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Evaluation Of The Efficiency Of A Designed And Fabricated Microfementer For The Production Of Banana Wine

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ABSTRACT

A microfermenter (prototype) was designed and fabricated to check on the possibility of a closed system for the production of wine from banana (Musa acuminata). Banana juice (must) was innoculated with Saccharomyces cerevisiae isolated from wild fermented banana and fermentation allowed to take place for 9 days at controlled temperature of between 2-6^{o}C. Physico-chemical parameters and yeast population were monitored throughout the course of fermentation. These parameters were; Colour, Yeast behaviour, Specific Gravity, Total Soluble Solids (^{0}Brix), pH, Titratable Acidity and Alcohol content. During must fermentation, the Specific Gravity, Total Soluble Solids and pH all decreased from 1.057 to 1.013, 14 to 3 Brix and 4.90 to 4.00 respectively, while the Titratable Acidity and Alcohol content increased from 0.094 to 0.773% and 0.00 to 6% respectively. The fermentation commenced with a gravity of 14^{0}Brix with a yeast population of 6 million per ml and terminated with a gravity of 3^{0} Brix and a yeast population of 8.7 million per ml to prevent yeast autolysis. The physico-chemical, microbiological and organoleptic properties of the banana wine (BWI) produced was compared with three (3) commercial wines made from grape. The commercial wines were "Vino de Italia Wine" (VIW), "Ocean Beach California" (OBC) and "Castilo de Espana" (CDE). Significant differences ($p<0.05$) were observed in all samples of wine for physicochemical parameters, but no significant difference ($p<0.05$) between the Specific Gravity of OBC and CDE. VIW had a Total Bacteria Count of 4×101CFU/ml while BWI and CDE had the same Total Bacteria Count of 3×101CFU/ml. VIW had a Total Fungi Count of 5×101CFU/ml, while BWI had a Total Fungi Count of 1×101CFU/ml. OBC had no Bacteria and Fungi. During organoleptic evaluation, significant differences ($p<0.05$) were observed in the colour and clarity of BWI and the colour of the three (3) commercial wines. There was no significant difference between the taste of BWI and CDE and no significant difference between the flavour of VIW and BWI. The overall acceptability of VIW (8.47) was the highest, followed by BWI (8.20), CDE (6.80) and OBC (6.53). The fermentation yield of BWI was 495.5kg/tonne with fermentation efficiency of 77%. Banana wine was successfully produced using the microfermenter (closed system) under hygienic condition.

KEY WORDS: Microfermenter, fermentation efficiency, Brix, Titrable acidity, Fungal yeast count and Bacterial count and Alcohol,

1. INTRODUCTION

A microfermenter is an apparatus (tank/vessel) where fermentation is performed (Wainwright, 1996). Fermenters vary from laboratory experimental models of one or two litres capacity, to industrial models of several hundred litres capacity, which refers to the volume of the main fermenting vessel (Maulik, 2007). There are many requirements that need to be met in the designing of a fermentation facility and aspects of design to be considered include; design yield

basis, operating schedule, media sterilization, fermenter vessel design, piping systems, Cleaning in Place (CIP)/ Sterilization in Place (SIP) and Current Good Manufacturing Practices (CGMP) compliance (Maulik, 2007). The fermenter must provide the following facilities for the process such as contamination free environment (asepsis), specific temperature maintenance, pH control, monitoring Dissolved Oxygen (DO), ports for nutrient and reagent feeding, ports for inoculation and sampling, fittings and geometry for scale up, minimize liquid loss and growth facility for wide range of organisms (El-Mansi et al, 2011). Asepsis is defined as protection against entry of unwanted microorganisms while containment is defined as a vigilance regarding prevention of escape of viable cells from the fermentation process (Stanbury et al., 1995). Both these environments must be provided by a fermenter where ever required. The original, old world commercial fermenters were open-air square tanks (open fermentation vats), but in recent years, cylindrico-conical tanks (CCT) are the predominant fermentation vessels in fermentation industries for beer production. Engineered with a cylindrical top and conical bottom, these vessels are usually three to four times taller than their diameter, fabricated from stainless steel and engineered with adjustable foot supports and racking (Gribbins, 2013).

Stainless steel is non toxic, corrosion proof, easy to clean and maintain, has a long life span, does not habour germs, aesthetically appealing and does not affect flavour of fermentation products (Karimi, 2014). Cylindroconical fermenters have many advantages- better mixing due to convection currents set up by rising bubbles, ease of cleaning and hygienic recovery of yeast from the base or (cone) (Gribbins, 2013). The cone's aperture is typically around 700, an angle that will allow the yeast to flow towards the cone's apex (Gribbins, 2013).

