The International Journal of Biotechnology
2019 Vol. 8, No. 1, pp. 48-58
ISSN(e): 2306-6148
ISSN(p): 2306-9864
DOI: 10.18488/journal.57.2019.81.48.58
© 2019 Conscientia Beam. All Rights Reserved.

MICROBIOLOGICAL AND PHYSICOCHEMICAL CHANGES IN PALM WINE SUBJECTED TO SPONTANEOUS FERMENTATION DURING STORAGE

Folake Titilayo Afolabi[1+]
Akeem Temitope Owoola[2]

[1,2]*Department of Microbiology, University of Ibadan, Oyo State, Nigeria.*
[1]*Email: folakeojo1@yahoo.com Tel: +2348034218552*
[2]*Email: ak.owoola@gmail.com Tel: +2348033611032*

(+ Corresponding author)

Article History
Received: 2 April 2019
Revised: 6 May 2019
Accepted: 10 June 2019
Published: 18 July 2019

Keywords
Palm sap
Palm wine
Spontaneous fermentation
Yeast
Saccharomyces cerevisiae
Storage time.

ABSTRACT

This work aimed to isolate and identify yeasts obtained from palm wine subjected to spontaneous fermentation during storage. A total of forty (40) yeast isolates were from spontaneously fermented palm wine. The yeast counts obtained ranged from 5.46 x 104 cfu/ml at day one to 3.00 x 102 cfu/ml at day twenty-two. Saccharomyces cerevisiae was isolated at all stages of fermentation. The pH dropped from 3.70 at 24 hours to 3.37 at 360 hours. Total titratable acidity of the wine increased from 2.28% at 24 hours to 4.50% at 528 hours. Total sugar of the stored palm wine from decreased from 4.0211g/10 ml at 24 hours to 0.6417g/10 ml at 528 hours was observed. The reducing sugar content of the stored palm wine decreased from 13% at 24 hours to 0.960% at 456 hours was also observed. Ethanol content of the stored palm wine increased steadily from 21.06 mg/ml at 24 hours to 88.99mg/ml at 456 hours as the storage time increases. Palm wine whose storage time is not beyond 120 hours is not injurious to the health of consumers.

Contribution/Originality: This study contributes to the existing literature by providing basic information for other researchers regarding the isolation of yeasts that are accountable for the spontaneous fermentation of palm wine during storage and also to determine its physicochemical characteristics.

1. INTRODUCTION

Palm sap is a sugary exudate obtained from palm plants like a silver date palm, Palmyra and coconut palms (Okafor, 1978; Chandrasekhar *et al.*, 2012). Worldwide, alcoholic drinks are prepared from the sap of palm trees. Example of such palm tree is the African oil palm and the botanical name is called *Elaeis guineensis* (Okafor, 1978). Freshly tapped palm wine is a sugary, pure, neutral, juice that contains about 10-18% sucrose, minimal sugar (less than 0.5%), and small amounts of proteins, gums and mineral (Okafor, 1987; Amoa-Awua *et al.*, 2007; Naknean *et al.*, 2010; Santiago-Urbina *et al.*, 2013). This makes it a rich substrate for the growth of different microorganisms. These microorganisms are responsible for the fermentation of palm sap into palm wine and prolonged fermentation changes the palm wine into vinegar (Okafor, 1987; Chandrasekhar *et al.*, 2012).

Palm wine is an alcoholic beverage which is obtained from the spontaneous fermentation of the sap of various palm plants. Fermentation starts immediately the sap leaves the palm tree because of the presence of natural yeasts in the air and fermenting microorganism in the gourd or container used for tapping. This fermentation has been attributed to the work of yeasts and bacteria (Okafor, 1978; Chandrasekhar *et al.*, 2012). The palm sap usually undergoes spontaneous fermentation for the multiplication of yeasts and bacteria to occur and for sweet substrate

© 2019 Conscientia Beam. All Rights Reserved.

conversion into several metabolites for example ethanol, lactic acid and acetic acid (Amoa-Awua *et al*., 2007; Stringini *et al*., 2009; Ouoba *et al*., 2012; Santiago-Urbina *et al*., 2013). *Saccharomyces cerevisiae* is the predominant yeast while *Lactobacillus plantarum* is the predominant Lactic acid bacteria (LAB) obtained from palm sap of *Elaeis guineensis* (Okafor, 1987). The palm wine changes quickly to alcohol. It is therefore very important to pasteurize the sap to prevent fermentation if the products to be produced are juice drink, sugar and syrup (Chandrasekhar *et al*., 2012).

