorded higher pH than the acid treated samples in all rice variety and throughout the experiment. The pH recorded in this study ranged between 5.8 ± 1.1 and 7.3 ± 0.3 and decreased with fermentation time. The drop in pH over time is as a result of the production of alcohol and organic acids in the wine (Chiang et al., 2006). The pH of wine depends on the acidity and sugar content of the wine (Karthikeyan et al., 2014). The rice wine pH recorded in this study are higher than those reported by Sanchez et al., (1988), 4.65 – 5.0, 3.3 – 4.0 by Chim et al., (2015) and 4.3 – 4.7 reported by Palaniveloo and Vairsppan, (2013). This difference may be attributed to the different rice varieties used, organism used for the fermentation and the method of starch hydrolysis.

The acid treated samples had higher reducing sugar than the control. The reducing sugar recorded in this study ranged between 0.00 ± 0.0% – 40.51 ± 5.1% and increased with fermentation time. This increase led to increase in the alcoholic content over time. This increasing trend was also observed by Shittu and Mohammed, (2019).

Total sugar content was higher in the acid treated samples than the control, increased all through the experimental period and ranged between 0.02 ± 0.0 % – 51.43 ± 4.6 %

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Parameters	Sample Source	Days 1	2	3	4	5
Colour	Control (Untr.)	Creamy	Creammy	Creamy	Creamy	pale brown
	Illa rice	Craeamy	Creamy	Creamy	pale brown	dark brown
	Ekpoma rice	Creamy	Creamy	Creamy	pale brown	dark brown
	Abakaliki rice	Creamy	Creamy	Creamy	pale brown	dark brown
Specific Gravity	Control (Untr.)	1.035±0.0[a]	1.013±0.0[a]	1.030 ±0.0[a]	1.025±0.1[ab]	1.015±0.2[b]
	Illa rice	1.045±0.1[ab]	1.028 ±0.2[b]	1.021 ±0.1[b]	1.009±1.1[bc]	1.005±1.2[bc]
	Ekpoma rice	1.055 ±0.1[b]	1.031±0.9[b]	1.023±1.3[c]	1.005±1.3[c]	1.001±1.1[c]
	Abakaliki rice	1.050±0.3[b]	1.027±0.1[b]	1.023 ±1.1[b,c]	1.010±1.7[c]	1.000±1.5[c]
pH	Control (Untr.)	7.3 ±0.3[a]	7.1 ±1.0[a]	6.9 ±1.6[a]	6.7±3.1[ab]	6.6±1.3[b]
	Illa rice	6.8 ±1.3[ab]	6.6 ±3.1[b]	6.5 ±2.3[b]	6.1±1.6[b]	5.9±1.6[b]
	Ekpoma rice	6.6 ±1.6[b]	6.3 ±2.7[b]	6.1 ±1.1[b]	5.8±2.1[b]	5.8±1.2[b]
	Abakaliki rice	6.7 ±14[b]	6.6 ±1.3[b]	6.4 ±2.5[b]	6.0±1.1[b]	5.8±1.1[b]
Alcohol content (%)	Control (Untr.)	0.01±0.0[a]	0.06 ±0.0[a]	0.59 ±0.1[b]	1.36±0.6[b]	2.34±1.3[b]
	Illa rice	0.21 ±0.1[b]	2.31 ±1.2[bc]	3.16 ±1.3[c]	4.21±1.1[c]	5.13±2.1[c]
	Ekpoma rice	0.32 ±0.5[b]	3.11±1.6[c]	4.23 ±2.1[c]	6.55±2.6[c]	6.99±2.5[c]
	Abakaliki rice	0.21 ±0.0[b]	3.0 ±1.3[c]	3.53 ±1.7[c]	5.11±2.3[c]	6.97±1.6[c]
Reducing sugar (%)	Control (Untr.)	0.00 ±0.0[a]	0.01 ±0.0[a]	0.36 ±0.1[a]	1.10±1.1[a][b]	4.13±1.1[a][b]
	Illa rice	1.08 ±0.7[ab]	3.08 ±1.7[ab]	8.14 ±2.6[b]	17.5±3.5[b][c]	31.15±2.5[c]
	Ekpoma rice	1.38 ±0.5[ab]	6.51 ±3.5[b]	16.11±3.9[b]	23.1±3.8[b][c]	40.51±5.1[c]
	Abakaliki rice	1.13±0.1[ab]	5.71 ±1.6[b]	10.83±3.1[b]	20.5±2.6[b][c]	38.5±2.5[c]
Total sugar (%)	Control (Untr.)	0.02 ±0.0[a]	0.05 ±0.0[a]	1.97±1.1[a]	2.05±1.0[a][b]	6.55±2.5[a][b]
	Illa rice	2.04 ±1.6[ab]	9.35±2.51[b]	14.31±3.2[b]	29.11±2.6[bc]	48.11±2.1[c]
	Ekpoma rice	2.58 ±1.3[ab]	6.81±3.11[ab]	23.11±4.9[b][c]	36.45±4.5[c]	51.43±4.6[c]
	Abakaliki rice	2.07 ±1.8[ab]	8.82 ±2.56[b]	18.5±1.5[b]	33.11±4.6[bc]	50.11±4.1[c]