The materials of construction must be such that they will not adversely affect, nor be adversely affected by the desired microbial activity, either by interaction with the fermentation media or by harbouring unwanted organisms (Wainwright, 1996). They must be resistant to corrosion by the nutrient medium and products, and to the effects of sterilization temperatures (Stanbury et al.,

1995). A smooth surface is required in the design of a microfermenter with no odour passing into the fermentation media (Wainwright, 1996). The fermentation medium is the raw ingredient (in this case banana), that is going to be metabolized by the fermenting microorganisms in the microfermenter and be transformed into the valuable fermentation products (wine) (Boulevard, 2008). Wines are healthy beverages that have been seen as a natural remedy for man‟s illness from early days and are said to aid recovery during convalescent period (Jay, 1996; Okafor 2007). Wine is obtained from fermentation of juices extracted from different comestible fruits (Reddy and Reddy, 2007; Samson, 1986). Any fruit with good proportion of sugar may be used in producing wine and the resultant wine is named after the fruit. The type of wine to be produced dictates the fruit and strain of yeast to be involved (Amerine and Kunkee, 2005). In contrast to most foods and beverages that spoil easily or that can spread diseases, wine does not spoil if properly stored.

This implies that the production of wine from fruit is a form of extending the shelf life of the fruit (Hassan, 2015). Banana is the common name used for the herbaceous plants of the genus Musa. It is cultivated in more than 100 countries throughout the tropics and subtropics, with an annual world production of about 98million tonnes, of which around a third is produced in each of the African, Asia Pacific, Latin American and Caribbean regions (Frison and Sharrock, 1999). Banana plants are monocotyledonous perennial and important crop in the tropical and Sub tropical world regions (Valmayor et al., 2000). It provides more than 25% of the carbohydrates and 10% of the calorie intake for approximately 70 million people in the

producing regions (Adejoro et al., 2007). Bananas are grown in nearly 130 countries; Uganda is the largest producer of banana in sub Saharan Africa (SSA), followed by Rwanda, Ghana, Nigeria, and Cameroon (IITA, 2009).

Bananas have become a key source of revenue as they are not only traded within the region where they are grown, but also exported to other countries in Europe (Ortiz and Vuylsteke, 1996). The status change from food to food/cash crop enhances its importance (Ortiz and Vuylsteke, 1996). Bananas have the potential to contribute to strengthening national food security and decreasing rural poverty (Adejoro, 2007). They are important staple foods in many developing countries, especially in Africa (IITA, 2009). It is readily available in Nigeria (Idise and Odum, 2011) and due to its high sugar content, it is suitable for the production of wine (Robinson, 2006).

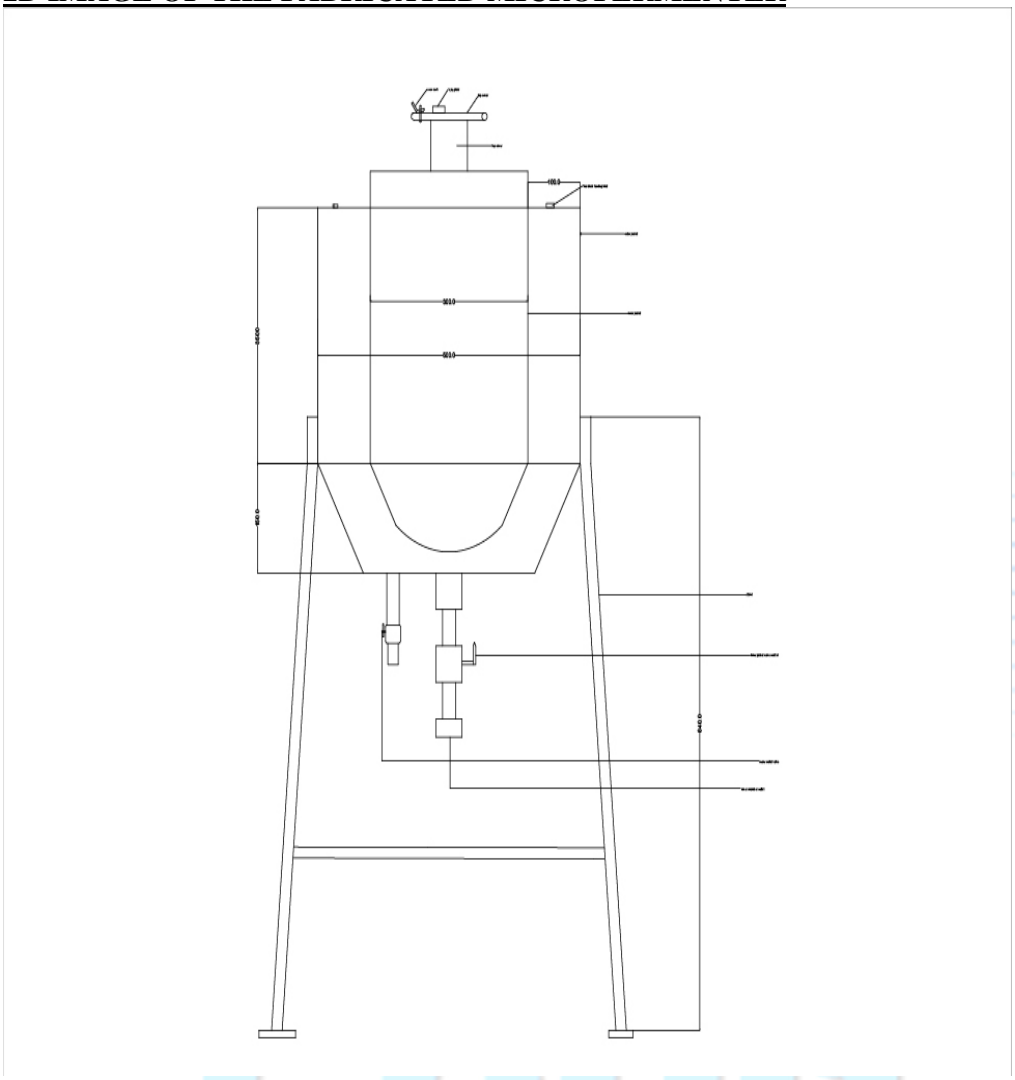
Banana wine is a fruit wine made from bananas. Commercially produced banana wine is a clear, slightly sparkling alcoholic beverage with a longer shelf-life than banana beer which spoils easily and therefore not stored for long periods (Mgenzi et al., 2010). Depending on the strain of yeast and amount of sugar added, the sweetness and alcohol level in the final product is variable (Bazirake et al., 2013). In Africa, production of banana wine is mostly at a small scale level, though, attempts have been made to bring it up to industrialized level (PTI, 2010) and since the early 2000"s, several attempts have been made to expand banana wine production to other countries where the crop is prevalent (Boromeo, 2005).