Physicochemical parameters of palm sap means its physical and chemical characteristics. These characteristics are known to occur rapidly during tapping and storage because of the unstable nature of palm sap as a result of the fermenting microorganisms. However, the characteristics of fresh unfermented palm sap are quite different from that of the fermented sap (palm wine) (Amoa-Awua *et al*., 2007). One of such characteristics is its pH which is almost neutral, approximately 7 to 7.4 for fresh unfermented palm sap but reduces with time as fermentation commences. This could be used to determine the freshness of palm sap (Ezeagu *et al*., 2003; Amoa-Awua *et al*., 2007; Karamoko *et al*., 2012).

The importance of palm wine especially in African societies cuts across the cultural and medical divide (Olasupo and Obayori, 2003; Chandrasekhar *et al*., 2012). Palm sap and palm wine are consumed as excellent food supplements because they contain several nutrients and minerals that are beneficial to the body (Chandrasekhar *et al*., 2012). Palm is also a source of inoculum for other fermentations (Chandrasekhar *et al*., 2012). This work therefore aimed at isolating the yeasts that are accountable for spontaneous fermentation of palm wine during storage and also to determine its physicochemical characteristics.

2. MATERIALS AND METHODS

2.1. Collection of Samples

Freshly tapped palm wine from different locations were obtained from tapping gourd using sterile, air-tight container and taken into the laboratory for analyses.

2.2. Fermentation of Palm Wine

This was done using 500 ml sterilized Erlenmeyer flask containing 250 ml fresh palm sap. The flask was maintained at room temperature and the fermentation was done spontaneously. The palm sap was stored for 22 days and samples were taken every 48 hours for analysis.

2.3. Yeast Enumeration

0.1ml sample was aseptically transferred from diluted samples on a solidified Yeast extract agar (YEA) which contain 0.1g chloramphenicol. Palm wine was dropped on the plates containing YEA with using sterile bent glass rod. Incubation was done at 28°C for 48 hours (Harrigan and McCance, 1976).

2.4. Identification of Yeasts

2.4.1. Microscopy

The microscopic examination test was carried out by inoculating yeast cultures into 10ml sterile liquid culture medium which is made up of (20g glucose, 5g yeast extract, 10g peptone) dissolved in 1liter of distilled water. The cultures were viewed under the microscope (Barnett *et al*., 1990) after incubation at 28°C for 72 hrs.

2.4.2. Sporulation Test

A smear was made using the method of (Barnett *et al*., 1990) and stained using 5% malachite green and counterstained using safranin. The stained smear was then viewed with a microscope. The sporulation test was done using the method of Barnett *et al*. (1990) using Yeast Extract Agar. The media was sterilized at 121°C for 15

© 2019 Conscientia Beam. All Rights Reserved.

minutes and was then slanted. The yeast was inoculated on the slant and then incubated at 28°C for 4weeks and was observed under the microscope.

2.4.3. Growth on Yeast Malt Agar at 37°C and 40°C

Yeast Malt Agar was dissolved in 1000ml distilled water was inoculated with fresh yeast culture and incubated at 37°C and 40°C (Harrigan and McCance, 1976).

2.4.4. Growth on Media Containing High Glucose Concentration

Fifty- six (56) grams of glucose was dissolved in 1% yeast extract solution, 3% agar was also added and was dispensed into test tubes. The medium was sterilized for 15minutes at

121°C.The yeast was inoculated into it and was then incubated at 25°C for 4 weeks .It was observed for growth (Barnett *et al*., 1990).

