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PRODUCTION OF WINE FROM PINEAPPLE AND WATERMELON USING YEAST ISOLATED FROM BURUKUTU

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ABSTRACT

Pale yellow wine was produced from watermelon and pineapple mixture using yeast isolated from burukutu. Three yeast strains, (Kloekera apiculata, Candida tropicalis and Saccharomyces cerevisiae) were isolated and subjected to various biochemical and potency estimation tests. Saccharomyces cerevisiae was found to possess the essential characteristics for wine production and was employed in the production of wine at regulated temperature of 15^{o}C ± 2^{o}C and the brix level was raised to 20^{o}. The final result gave a wine with an alcoholic content of 8.3%, final pH 4.19, titratable acidity of 0.85 and brix level of 8.0^{o} sensory evaluation conducted by panel of judges showed that the wine was not well accepted.

Keywords: *Wine, Pineapple, Watermelon, Yeast Isolated, Production.*

Introduction:

Wine is an alcoholic beverage made by fermentation of fruit juice of ripe grapes using (*Saccharomyces cerevisiae*); other sugar rich fruits can also be used. It has been produced for thousands of years since ancient civilization to modern times, and is enjoyed by people from peasants to kings (Michael, 2000).

Wine has been enjoyed for over 7,000 years and through the centuries it has been the preferred drink of the Egyptians, Romans and Mesopotamians. It has played a very key role in religion and cross-cultural trade, but only in the past 150 years has science and

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technology become a part of the wine making process. Louis Pasteur's discovery of germ theory led to the modern process of wine making, growing and harvesting, crushing and pressing, fermentation, clarification, aging and bottling.

Wine is a good food with a flavor like fresh fruit which could be stored and transported under the existing conditions. Being fruit based fermented and undistilled product, wine contains most of the nutrients present in the original fruit juice. The nutritive value of wine is increased due to release of amino acid and other nutrients from yeast during fermentation (Thakor, 2008).

Tropical wines are subjectively perceived as inferior in quality on the basis of flavour, aroma, odour and colour. Many different factors influence the fermentation process and determine the end products obtained. The quality of wine produced greatly depends on the yeast strains (Kasekar, 2012). Fruit wine can be made from virtually any plant matter that can be fermented.

Most fruits and vegetables have the potential to produce wine. Few foods other than grapes have the balanced quantities of sugar, acid, tannin, nutritive salts for yeast feeding and water to naturally produce a stable, drinkable wine, so most wines are adjusted in one or more respects at fermentation. However, some of these products do require the addition of sugar or honey to make them palatable and to increase the alcoholic content sugar is converted to alcohol in the fermentation process (Solomon, 2013).

Fruit is a structural part of plant that contains seeds, normally fleshy, sweet and edible in the raw state, they include; orange, grape, straw berries, juniper berries, pineapple, and watermelon, among others (Mauseth, 2008). Most fruits are eaten as desserts and they can be processed into liquid product which includes fruit juices, wines and other preserved like marmalade, jams and jellies.

Spoilage of fruits usually occurs during storage, transportation and while waiting to be processed. It has been recognized for many years that fruits continue undergoing biochemical changes even after harvest until spoilage occurs by micro-organisms. This contributes to high post-harvest losses

(Akande, 1995). An effective method of fruits preservation should retain the original characteristics of fruit as convenient as possible. The main method of fruit preservation includes; Modified Atmosphere Storage (MAS), controlled Atmosphere storage (CAS), use of preservatives, use of irradiation, us of heat, chilling and processing, all of which extend the shelf life of fresh fruit produce (George, 1999).

Imported grape fruits for wine production are expensive and only available for the privileged, therefore, there is need to use locally available fruits which possess the characteristics of grapes. This will reduce the cost and promote our economy.

METHODOLOGY

Sample Collection

The pineapple and water melon (*Ananas sativus* and *Citrullus lanatus*) were purchased in fresh and healthy states from Wunti Market in Bauchi while the burukutu was purchased at Yelwa Market.