TABLE 1: EXAMPLES OF WINE EVALUATION TERMS

Sight	Clarity: clear, bright, brilliant, gleaming, sumptuous, dull, hazy, cloudy. Colour intensity: pale, subdued, faded, deep, intense. White wine: water clear, pale yellow, yellow with green tinges, straw, gold, deep yellow, brown, Maderised. Rose wine: pale pink, orange-pink, onion-skin, blue-pink, copper. Red wine: purple, garnet, ruby, tawny, brick-red, mahogany.
Smell (nose, aroma, bouquet)	Condition: clean – unclean. Intensity: weak, - pronounced. Other aroma descriptors: fruity, perfumed, full, deep, spicy, vegetal, fine, rich, pleasant, weak, nondescript, flat, corky.
Taste	Sweetness/dryness: bone dry, dry, medium dry, sweet, medium sweet, luscious. Acidity: low – high. Tannin: low – high. Body: thin, light, medium, full-bodied. Length: short – long. Other taste descriptors: fruity, bitter, spicy, hard, soft, silky, floral, vegetal, smooth, tart, spritzig/petillant (slightly sparkling).
Conclusion	Summing up: well-balanced, fine, delicate, rich, robust, vigorous, fat, Flabby, thick, velvety, harsh, weak, unbalanced, insipid, for laying down, just right, over the hill. Overall quality/value: poor – acceptable – good – outstanding.

Source: (Lillicrap and Cousins, 2006).

2D IMAGE OF THE FABRICATED MICROFERMENTER



3.0 MATERIALS AND METHODS

3.1 Typical Fabricated Microfermenter

The microfermenter was fabricated from 1.8mm stainless steel plate at Adis Engineering, No 26, Abimbola Street, Isolo, Lagos State, Nigeria. It has the following design specifications and dimensions.

3.1.1 Shape

The shape of the microfermenter is cylindroconical

3.1.2 Dimensions

It has the following dimensions and geometry;

i Top cover lid- (26×26)mm

ii Spy glass- (10.5×7)mm

iii Cover lock knob- 1mm

iv Iced block feeding inlet and Lid- (10×6.5)mm
v Hollow Thickness- 100mm
vi Diameter of Inner vessel- 300mm
vii Diameter of Outer vessel- 500mm
viiiInner vessel outlet valve- (Liquid control valve)
ix Outer vessel outlet valve- 1 inch
x Cone Height (h)- 150mm
xi Cylinder Height (H)- 350mm
xii Stand Height (Hs)- 840mm
xiii Brace Length (Lb)- 57mm

3.1.3 Microfermenter Design Parameters and Calculations

1. CROSS-SECTIONAL AREA OF INNER VESSEL:

$Ai = \pi di2 /4$ (1) Where Ai= Cross- Sectional Area of Inner Vessel (mm2)
di = Diameter of Inner Vessel (mm)
di = 300mm
$Ai= \pi(300mm)2/4$
$=3.142 X 90000mm2/4$
$=282780/4 = 70695mm2$

2. CROSS-SECTIONAL AREA OF OUTER VESSEL:

$AO = \pi DO2 /4$ (2)
Where; AO = Cross- Sectional Area of Outer Vessel (mm2)
DO = Diameter of Outer Vessel (mm)
DO = 500mm
$Ai= \pi(500mm)2/4$
$=3.142 X 250000mm2/4 =785500/4 = 196375mm2$