2.4.5. Glucose fermentation

Test tubes were filled with 15 mls of yeast extract broth containing 2% glucose with inverted Durham tubes and then sterilized at 121°C for 15minutes. The broth in the test tubes were inoculated with yeast and was incubated at 25°C for 3 weeks. It was observed for gas bubbles (Barnett *et al*., 1990).

2.4.6. Assimilation of Carbon Compounds

Glucose, Sucrose, Galactose, Lactose, Raffinose, Melibiose

Test tubes were filled with 10ml sterile medium made up of 0.5% peptone water with- 4% test sugar. The tubes were then inoculated with the yeast cultures in duplicate. A Vaseline- paraffin (Vaspar) layer, 2cm deep, was added to the top surface of the medium in the tube. It was incubated at 25°C for 5days. Fermentation was observed by the lifting of the Vaseline-paraffin layer (Kiss, 1984).

2.4.7. Urease Test

Christensen's Urea agar (2.4g) was suspended in 95ml of distilled water and boiled to dissolve completely. It was sterilized at 121°C for 15minutes and cooled to 50°C. 5ml of sterile 40% Urea solution was then aseptically introduced and distributed in 10ml amounts into sterile test tubes and allowed to set in the slanting position. The medium was inoculated and incubated at 37°C. The tubes were examined every half hour for a change of colour from orange to pink which is an indication of urease activity (Christensen, 1946).

2.4.8. Cycloheximide Resistance

Fifteen (15) ml of yeast extract broth containing 2% glucose with the addition of 0.1% cycloheximide was prepared. The medium was sterilized at 121°C for 15 minutes. The test tubes were inoculated with a fresh yeast suspension and was incubated at 25°C for 3 weeks. It was then observed for growth (Barnett *et al*., 1990).

2.5. Physicochemical Parameters

2.5.1. Determination of Moisture Content

A crucible was washed and dried in the oven at 100°C and later cooled in a desiccator. The crucible was weighed. 10 ml of the palm wine samples was added to it. The weight of the crucible with palm wine samples was also recorded and then transferred to hot air oven at 70-80°C for 2 hours and 105°C for 4 hours until a constant weight is achieved. The crucible was cooled in a desiccator and the weight of crucible with the dry wine sample was recorded. The moisture content was measured using the formula below:

© 2019 Conscientia Beam. All Rights Reserved.

$$\%\ moisture = \frac{\text{weight of moisture}}{\text{weight of sample}} \text{X } 100 \text{ (AOAC, 1990).}$$

2.5.2. Determination of Ash Content

Ten mls of samples was added into a clean, dry crucible and weighed. The palm wine sample was then charred on a Bunsen flame in a fume cupboard. The crucible was then transferred into a preheated muffle furnace at 600°C and heated for 2 hours. The crucible was allowed to cool in a desiccator and the weight of the ash was obtained by the formula below:

$$\%\ Ash = \frac{\text{weight of Ash(gm)}}{\text{weight of sample(gm)}} \text{X } 100 \text{ (AOAC, 1990).}$$

2.5.3. Determination of Crude Protein Content

Two mls of palm wine was weighed into a Kjeldahl flask. 5 g of anhydrous sodium sulphate was added to the wine sample. One (1) g copper sulphate and a speck of selenium as catalysts, 25 cc concentrated sulphuric acid and 5 glass beads were then added to the mixture. The mixture was then heated in a fume cupboard, very gently at first and then with increased heat with occasional shaking till the solution turned to a green colour. The mixture was then cooled. The mixture was reheated gently at first and then with increased heat until the green colour disappeared and then allowed to cool. The digest was transferred into a 250ml volumetric flask and was filled up with distilled water. Distillation was then performed using Markham distillation apparatus.

The crude protein con was calculated by the formula below:

$$\%\ Nitrogen = \frac{Vs - Vb \times Nacid \times\ 0.01401\ \times\ 100\ \times X}{\text{W}\ \times\ \text{Y}}$$

X= Total volume of digested wine sample

Vs = Volume of acid required to titrate sample (ml

Vb= Volume of acid required to titrate blank (ml)

N acid= Normality of acid 0.01N

W= Weight of samples (g)

Y= Volume pipetted during distillation

For crude protein calculation, % Nitrogen is multiplied by a general factor whose value is 6.25 (AOAC, 1990).