Isolation Procedure

Dilution of burukutu was made up to 10^{-6}. A volume 0.1ml each of the dilution pipetted into the surface of the prepared Potato Dextrose Agar contained in a sterile petri-dish. The sample was spread over the surface of the medium using a sterile bent glass rod and then incubated at 37^{o}C for 48 hours. Various colonies were picked and stained with carbol fushin (yeast indicator) then observed at x40 magnification under a light microscope, the colonies that gave positive result were sub-cultured by streaking on the prepared Potato Dextrose Agar and then pure colonies were streaked on Potato Dextrose Agar slants and stored.

Yeast Analysis

The identification of the yeast strains was based on morphological and physiological properties of the yeast.

Morphological Observation

The general morphology of the yeast and demonstration of the nucleus was carried out using Aiquinet(1999) method. Smear of the yeast strain was flooded with 40% ethanol after previous fixing by heat.
The yeast nucleus was hydrolized with potassium hydroxide solution for 1hr before staining by the addition of the methylene blue stain. Observation was done under low power magnification of x40. The criteria used in the morphological identification were based on the shape, size and the presence of mycelium or pseudo-mycelium (Jack, 2011).

Sugar Assimilation Test

The composition and method of preparation of the media was based on the manufacturer's instruction. Each set of four sugars named was involved with the isolated species and incubated at 37^{o}C for 18 to 24hrs, positive assimilation reaction was indicated by the presence of gas bubbles in durham tubes contained in the test tube(Celmedo and Conde,1998).

Flocculence

The following ability of the yeast was determined by measuring the rate of sedimentation of washed cells in a sugar solution. In the test the yeast was grown for 2days and harvested by centrifugation at 5000rpm for 10 minutes. It was washed twice with sterile distilled water, and the centrifuged yeast (wet weight) was suspended in 10ml of distilled water and 1ml of acetic buffer (pH 4.5) was shaken thoroughly in a 15ml centrifuge tube and allowed to stand for10 minutes. The amount of sediment formed after 10 minutes indicated whether or not the yeast was flocculent (Romano,2006).

Ethanol tolerance

This method was based on the visual assessment of growth in a test tube. The isolated yeast strain was inoculated into medium containing (W/V) 2% sucrose, 0.2% yeast extract and 0.7% peptone, the growth was examined after 48hours. The growth of 0.5mls of a growing culture of yeast was added into the test tubes containing concentrations (8%, 10%, 12%,

14%, 16%) of ethanol respectively. The yeast was incubated at 37°C for 24hours (Daglia,2007).

Fermentation of Sucrose at 15 °C and 28 °C

A solution of 1% sucrose, 0.5% yeast extract and 0.7% bacteriological peptone water was prepared from which 4ml each of the solution was dispensed into sterile test tube. Bromothymol blue indicator was added into the test tube until the solution in them became intense blue in colour. Durham tubes were then inserted into the test tubes and the solution sterilized at 121°C for 15 minutes.

The solution of sucrose was prepared. From such solution 2ml was pipetted into the cooled solutions respectively and mixed well using sterile glass rod. These were then inoculated with isolated yeast strains, into test tubes of the solution containing the mixture of the sucrose, yeast extract and bacteriological peptone water. Incubated at 15^{0}c for 24hrs.Testing the yeast for ability to ferment sucrose was determined.

Estimation of Yeast Potency

This was done according to the method of Zoecklein *et al*, (1990) .

Viability Test of Yeast Strain

The yeast suspension was fixed on a microscope slide and the smear was stained with methylene blue solution and covered with a cover slip. Then it was examined under the microscope, to determine the percentage of living yeast cells in the yeast mass. Viable yeast cells appeared colourless while the dead cells appeared blue.

Test for Presence of Glycogen in Yeast Strain

Suspension of yeast cell was fixed on a microscopic slide and covered with lugol's iodine and viewed under the microscope. This was done to determine the state of nourishment the yeast cells. Yeast cells without glycogen stained yellow. If less than two third ones stained brown, it indicated insufficient nourishment.