3. AREA OF TOP LID (AT) AT = L2 (3)
Where; AT = AREA OF TOP LID (mm2)
L= LENGTH OF TOP LID (mm)
AT = (26 X26) mm2 = 676mm2

4. AREA OF SPY GLASS (Asg)

Asg= LxB (4)
Where; Asg = AREA OF SPY GLASS (mm2)
L = LENGTH OF SPY GLASS (mm)
B = BREADTH OF SPYGLASS (mm)
L =10.5mm; B = 7mm
Asg = 10.5 x 7 = 73.5mm2

5. VOLUME OF THE CYLINDRICAL PART (VC)/ CAPACITY OF THE CYLINDER PART

$VC = \pi R2H$ (5)
Where; VC = CAPACITY OF THE CYLINDER PART (mm3)
R = RADIUS OF THE CYLINDER PART (mm)
H = HEIGHT OF THE CYLINDER (mm)
D =2R
R = D/2
$VC = \pi (D/2)2xH$
$VC = 3.142 X (300/2)2x350$

VC = 3.142x(150)2x350
VC = 24743250mm3.
Vc= 24.74 litres (recall 1mm3 = 1x10-6L)
6. CAPACITY OF THE CONICAL PART (VCO)
VCO= π R2H/3 (6)
= π (D/2)2H/3
= 3.142x (300/2)2x150/3
= 10604250/3
= 3534750mm3
= 3.53 litres (recall 1mm3 = 1x10-6L)
7. HEADSPACE VOLUME: (25% OF CYLINDRICAL VOLUME)
= 6.185litres
8. HEADSPACE HEIGHT
V = πR2H (7)
H = V/πR2
H = 6.25 x 1000000/3.142(150)2
H = 6250000/70695
H = 88.41mm
6. CAPACITY OF THE CYLINDROCONICAL VESSELS (CCV)
= 24.74 + 3.53 = 28.27 litres

3.2 Materials

3 bunches (30 fingers each) of matured ripe banana (Musa acuminata; dwarf carvendish) were purchased at the National Horticultural Resarch Institute (NIHORT), Jericho, Ibadan, Oyo State, Nigeria.

3.3 Propagation of yeast

3.3.1 Isolation of yeast from wild fermentation of banana fingers of over ripe banana were peeled, sliced and mashed. 30g of the mashed banana was poured in a clean container and 2.7litres of distilled water was added. The container was then placed in a clean environment and exposed to wild fermentation for 3 days. Yeast growth was seen on the surface of the water after 3 days and a pure culture of yeast was obtained using serial dilution.

3.3.2 Preparation of yeast pure culture

Serial dilution was carried out using the method as described by (Brown, 2001) to get a pure culture of yeast. 5 Mcartney bottles were sterilized and labeled with dilutions 1:10, 1:100, 1:1,000, 1:10,000 1:100,000. 6 plates with the dilutions 1:10 (10-1), 1:100 (10-2), 1:1,000 (10-3), 1:10,000 and 1:100,000 (10-5) were also labeled. 9ml of distilled water was pipetted into each Mcartney bottle. 1ml of the yeast broth from the wild fermentation of banana was pipetted into the first dilution blank bottle and the mixture stirred well. 1ml of the first dilution blank was also transferred to the second dilution blank (1:100). The same procedure was repeated until the original broth had been diluted to 1:100,000. 0.1ml of sample in each bottle was then transferred into each corresponding plates. 3.9g of Potato Dextrose Agar was diluted with 100ml of distilled water inside a conical flask, the flask was then plugged with cotton wool and wrapped with foil paper and placed inside an autoclave (EquitronR) at 1210C for 15minutes. After the mixture had cooled to 450C, it was poured into each of the plates. The plates were then incubated at 270C for 72hours.

3.3.3 Preparation of yeast subculture

A yeast subculture was prepared to get isolated colonies that grew from a single yeast cell. 3.9g of Potato Dextrose Agar was diluted with 100ml of distilled water and the mixture was placed

inside the autoclave at 1210C for 15 minutes. The agar was then poured into 5 plates and allowed to solidify. An inoculating loop was sterilized by placing it at an angle over a flame. A rounded colony of yeast was picked from the cultured plates using the loop and streaked in a zig-zag horizontal pattern on a solid agar plate. This was done on the other 4 plates and the plates were incubated at 270C for 72hours.

3.3.4 Identification of yeast isolate

This was carried out as described by (Fawole and Osho, 2007). A thin smear was prepared by taking a speck of the isolate from the subcultured plate and placing it on a sterilized slide. A drop of lactophenol blue was added and a cover slip placed on the slide and observed under the microscope at x 40 objective.