2.5.4. Determination of Crude Fibre Content

One ml of the palm wine sample was transferred into a 500 ml conical flask. 100 ml of digestion reagent was added. The mixture was boiled and refluxed for 40 minutes. The conical flask was removed from the heater and cooled under cold tap. The mixture was filtered through 15cm of No 4 Whatman paper. The filter paper was then washed 6 times with hot water and once with methylated spirit. The filter paper was opened and the fibre residue was removed and transferred to a dish containing silica. The fibre residue was then dried overnight at 105°C, transferred to a desiccator and weighed. It was then ashed at 600°C overnight in a muffle furnace, cooled and weighed.

$$\%\ Fibre = \frac{\text{difference in weighing}}{\text{weight of sample}} \text{x } 100 \text{ (AOAC, 1990).}$$

2.5.5. Determination of Fat content

Cleaned 250 cm^3 flasks were dried in the oven at 105°C for 30 minutes and was put in a desiccator and was allowed to cool. Two mls of the palm wine sample was transferred into labeled extraction thimbles and then plugged lightly with cotton wool. The boiling flasks were weighed and were filled with about 300 cm^3 of petroleum ether. Soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimbles were then removed.

© 2019 Conscientia Beam. All Rights Reserved.

After the flasks were free of petroleum ether, they were then removed and dried at 105°C for 1 hour. The flasks were put into a desiccator to cool and were then weighed.

$$\% Fat = \frac{\text{weight of fat}}{\text{weight of sample}} \text{X } 100$$ (AOAC, 1990).

2.6. Determination of Total sugar

Anthrone reagent was prepared by dissolving 200 mg anthrone in 100mL of ice-cold 95% sulphuric acid. Standard glucose stock was prepared by dissolving 100mg glucose in 100mL distilled water. The working Standard was 10ml of the stock diluted to 100mL with water. Standards were prepared by pipetting 0.2 mL-1.0mL of the working standard into different test tubes and made up to 1.0 mL with water. 4mL of anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath. The value obtained is multiplied by a factor 0.9 to arrive at the total sugar content (Hodge and Hofreiter, 1962).

2.6.1. Determination of Reducing Sugar

The Fehling's solution used was standardized. Ten mL (10mL) Fehling's solution A and 10 mL Fehling's solution B were transferred into Erlenmeyer flask. 30 mL DDI water was added and mixed properly. A burette was filled with the dextrose standard. The flask was placed on a hot plate and was heated to at least 70°C but below boiling. Throughout titration, temperature of the solution was maintained above 70°C. The dextrose standard was added until the blue color nearly disappears. One or two drops of methylene blue was added. The dextrose standard was added to the mixture till the blue color disappears and only a colourless solution with a red precipitate was left (Browne and Zerban, 1948; Mendham *et al*., 2000).

2.6.2. Refractive Index, Total Soluble Solids and Sucrose Quantification

This was measured using a refractometer and is referred to as the degrees Brix (° Brix). The total soluble solids, refractive index and sucrose concentration of palm wine sample were determined using the refractive indices/ Sucrose/ total soluble solids table (Echeverria and Ismail, 1987; Dongare *et al*., 2014).

2.7. Turbidity Quantification

This was determined by measuring the sample at 650nm using a spectrophotometer (Talapaiboon, 2004).

2.7.1. pH Determination

This was determined by measuring the palm wine sample with a pH meter which was calibrated at pH 4.0 and 9.0 (AOAC, 1984).

2.7.2. Total Titratable Acidity Determination

The palm wine sample was titrated against 0.1M Sodium Hydroxide (NaOH) and phenolphthalein was used as an indicator. It was calculated in term of lactic acid (Zoecklein *et al*., 1990; Nielsen, 2014).