Test for the Presence of Lipid in the Yeast Strain

Suspension of yeast cells were fixed on a microscopic slide and covered with Sudan III solution, these were covered with cover slip and observed under the microscope. Yeast cells containing lipids were stained red while the one without lipid were colourless. If less than two third of the yeast cell were stained red indicated insufficient nourishment.

Inoculum Propagation

Pineapple and watermelon Must was used as medium for the production of inoculums. The Must was first obtained by first washing the pineapple and watermelon purchased with water to remove any possible contaminant. The skins were then peeled off for the pineapple and watermelon. The juice called the Must was obtained using a blender. This was then filtered and fortified with sodium metabisulphate. The pineapple and watermelon Must Brix was standardized by raising the brix value to 20^{o} Brix using inverted sugar. The standard pineapple and watermelon Must was analyzed for pH Brix level, titratable acidity and colour. It was then pasteurized at 68^{o}C for 15minutes to reduce its microbial load. It was allowed to cool and ready for inoculation with the yeast. (Uzochukwu, 2014).

Fermentation

Based on the result that was obtained in the characterization of the yeast for attributes important for wine production, *Saccharomyces cerevisiae* was used. About 8 litres of the standardized fruit Must (pineapple and watermelon) were pitched with 400ml of propagated Burukutu yeast (2% v/v of yeast). The Must was fermented at $15^{o}C \pm 2^{o}C$ for 2weeks to obtain the wine. Sample of fermenting Must was collected at 24hours that period for chemical analysis such as, Colour determination, pH determination, Titratable Acidity, Brix level determination and Percentage alcohol determination.

Colour Determination

The colour was determined using visual examination.

Determination of pH

The pH meter of a highly sensitive electrode instrument was used to determine the pH of the wine during fermentation.

Titration Acidity Determination

A volume of 20cm^3 of the wine was measured into a conical flask with 200ml of distilled water, 20ml of the diluent was titrated with 0.1N of sodium hydroxide and 10ml of it was poured into conical flask using phenolphathalein as the indicator.

$$\%\text{T.T.A} = \frac{0.073\ X\ MI\ X\ 100\ X\ V2}{VI\ (ML)}$$

Where M1 = Molarity of NaoH

V2 = Titre value

V1 = Volume of the sample (20ml)

(Akubor *et al*., 2003)

Brix Determination

Refractometer was used for Brix determination the instrument was placed on the bench facing the natural light source from the window. A drop of sample was placed on the lower prism and locked up with the upper prism. Brix value reading for the sample was made accordingly Akingbala *et al*, (1992).

Percentage Alcohol Determination

A volume of 100mls of the wine was produced with the yeast strain which was measured into a measuring cylinder then the hydrometer was put into the measuring cylinder containing wine produced. The hydrometer was allowed to balance without touching the body of the measuring cylinder then the reading was taken. It was repeated for about two weeks.

The formula for calculating percentage alcohol:

$$\%\text{ABV} = \frac{ORIGINAL\ SG - FINAL\ SG\ X\ 100}{7.36}$$

(Divate, 2014)

Determination of Wine Stability

The freeze test for wine stability was adapted from Zoecklein, *et al* (1990). The method involved freezing or chilling the wine samples at 6^{o}C for one

week after which the wine samples were then examined for the presence of crystals. If present the wine was judged as "unstable" and where absent the wine was judged to be stable.

Sensory Evaluation

The samples of the wine produced were evaluated by a taste panel of judges familiar with taste. The samples were served in wine glass. The precise instruction about the taste was given to each taster. Tasters were not allowed to discuss their reactions.

RESULTS

Yeast Strain Identified

Based on the result carried out, morphological characteristic and sugar assimilation, 3 yeast strains were identified. These yeast strains are *Saccharomyces cerevisiae*, *Candida tropicalis* and *Kloeckera apiculata*. The respective yeast strains together with their morphology and physiological characteristic and the source of isolation are presented in Table I.