3.4 Preparation of Fermentation Medium

The method of (Uraih, 2003) was employed with some modifications. The ripe bunches of banana were cleaned to remove dirts, peeled and sliced aseptically. 6.4kg of the sliced banana was added to 11litres of distilled water and the mixture boiled for 45minutes. The purpose of boiling was to gelatinize the starch and also to sterilize the medium. After boiling, the mixture was filtered and 600g of sugar added to make it up to a Brix value of 140Brix.

3.5 Preparation of fermentation starter culture medium

A loop full of yeast colonies from the sub cultured plate was inoculated into a 10ml banana must inside a test tube. The test tube was then covered with a foil paper and a hole made to allow for aeration and escape of carbon dioxide and was placed at room temperature. A krausen (foaming activity) was seen after 4 days. The culture was then transferred into a 100ml starter and then to a 1litre starter.

3.6 Sterilization of the microfermenter

The microfermenter was sterilized using the method as described by (Wainwright, 1999). A 3% caustic soda solution and 3% hydrochloric acid solution were prepared. The fermenter was first sterilized with caustic soda in hot water, rinsed with the hydrochloric acid and then rinsed with a lot of distilled water. A litmus paper test was used to check if it was still acidic or not.

3.7 Production of Banana wine

The method of (Uraih, 2003) was adapted and modified. Starter culture (1litre) was transferred into 10litres must and the mixture stirred vigorously. The mixture was then
pitched into the microfermenter. Ice block was introduced into the water jacket and the temperature maintained at 2-6^{0}C. Fermentation was then allowed to take place for 9 days. At every 24 hours, sample was
aseptically drawn from the microfermenter through the valve and fermentation parameters determined. After 9 days, the liquor (banana wine) was collected through the valve, filtered and clarified.

3.7.1 Clarification of the produced banana wine

The wine was clarified using kieselghur (diatomaceous earth) using the method employed by (Wainwright, 1999).5g of kieselghur was added to 1 litre of banana wine and the mixture stirred very well to get a homogenous mixture. The mixture was then filtered by passing it through 4 layers of filter paper (185mm Whattman filter paper) using a vacuum pump (Jinteng Diaphragm vacuum pump).

3.7.2 Bottling and Pasteurization

The banana wine was bottled (using sterilized glass bottles) and pasteurized using a water bath (heated to 750C and held for 50secs). After pasteurization, it was cooled and corked and aged.

3.7.3 Accelerated ageing

Accelerated ageing was carried out on the wine by placing it inside the refrigerator at 60C and then placed where there is ray of light for 24hours simultaneously. This was done for a period of one month.

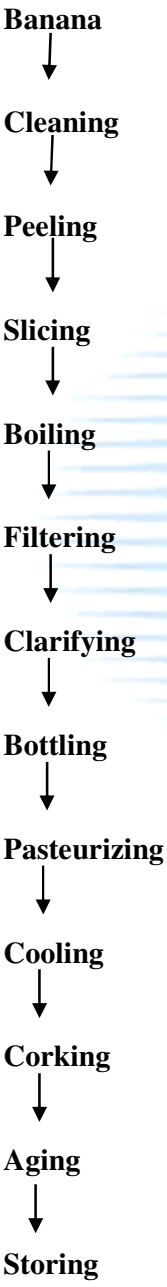


Figure 1: Flow diagram for the production of banana wine (closed fermentation process). (Adapted from Uraih, 2003).

3.7 METHOD OF ANALYSES

3.8 Physico-chemical parameters

3.8.1 Specific gravity

The specific gravity before the start of fermentation (original gravity), the specific gravity during fermentation and the specific gravity at the end of fermentation (final gravity) were determined using the specific gravity bottle at 20oC.

3.8.2 Yeast behavior determination

The nutrient available to the yeast was used to determine the yeast behaviour and fermenting ability during the fermentation process.

3.8.3 Colour at original and final gravity

The colour at original gravity, during fermentation and colour at final gravity was viewed with the naked eyes.

3.8.4 Brix value determination

The Brix value (0Brix) at original gravity, during fermentation and at final gravity was measured using Palm Abbe TM Refractometer.

3.8.5 pH determination

The pH at the original gravity, during fermentation and at final gravity was measured using the pH 211 Microprocessor pH meter

3.8.6 Titratable Acidity (TA) Determination

The Titratable Acidity on each day of fermentation was determined with reference to the method of (AOAC, 2003). The wine sample (10mls) was poured into a dried conical flask, 3 drops of phenolphthalein indicator was then added and titrated against 0.1N Sodium hydroxide (NaOH) until a faint pink colour was obtained. The percentage Titratable Acidity of the wine was then calculated using the formula:

$$\% \text{ TA} = \frac{\text{No of mls of NaOH x Normality of NaOHx milli equivalent factor (0.067)} \times 100}{\text{No of mls of sample}}$$