2.8. Glucose Quantification

A sucrose stock solution was prepared by dissolving 1000mg sucrose in 1dL. Five diluted sucrose stock solution samples were prepared as standards. Diluted palm wine sample was also prepared. 2 mL of each sucrose standard was pipetted into test tubes. Two mLs of the diluted palm wine samples was pipetted into a test tube. 2 mLs of Deionized water was pipetted into another test tube as blank. 2 mL of 6M HCl was added to the test tubes (standards, sample and blank). The test tubes were placed in boiling water for 10 minutes. 8 mL of 2.5 M NaOH

© 2019 Conscientia Beam. All Rights Reserved.

solution was added. 2 mL of 0.05 M DNSA solution was also added and the test tubes were covered with parafilm and shaken well to mix (Miller, 1959).

2.8.1. Organic Acid Quantification

The organic acids and ascorbic acid contents of the palm wine were determined using High Performance Liquid Chromatography (Nollet, 1992; Moreno and Salvado, 2000; Staroverov *et al*., 2004).

2. 9. Alcoholic Content Quantification

The alcoholic content of the palm wine sample was determined using Gas Chromatography Flame Ionization Detector method.

3. RESULTS

A total number of forty (40) yeast isolates were obtained from the spontaneously fermented palm wine during storage. As a result of the colonial and cell morphology of the isolates on Yeast Malt Agar and 50% glucose as well as the assimilation of eight (8) carbohydrates and glucose fermentation. All the yeast isolates fermented glucose, assimilated xylose, monosaccharides such as glucose, fructose, galactose, and sugar alcohol such as mannitol failed to assimilate disaccharides such as sucrose, maltose, and lactose. The results showed that only one genera of yeast was isolated from all stages of storage and this was *Saccharomyces cerevisiae*. Hence, the isolates (AI through D10) were all identified as *Saccharomyces cerevisiae* during the spontaneous fermentation of palm sap during storage Tables 1, 2a and 2b.

A variation in the yeast count of the stored palm wine with age was observed. As the storage time (age) of the palm wine increases, there was an initial increase in the growth of yeasts from 5.46 x 10^4 cfu/ml at 24 hours to 2.10 x 10^5cfu/ml at 120 hours, followed by a steady decline in their growth till 3.00 x 10^2 cfu/ml at 528 hours as illustrated in Table 3.

Variations in the nutritional contents of the stored palm wine with age were also observed as shown in Table 3. The storage time of palm wine increased and the moisture content of the palm wine was also increased from 88.39% at 24 hours to 97.81% at 528 hours. The crude proteins content of the palm wine increased from 36 mg/10 ml at time 24 hours to 53 mg/10 ml at 120 hours and then decreased from this time to a value of 42 mg/10 ml at 528 hours. The ash content of the palm wine also decreased from 61 mg/10 ml at 24 hours to 36 mg/10 ml at 528 hours. A continuous decrease in the carbohydrate content of the stored palm sap throughout storage was observed. The carbohydrate content of the stored palm wine decreased from 106.4 mg/ml at 24 hours to 14.10 mg/ml at 528 hours. The stored palm sap did not contain any crude fat or crude fibre. The vitamin C content of the stored palm wine increased from 17.2 mg/100 ml at 24 hours to 19.7 mg/100 ml at 120 hours and then decreased from this value to 2.7mg/100ml at 528 hours.

Variations in the physicochemical properties of the stored palm wine with age were also observed as shown in Table 4. There was an initial increase in temperature of the stored palm wine from 30°C at 24 hours to 32°C at 72 hours. From 72 hours onwards, the temperature stabilized at 32°C until 528 hours when it increased to 33°C. A variation in the pH of stored palm wine with time was also observed. The pH drops steadily from 3.70 at 24 hours to 3.37 at 360 hours and then stabilized at this value till 528 hours. Total titratable acidity of the stored palm wine also varied with age. The total titratable acidity of the wine from 2.28% at 24 hours to 4.50% at 528 hours was observed. A steady decrease in the total sugar of the stored palm wine from 4.0211g/10 ml at 24 hours to 0.6417g/10 ml at 528 hours was observed. A steady decrease in the reducing sugar content of the stored palm wine from 13% at 24 hours to 0.960% at 456 hours was also observed. Ethanol content of the stored palm wine increased steadily from 21.06 mg/ml at 24 hours to 88.99mg/ml at 456 hours as the storage time increases. Lactic acid content of the palm wine increases steadily from 3.197% at 24 hours to 8.577% at 528 hours as storage time