Estimation of Inoculums Potency

The viability, glycogen and lipid test performed on the physiological state of the inoculums showed that for viability test, the number of dead yeast cells were less than 2% for *Saccharomyces cerevisiae*, glycogen and lipid, the number of living yeast cells were more than the dead yeast cells.
After the propagation of yeast strain, the result of the analysis on the potency of the yeast cells obtained are shown in Table II.

Characteristics of the Yeast Strain Based on Attributes for Wine Production.

Table III represents the performance of the yeast strain when they were tested with respect to certain parameters considered to be potentially useful in industrial wine fermentation. This parameter includes the floculative ability and tolerance to grade percentage of ethanol concentration *Saccharomyces cerevisiae* fermented sucrose at 15^{o}C.

Analysis of Must before Fermentation

Table IV shows that result of must analyzed before fermentation and various parameters such as pH, % Brix (soluble solids), titratable acidity and colour. Table V shows the result of the wine during fermentation.

Table I: Morphological and physiological differentiation of the identified yeast.

Source	Cell shape	Ps	My	Glu	Suc	Mal	Lac	Growth on ethanol	Yeast species
Bkt	Egg shape	+	-	+	+	+	-	+	*Saccharomyces cerevisiae*
Bkt	Lemon shape	+	-	+	+	+	-	+	*Kloeckera apiculata*
Bkt	Ovoid shape	+	-	+	+	+	-	+	*Candida tropicalis*

Key to the table

Glu = Glucose, Suc = Sucrose, My = Mycelia, Ps = Pseudomycelia , Lac = Lactose Mal = Maltose Bkt = Burukutu + = Growth produced, presence of gas bubble, change of colour

Table II: Estimation of inoculums potency

Test Performed	Colour of Cell	*Saccharomyces cerevisiae*
Viable Cell	colourless	>90
Dead Cell	blue	< 22
Cells With Glycogen	dark blue	>75
Cell without Glycogen	yellow	< 24
Cell with lipid	red	> 85
Cell without lipid	colourless	< 20

Keys: > greater than
< less than

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Table III: Characteristics of Yeast obtained based on attributes for wine production

Yeast specie	Flocculation rate	Ethanol tolerance concentration 8%	10%	12%	14%	16%
S. Cerevisiae	0.76	+	+	+	+	_

Table IV: Analysis of MUST before fermentation.

Parameter	Pineapple	Watermelon	Watermelon and pineapple
Colour	Yellow	Red	Orange
PH	4.25	5.19	4.48
Brix°	18%	7%	12%

Table V: Analysis of Wine during Fermentation

Day	% Brix (°)	PH	Temp °C	% Alcohol
1.	20	6.20	15	0.00
2.	19	5.31	15	0.8
3.	18	5.23	15	1.0
4.	18	5.28	15	1.3
5.	16	5.22	15	1.6
6.	15	5.10	15	2.0
7.	14	5.8	15	2.4
8.	14	4.75	15	2.7
9.	12	4.53	15	3.3
10.	11	4.48	15	3.8
11.	10	4.55	15	4
12.	9	4.32	15	5.4
13.	9	4.26	15	6.1
14.	8	4.19	15	8.3

Table VI:

Table of test, different analysis of two samples result.

Panellists	Taste	Colour	Clarity	Flavour
1	+	+	+	-
2	+	+	+	+

3	+	+	+	+
4	-	-	-	-
5	+	+	+	+
6	+	-	-	+
7	+	+	+	-
8	+	+	+	+
9	+	-	-	+
10	+	-	+	+
	9	6	7	6

($P<0.05$)

Key + = correct judgment

\- = wrong judgment

Table VII: Acceptability of the two samples result.