3.8.7 Crude Protein Determination

The Crude protein at original gravity (before the start of fermentation) was determined using the Kjeldahl method of protein analysis as described by (AOAC, 1990). The sample was first digested using the Foss Tecator TM Digestor, distilled using the Kjectec 2200 Distillation Apparatus and titrated using the Automated Titre equipment. The percentage Nitrogen was then calculated using the formula:

$$\%\text{Nitrogen} = \frac{(T-B) \times 14.007 \times N \times 100}{\text{Weight of sample in mg}}$$

Where:

T is the titre value of the sample

B is the titre value of the blank (catalyst + acid) = 0.03

N is the Normality of acid used in titration (0.1N)

The percentage Crude Protein was then calculated as:

%CP= % Nitrogen × conversion factor (6.25)

3.8.8 Alcohol content

The alcohol content was calculated using the formula as described by (Williams, 2013).

$$\% \text{ Alcohol by Volume (ABV)} = \frac{\text{Original Specific Gravity – Final Specific Gravity} \times 100}{0.736}$$

3.9 Microbiological analysis

3.9.1 Yeast count

Serial dilutions were prepared using the method of (Brown, 2001). Potato Dextrose Agar was

inoculated with 0.1ml of the diluted banana wine. The agar plates were incubated at 270C for 72hours. Colonies were counted and multiplied by the dilution factor.
Yeast load count (cfu/ ml) = N x D
Where:
N = Number of colonies counted
D= Dilution factor
3.9.2 Total bacteria count
Pour plate technique as described by (Koch, 2005) was used. 1ml of sample was aseptically transferred to 9ml sterile water in a tube and mixed vigourously. 1ml of the resulting mixture was transferred up to the 5thdiluent (10-5). Nutrient Agar (NA) was then inoculated with 0.1ml of the diluted banana wine and incubated at 370C for 24hours. Colonies were counted and multiplied by the dilution factor.
Bacteria load count (cfu/ ml) = N x D
Where:
N = Number of colonies counted
D= Dilution factor
3.9.3 Total fungal count
The fungal load was determined in a similar way as the bacterial count. However, Potato Dextrose Agar was used. The plates were incubated at 270C for 72hours.
Fungi load count (cfu/ ml) = N x D
Where:
N = Number of colonies counted
D= Dilution factor
3.10 Organoleptic evaluation
Organoleptic evaluation was carried out using a nine point Hedonic Scale as outlined by (Ihekoronye and Ngoddy, 1985). The banana wine was compared with three (3) commercial wines (Vino d‟ Italia Italienischer Wein, Ocean Beach California and Castillo de Espana) using a 15 member trained panelists who are regular consumers of wine. The samples were rated in terms of colour, clarity, taste, flavour and overall acceptability on a 9-point scale ranging from 9 like extremely to 1-dislike extremely.
3.11 Statistical analysis
Data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science V.17.0.
TABLE 3: PHYSICOCHEMICAL PARAMETERS OF THE SAMPLES OF WINE

SAMPLE	TOTAL SOLUBLE SOLIDS (^{0}BRIX)	SPECIFIC GRAVITY	pH	TITRATABLE ACIDITY (%)	ALCOHOL CONTENT
VIW	9.81±0.15	1.04±0.00			

4.0 RESULTS AND DISCUSSION
4.1 Isolation and Identification of yeast
The microscopic appearance of the yeast using lactophenol blue showed the colour of the yeast isolated from the wild fermentation of banana as cream, the shape as circular and the size as moderate. This result concluded that the yeast isolated was the top fermenting *Saccharomyces*

cerevisiae which was characterized by a foaming activity at the top of the fermentation medium (banana juice) during fermentation.

4.2 Crude protein at Original Gravity

The crude protein content of the banana juice (must) at Original Gravity was 3.13%. The crude protein was a necessary nutrient in the must which was needed for yeast multiplication.

4.3 Yeast Behaviour

The type of yeast used and the nutrient available to the yeast determined the yeast behaviour and fermenting ability during the fermentation process. The fruit must provided the nutrients necessary in the completion of fermentation by *Saccharomyces cerevisiae*.

4.4 During fermentation of banana juice (must)

The value of the various parameters; yeast count, Specific Gravity, Total Soluble Solids, pH, Titratable Acidity and Alcohol content were monitored throughout the fermentation of banana juice (must).Three (3) replicate samples were produced and the values of the parameters expressed as average of triplicate samples as shown in Table 2. The yeast count was 6×106CFU/ml at Original Gravity and it increased exponentially till the 4th day to 9×106CFU/ml. The increase in yeast count was attributed to the presence of utilizable sugar and nutrients in the must. Yeast count was 9.5×106CFU/ml on the 5th day, it gradually decreased thereafter and at Final Gravity, yeast count was 8.7×106CFU/ml. The Total soluble Solids decreased from 140Brix to 3.00Brix.