© 2019 Conscientia Beam. All Rights Reserved.

increases. Total soluble solids content of stored palm wine decreases steadily from 118.89 kg/m^3 at 24 hours to 56.03 kg/m^3 at 360 hours and then stabilized at this value till 456 hours.

ANOVA showed that no significant difference was observed in the treatments while there is significant difference in the blocks of Table 4. Using the Least Significant Difference method to compare values in the block in order to determine significant differences between them shows that, within each column, the values with different superscript letters are different from one another. The treatment (storage) of the palm wine samples brings about a significant change in its microbiological, nutritional contents and physicochemical properties of the stored palm wine.

Table-1. Morphological characteristics for the identification of yeast isolates obtained at different stages of spontaneous fermentation of palm sap.

Isolates Code	Time of Stg. (hrs.)	Colour on YEA	Form on YEA	Elevation on YEA	Margin on YEA	Shape under microscope	Vegetative Growth	Sporulation
AI-D1	24	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A2-D2	72	Creamy White	Circular	Convex	Entire	Circular	Budding	-ve
A3-D3	120	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A4-D4	192	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A5-D5	240	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A6-D6	288	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A7-D7	360	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A8-D8	408	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
A9-D9	456	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve
*AI0-D10	528	Creamy white	Circular	Convex	Entire	Circular	Budding	-ve

YEA – Yeast Extract Agar, -ve– Negative, Stg – Storage, A- D= *Saccharomyces cerevisiae.*

Table-2a. Biochemical characteristics of yeast isolates obtained at different stages of spontaneous fermentation of palm sap.

Isolates code	Time of Stg (hrs.)	Growth on YMA at 37°C	Growth on YMA at 40°C	Growth in 50% glucose	Growth in 60% glucose	Urea hydrolysis	Probable Organism
AI-D1	24	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A2-D2	24	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A3-D3	24	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A4-D4	24	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A5-D5	72	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A6-D6	72	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A7-D7	72	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A8-D8	72	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
A9-D9	120	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*
AI0-D10	120	+ve	+ve	+ve	-ve	+ve	*Saccharomyces cerevisiae*

YMA – Yeast Malt Agar, Stg – Storage, Iso – Isolates, A- D= *Saccharomyces cerevisiae*, +ve=Positive, -ve=Negative.

© 2019 Conscientia Beam. All Rights Reserved.

Table-2b. Biochemical characteristics of yeast isolates obtained at different stages of spontaneous fermentation.of palm sap (Contd.).

Isolates code	Time of stg (hrs.)	Glu Ferm	Glu Ass	Fruc Ass	Gal Ass	Lac Ass	Mal Ass	Suc Ass	Xyl Ass	Mann Ass	Probable Organism
AI-D1	24	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A2-D2	72	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A3-D3	120	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A4-D4	192	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae.*
A5-D5	240	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A6-D6	288	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A7-D7	360	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A8-D8	408	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
A9-D9	456	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*
AI0-D10	528	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	*Saccharomyces cerevisiae*

Stg - Storage, Ferm- Fermentation, Ass - Assimilation, Glu – Glucose, Fruc – Fructose, Gal - Galactose, Lac – Lactose, Mal – Maltose, Suc – Sucrose, Xyl – Xylose, Mann– Mannitol , A- D = *Saccharomyces cerevisiae* +ve=Positive,-ve=Negative.

Table-3. Microbiological and nutritional changes in stored palm wine subjected to spontaneous fermentation.