Panellists	Taste		Colour		Clarity		Flavour	
	Odd Sample	Dup. Sample	Odd Sample	Dup. Sample	Odd Sample	Dup. Sample	Odd Sample	Dup. Sample
1	√		√		√		√	
2		√	√	√	√	√	√	
3	√			√	√		√	√
4	√					√		
5	√	√	√		√		√	
6	√	√			√		√	√
7				√	√	√		
8	√		√	√		√	√	
9			√	√				√
10	√							√
Total	7	3	5	5	6	4	6	4

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DISCUSSION

For an industrial production of wine, the use of yeast with attributes potentially useful for its production is necessary. The yeast used in the production of this wine was a pure culture of *Saccharomyces cerevisiae* from burukutu. The yeast was obtained based on morphological appearance and biochemical characteristics which are consistent with the report of Okafor, (1993) who research used a local yeast strain for ethanol production.

In a related development, the yeast cells were found to be actively growing, metabolizing and well nourished. The presence of more than 75% of the viable cells' Piton (1998), reported that these reserve material contribute to wine flavour. More over they have been found to play an important role in ethanol tolerance of fermenting yeast cells. Although 3 yeast strains were isolated in this study, only one had considerable industrial application. They combine three desirable attributes namely flocculation ability, 8-16% ethanol tolerance.

Dioanin (2000) also showed that these reserve materials (lipid and glycogen) of the yeast strain determines the growth, viability and fermentation ability of the yeast. The species of *Saccharomyces cerevisiae* fulfilled the requirement for organism that can be used for ethanol production the inability of *Kloeckera apiculata, Candida tropicalis* to give one or more of the attribute may not being acceptable for the production of wine.

During the primary wine fermentation, it was observed that the percentage total sugar (%brix) decreased from 20^{o} to a final brix of 8.0^{o} with an alcohol percentage of 8.3, produced at a temperature of $15^{0}C$ and the titrate acidity (TTA) changed with fermentation. However, the role of TTA increased as the pH decreased, the comparable and agreed with the finding obtained by Benitex (2004) as their result indicated that TTA increased as the pH decreased.

The result of sensory evaluation showed that wine produced from pineapple and watermelon at $15^{0}C \pm 2^{0}C$ with the yeast *Saccharomyces cerevisiae* isolated from burukutu has a good reception but the wine was significantly different in taste, aroma, colour and general acceptability

from the imported wine. Alcohol at low level suggest lack of hotness of wine which according to Snow, (1995) was a desirable characteristic in selecting wine yeast for fermentation.

Sensory Evaluation Taste Using Tabulated Table of Triangle Test Method showed result for taste. The number of panelists that answered correctly 9 is greater than tabulated value 7 at 5% significant level. This showed that a significant difference between the odd and duplicate sample on the taste.

Taste

The number of panellists that answer correctly 9 is greater than tabulated value 7 at 5% significant level. This showed that a significant difference between the odd and duplicate sample based on the taste.

Colour

The number of panellists was 6 and tabulated was 7 at 5% confidence level. The correct answer on colour showed no significant difference between odd and duplicate sample.

Clarity

The number of panellists was 8 and tabulated was 7 at 5% confidence level. The correct answer on clarity showed that a significant difference between the odd and duplicate sample.

Flavour

The number of panellists was 6 and tabulated was 7 at 5% confidence level. The correct answer on flavour showed that no significant difference between the odd and duplicate sample.

General Conclusion

The result of the taste and clarity showed that there was significant difference between the odd and duplicate sample. The colour and flavour result showed that there was no significant difference between the odd and duplicate sample. Percentage acceptability of the panellists that preferred the odd sample than the duplicate sample.

CONCLUSION

From the result obtained, it could be seen that a good wine can be produced from composited mixture of pineapple and watermelon under proper fermentation. Therefore the production of wine from pineapple and watermelon would be a profitable outlet for utilizing these fruits since they cannot be preserved for years in their original conditions. This is also another means of using a locally available materials instead of the international grape which does not grow in Nigeria in other to prevent waste of these tropical fruits.

RECOMMENDATIONS

The commercial wine in Nigeria is not as popular as the imported wines. However if the marketing of watermelon and pineapple wine is exploited commercially, it will help to reduce the cost of importation of wine produced with grape fruit which is not cultivated in Nigeria and increase the income of local farmers that cultivate watermelon and pineapple fruits, it will also create job opportunities for many.

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