The Specific Gravity decreased from 1.057 to 1.013. The reduction in Specific Gravity was due to the microbial utilization of nutrients (primary sugars) for microbial activities by the fermenting yeast. This agrees with the reports of (Uraih, 2003) and (Okafor, 2007). Reports have also shown that the major problem associated with the use of tropical fruits for the production of wine are their low sugar content (Alobo and Offonry, 2009). In other to supplement the sugar of the must, 600gms of sugar was added. The continuous decrease of Specific Gravity was relative to the Total Soluble Solids decomposition by *Saccharomyces cerevisiae.*

The pH decreased from 4.9 to 4.0. The determination of pH during fermentation was important because the metabolism of yeast is pH dependant (Uva, 2013).The Titratable Acidity increased from 0.094% to 0.773%.

The Alcohol content increased from 0.00 to 6%.The amount of alcohol in the wine was directly proportional to the amount of sugar present in the must. The yeast utilized acetaldehyde as hydrogen acceptor which served as the terminal electron. Pyruvate was first decarboxylated by decarboxylase to yield acetaldehyde; acetaldehyde was then reduced to ethanol by alcohol dehydrogenase which was the enzyme present in Saccharomyces cerevisiae thus generating NAD+.

The decrease in Specific Gravity, Total Soluble Solids, pH and increase in the Titratable Acidity

and Alcohol content were in agreement with the reports of other researchers (Akinwale, 1999; Aroyeun et al., 2005; Ogunjobi and Ogunwolu, 2010; Hassan, 2015).

4.5.1 Physicochemical properties of the samples of wine Table 3 shows the mean±standard deviation of the physicochemical parameters of the samples of wine. There were significant differences ($p<0.05$) in the physicochemical parameters of all the samples. This was because all the samples were produced by different manufacturers. There was however no significant difference ($p\leq0.05$) between the Specific Gravity of “Ocean Beach California” and “Castillo de Espana”.“Vino d Italia Wein” had the highest Total Soluble Solids of 9.810Brix while Banana wine had the lowest Total Soluble Solids of 3.010Brix. “Vino d Italia

Wein" had the highest specific gravity of 1.04, "Ocean Beach California" and "Castillo de Espana" had the same Specific Gravity of 1.03 while Banana wine had the lowest Specific Gravity of 1.02. Banana wine had the highest pH of 4.01 while "Vino d Italia Wein" had the lowest pH of 3.20. This indicated that "Vino d Italia Wein" was the most acidic out of all the four (4) samples of wine while Banana wine was the least acidic.The percentage Titratable Acidity of "Vino d Italia Wein", "Ocean Beach California", Banana wine and Castillo de Espana were 1.09%, 0.99%, 0.77% and 1.06% respectively. The colour of "Vino d Italia Wein", Ocean Beach Califonia, Banana wine and Castillo de Espana were light yellow, light yellow, yellow and light yellow respectively. Ocean Beach California had the highest Alcohol content of 11.51%, followed by Castillo de Espana with Alcohol content of 10.51%, then "Vino d Italia Wein" with an Alcohol content of 9.50%. BWI had the lowest Alcohol content of 6.01%. Banana wine had the lowest Specific Gravity, Total Soluble Solids, Titratable Acidity and Alcohol content and it had the highest pH. The low Alcohol content of BWI was as a result of the Total Soluble Solids at Original Gravity which was 140Brix. If a higher Total Soluble Solids had been started with, a
higher Alcohol content would have resulted.

4.5.2 Microbiological properties of the samples of wine.

The Total bacteria count and Total Fungi count of the samples of wine are represented in Table 4. "Vino d Italia Wein" had the highest Total Bacteria Count of 4×101CFU/ml while Banana wine and Castillo de Espana had the same Total Bacteria Count of 3×101CFU/ml. "Vino d Italia Wein" had the highest Total Fungi Count of 5×101CFU/ml, followed by Castillo de Espana with Total Fungi Count of 2×101CFU/ml and then Banana wine with a Total Fungi Count of 1×101CFU/ml
.Ocean Beach California had no bacteria and fungi count. The bacteria and fungi count of the samples of wine were within the general range of acceptance for countable numbers of colonies on a plate which is 30-300.

4.5.3 Organoleptic properties of the samples of wine

Table 6 shows the mean±standard deviation of the organoleptic properties of the samples of wine. It was observed that there were significant differences ($p<0.05$) as well as no ignificant differences ($p\leq0.05$) among some samples of wine. For colour, the colour of "Vino d Italia Wein" and Ocean Beach California were not significantly different from each other; the colour of Ocean Beach California and Castillo de Espana were not significantly different from each other; the colour of Vino d Italia Wein and Castillo de Espana were significantly different from each other, while the colour of Banana wine was significantly different from the colour of all the other samples. The colour of Banana wine was mostly accepted by the panelists while the colour of Castillo de Espana was the least accepted. For clarity, the clarity of all the samples was significantly different from each other; Vino d Italia Wein, Ocean Beach California, Banana wine and Castillo de Espana had values of 8.07, 5.47, 8.87 and 6.8 respectively. The clarity of Banana wine was mostly accepted by the panelists while the clarity of Ocean Beach California was l 9×106CFU/ml. The increase in yeast count was attributed to the presence of utilizable sugar and nutrients in the must. Yeast count was 9.5×106CFU/ml on the 5th day, it gradually decreased thereafter and at Final Gravity, yeast count was 8.7×106CFU/ml. The Total soluble Solids decreased from 140Brix to 3.00Brix.