Key: Untr.(untreated)

Table 1: Variation in physicochemical properties of rice wine fermentation over five days

Alphabets a, b, c represents equal level of significance difference with respect to control (rice not treated with mineral acid) using Tukey's multiple comparism at $P<0.01$

The result of the proximate analysis of the rice wine is shown in table 2.0. Moisture content was higher in the control than the acid hydrolysed samples, decreased with fermentation time and ranged between 77.5% – 98.6%

The protein content recorded in the study was higher in the treated samples than the control, increased with fermentation time and ranged between 0.14% – 1.49%. The protein contents recorded in this study are within the range recorded by Tharmabalan et al., (2013).

Parameters	Sample Source	Days 1	2	3	4	5
Moisture (%)	Control (Untr.)	98.6±8.6	97.1±3.3	95.8±5.1	94.1±6.3	91.5±5.6
	Illa rice	97.5±4.1	94.3±5.1	93.1±3.6	88.3±4.7	77.8±3.9
	Ekpoma rice	97.1±9.3	93.2±6.1	88.6±7.1	83.1±6.1	76.1±4.9
	Abakaliki rice	97.8±5.	95.1±3.	93.5±3.6	85.1±5.5	77.5±5.5
Protein (%)	Control (Untr.)	0.14±0.0	0.19±0.1	0.15 ±0.0	0.31±0.3	0.71±0.4
	Illa rice	0.16±0.1	0.28 ±0.1	0.51 ±0.1	0.81±0.1	1.05±1.0
	Ekpoma rice	0.16±0.1	0.32±0.2	0.56±0.3	0.85±0.5	1.11±1.1
	Abakaliki rice	0.15±0.0	0.29±0.1	1.49 ±0.1	0.85±0.7	1.12±1.1
Carbohydrate (%)	Control (Untr.)	1.6±3.5	3.1±1.6	6.5 ±1.6	13.7±3.3	21.6±5.1
	Illa rice	11.1 ±1.8	14.5 ±3.1	21.5 ±2.5	34.1±7.5	42.9±3.6
	Ekpoma rice	10.6 ±3.6	18.3 ±4.6	23.1 ±4.3	35.6±5.1	45.8±9.1
	Abakaliki rice	12.7 ±2.4	15.6 ±3.3	23.1 ±3.9	35.2±5.0	48.4±7.3
Fats (%)	Control (Untr.)	0.03±0.0	0.03 ±0.0	0.02 ±0.0	0.02 ±0.0	0.01 ±0.0
	Illa rice	0.02 ±0.0	0.02±0.0	0.01 ±0.0	0.00±0.0	0.00 ±0.0
	Ekpoma rice	0.01 ±0.0	0.01±0.0	0.01 ±0.0	0.00±0.0	0.00 ±0.0
	Abakaliki rice	0.02 ±0.0	0.02±0.0	0.01 ±0.0	0.01±0.0	0.00 ±0.0
Ash (%)	Control (Untr.)	0.7 ±0.3	0.6±0.3	0.5 ±0.3	0.3±0.3	0.4 ±0.1
	Illa rice	0.3 ±0.2	0.4 ±0.2	0.3 ±0.2	0.8 ±0.2	0.8 ±0.2
	Ekpoma rice	0.3 ±0.1	0.5 ±0.1	0.3±0.1	0.8 ±0.1	0.9±0.2
	Abakaliki rice	0.2±0.3	0.5 ±0.3	0.2±0.1	0.7 ±0.3	0.8±0.3

Key: Untr.(untreated)

Table 2: Variation in proximate assay of rice wine fermentation over five days

The carbohydrate content was higher in the acid treated samples than the control, increased as fermentation time increases and ranged between 1.6% - 48.4%

The ash content was higher in the control sample than the acid treated samples up to day 3 after which the acid treated samples had higher ash content than the control. Ash content ranged between 0.20% - 0.90%.

The fats content of the wine was higher in the control sample, decreased over time and ranged between 0.00% - 0.03%.

The increase in carbohydrate, protein content, reducing sugar and total sugar in the wine over time is because fermentation increases the nutritive constituents of foods. Fermentation enriches the rice, supplements it with different essential amino acids, vitamins, minerals, prebiotics, probiotic organisms, and degrades anti-nutrients (phytic acid, tannins, and polyphenols). Thus, its nutrition, energy contents, and therapeutic potentialities are increased (Mohan et al., 2014).

Among the three rice variety utilized in the study, Ekpoma rice is best for the production of rice wine because it scored the highest physicochemical parameters. The quality of rice wines is influenced by the type of rice fermented and its physiochemical characteristics (Vairappan and Kishneth, 2013; Kadiri *et al.*, 2014).

IV. CONCLUSION

The physiochemical properties of the rice wine produced using acid hydrolysis reported in this study compares favourably with those from rice wine produced using enzyme hydrolysis reported in other studies. Acid hydrolysis is a

simple and cheap method that can be employed in starch hydrolysis for rice wine production.

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