Time of storage (hrs.)	Crude proteins (%, w/v)	Carbohydrate (%, w/v)	Vitamin C (%, w/v)	Moisture Content (%, w/v)	Yeast Count (x 10^2) (CFU/ml)
24	0.36^a	10.64^a	0.0172^a	88.39^a	546^a
72	0.52^a	3.95^b	0.0181^a	95.17^b	1100^b
120	0.53^a	2.90^b	0.0197^a	96.21^b	2100^c
192	0.52^a	2.61^b	0.0191^a	96.54^b	500^a
240	0.51^a	1.49^b	0.0128^a	97.51^b	150^d
288	0.27^a	1.79^b	0.0058^a	97.51^b	60.50^d
360	0.43^a	1.57^b	0.0127^a	97.55^b	26.80^d
408	0.43^a	1.56^b	0.0100^a	97.57^b	16.60^d
456	0.43^a	1.49^b	0.0070^a	97.56^b	4.40^d
528	0.42^a	1.41^b	0.0027^a	97.81^b	3.00^d

Different superscript letters within each column indicate significant differences at $\alpha = 0.05$, according to Least Significant Difference method.

Table-4. Physicochemical changes in stored palm wine subjected to spontaneous fermentation.

Time of storage (hrs.)	Temp (°C)	pH	Total Titratable Acidity (%)	Total sugar (%,w/v)	Red. Sugars (%,w/v)	Ethanol (%, w/v)	Lactic Acid (%, w/v)	Total Soluble Solids (kg/m^3)
24	30^a	3.70^a	2.28^a	40.21^a	13.000^a	2.11^a	3.197^a	118.89^a
120	32^b	3.54^a	2.64^a	36.48^a	4.469^b	4.64^a	4.105^a	77.01^b
240	32^b	3.48^a	4.23^a	25.84^b	1.440^b	6.99^b	5.193^a	63.34^c
360	32^b	3.37^a	4.38^a	10.93^c	0.968^b	8.09^b	6.851^a	56.03^d
456	32^b	3.37^a	4.20^a	8.40^c	0.960^b	8.90^b	7.093^a	56.03^d

Red.Sugars – Reducing Sugars.
Different superscript letters within each column indicate significant differences at $\alpha = 0.05$, according to Least Significant Difference method.

4. DISCUSSION

The yeast isolates A1 through D10 were all identified as *Saccharomyces cerevisiae.* Amoa-Awua *et al.* (2007) who worked on growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm in Ghana discovered that *Saccharomyces cerevisiae* was the dominant yeast and was the only yeast isolated from the samples of the palm wine. The yeast growth pattern could be as a result of an initial abundance of palm total sugars of the wine followed by its reduction as storage progressed. The palm sugars serves as substrate for the growth of yeast. The initial increase in growth of yeast from 24 hours to 120 hours, where the growth was maximum, could be as a result of the initial abundance of the palm total sugars. Reduction in the palm total sugars

© 2019 Conscientia Beam. All Rights Reserved.

as storage progressed leads to a reduction in the substrate and subsequently a reduction in the growth of yeasts. This growth pattern could also be as a result of an increasing amount of ethanol present in the stored palm wine sample as the growth of yeast is known to reduce in the presence of excess ethanol when its ethanol tolerance level is exceeded. When the ethanol content of the palm wine was at minimum, there was an initial increase in growth of yeast. The reduction in growth of the yeast, after an initial increase, could be as a result of accumulation of Ethanol beyond the Ethanol tolerance level of the yeast growing in the stored palm wine. Increase in the moisture content of the stored palm sap could also be the reason why the yeast growth reduced after 120 hours as fungi generally prefer to grow on substances of low water activity and grow sparsely on substances of high water activity. The increase in the moisture content of the stored palm wine as storage progressed could be as a result of the utilization of palm sugars by various microorganisms during storage. The utilization of these sugars leads to the precipitation of the said sugars out of the solution that constitutes palm wine. This leads to an increased value of water (moisture) content of the palm wine during storage. Also, the increase in water content of the palm wine during storage could be as a result of the various microorganisms present inside it undergoing cellular respiration which leads to the production of water as a by – product of respiration. Nwachukwu *et al.* (2006) who worked on investigation of some physicochemical and microbial succession parameters of palm wine from Raffia palm and oil palm recorded increase in moisture content of wines equally the holding time of the wines increased. Obi *et al.* (2015) who worked on assessment of microbial growth and survival in fresh Raffia palm wine from Abia State, Nigeria also recorded that the moisture content was increased as the holding time of the wines increased. The moisture content of the stored palm sap increased which was also an indication that the stored palm wine has a short shelf life as shelf life of food is inversely proportional to its moisture content. Hence, the stored palm wine sample gets spoilt easily as a result of its increasing moisture content during storage.