The Specific Gravity decreased from 1.057 to 1.013. The reduction in Specific Gravity was due to the microbial utilization of nutrients (primary sugars) for microbial activities by the fermenting yeast. This agrees with the reports of (Uraih, 2003) and (Okafor, 2007). Reports have also shown that the major problem associated with the use of tropical fruits for the

production of wine are their low sugar content (Alobo and Offonry, 2009). In other to supplement the sugar of the must, 600gms of sugar was added. The continuous decrease of Specific Gravity was relative to the Total Soluble Solids decomposition by *Saccharomyces cerevisiae.*

The pH decreased from 4.9 to 4.0. The determination of pH during fermentation was important because the metabolism of yeast is pH dependant (Uva, 2013).The Titratable Acidity increased from 0.094% to 0.773%.

The Alcohol content increased from 0.00 to 6%. The amount of alcohol in the wine was directly proportional to the amount of sugar present in the must. The yeast utilized acetaldehyde as hydrogen acceptor which served as the terminal electron. Pyruvate was first decarboxylated by decarboxylase to yield acetaldehyde; acetaldehyde was then reduced to ethanol by alcohol dehydrogenase which was the enzyme present in Saccharomyces cerevisiae thus generating NAD+.

The decrease in Specific Gravity, Total Soluble Solids, pH and increase in the Titratable Acidity and Alcohol content were in agreement with the reports of other researchers (Akinwale, 1999; Aroyeun et al., 2005; Ogunjobi and Ogunwolu, 2010; Hassan, 2015).

During the fermentation process, it was observed visually that the colour of the banana juice (must) at Original Gravity was cream and the colour changed to light brown at Final Gravity. After clarification, the colour of the wine changed from light brown to yellow. During accelerated ageing, the colour, odour and flavour were enhanced with sediments settling at the bottom of the wine bottle.

TABLE 2: Changes in fermentation parameters of banana juice (must)

Fermentation time in days	Yeast population (CPU/ml)	Total Soluble Solids (^{0}Brix)	Specific Gravity	Colour	pH	Titrable Acidity (%)	Alcohol Content (%)
1	6x106	14	1.057	Cream	4.9	0.094	0
2	7x106	11.67	1.045	Dirty Cream	4.73	0.179	1.57
3	8x106	9.53	1.040	Light brown	4.62	0.337	2.31
4	9x106	8.10	1.032	Light brown	4.54	0.368	3.41
5	9.5x1066.43	6.43	1.025	Light brown	4.43	0.447	4.30
6	9.4x1064.23	4.23	1.018	Light brown	4.30	0.562	5.28
7	9.2x1063.73	3.73	1.016	Light brown	4.21	0.622	5.64
8	9.1x1063.43	3.43	1.014	Light brown	4.10	0.699	5.87
9	8.7x106	3.0	1.013	Light	4.00	0.773	6.00

4.6 FERMENTATION YIELD

Fermentation yield was measured in litres of absolute alcohol in wine per ton of sugar in molasses and was given by

$Yf = Vb.ab$ (8)

Where; Yf = fermentation yield

Vb = volume of wine (litre)
ab = alcohol content of wine
Mm = mass of molasses (tonne)
fSm = fermentable sugar content of molasses
Vb = 7 litres
a_w= 6.01%
M_w = 600g = 0.0006 tonnes
fS_m = 141.43g

$$Yf = \frac{7x6.01}{0.00006x141.43}$$

= 495.524
= 495.5/tonne
Fermentation yield was equal to 495.5kg/tonne

4.7 FERMENTATION EFFICIENCY

Fermentation efficiency was an expression of how much alcohol was actually produced in the wine relative to the amount that could be theoretically produced and was given by

$$E_f = Y_f \frac{0.794}{\frac{0.5111x(100)}{1000}}$$

E_f is fermentation efficiency
Y_f is fermentation yield

$$E_f = \frac{3993.427}{0.5111x0.1}$$

= 769.76521x.01
= 76.98%
=77%
The fermentation efficiency was equal to 77%

5.0 CONCLUSION

5.1 CONCLUSION

Banana wine was successfully produced using the microfermenter (prototype) and it compared favourably with three (3) other commercial wines made from grape.
The efficiency of the fermentation (77%) was in line with what is recorded in literature as above 70%, therefore, there is possibility of adopting a closed system for the production of wine. Apart from hygiene condition, a microfermenter assists in monitoring yeast population and other parameters as well as control on fermentation temperature compared to fermentation in open vats.

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