The initial increase in crude proteins contents of the stored palm wine followed by its gradual decline till the end of storage time could be as a result of the initial increase in the growth of yeasts till 120 hours and their growth decline beyond this time. Yeasts are known to be good producers of proteins (Chandrasekhar *et al.*, 2012).

A decrease in carbohydrate of the stored palm sap as storage progressed was as a result of the conversion of the palm carbohydrate (sugars) into ethanol and organic acids by microorganisms present in the palm wine sample during storage. Decrease in pH of the stored palm wine was as a result of the various organic acids produced by the microorganisms present in the stored palm wine. These organic acids lower the pH of the palm wine.

The result obtained in this study agreed with the work of Nwachukwu *et al.* (2006) who worked on investigation of some physicochemical and microbial succession parameters of palm wine from Raffia palm and oil palm. They discovered that the pH of palm wine samples reduced with increase in ages of wines. This observation is also in agreement with the work of Amoa-Awua *et al.* (2007) who worked on growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm in Ghana. They also recorded reduction in the pH of palm wine samples with increase in ages of the wine.

An increase in total titratable acidity of the stored palm wine resulted in the production of various organic acids by microorganisms present in the stored palm sap. These acids increase the total acidity of the medium (palm wine). Nwachukwu *et al.* (2006) investigated on some physicochemical and microbial succession parameters of palm wine from Raffia palm and oil palm, also discovered that there was increase in the total titratable acidity of palm wine samples with increase in ages of wines. The decrease in the total sugar content of the stored palm wine was as a result of yeasts and other microorganisms present in the stored palm sap converting the palm sugars into organic acids and ethanol. The palm total sugars content, which is made up of sucrose, glucose and reducing sugars, steadily decreased in value as the time of storage of palm sap increased. Nwachukwu *et al.* (2006) who worked on palm wine from Raffia palm and oil palm also discovered that the reducing sugar content of the palm wine decreased with the age of the wines. The increase in ethanol content of the stored palm wine as storage progressed was as due to the conversion of the palm wine sugars into ethanol by yeast and bacteria present in the palm sap. Obi *et al.* (2015) also

© 2019 Conscientia Beam. All Rights Reserved.

recorded increase in ethanol contents of wine as the holding time of the wine increased. Decrease in the total soluble solids of the stored palm wine as storage progressed could be as a result of the utilization of palm sap soluble solids (soluble sugars such as glucose, sucrose) by microorganisms present in the sap. The leads to the reduction of the total soluble solids content of the stored palm wine with time. This is contrary to the work of Nwachukwu *et al.* (2006) who worked on investigation of Raffia palm and oil palm. They discovered that the percentage total soluble solids of the two wine samples increased with the age of the wines.

5. CONCLUSION

In conclusion, palm wine whose storage time is not beyond 120 hours is a good source of carbohydrate, crude proteins, vitamins, moisture, yeast and minimal alcohol (4.64 %) which is not injurious to the health of consumers. Beyond this time of storage, there was a decrease in the carbohydrate, crude proteins, vitamins, moisture and yeast contents of the stored wine and also increase in the ethanol content of the palm wine that was stored. Also, beyond this time of storage, the stored palm wine become more susceptible to spoilage microorganisms as the shelf life of food reduces with increase in its moisture content.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Acknowledgement: Both authors contributed equally to the conception and design of the study.

REFERENCES

Amoa-Awua, W., E. Sampson and K. Tano-Debrah, 2007. Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. Journal of Applied Microbiology, 102(2): 599-606.Available at: https://doi.org/10.1111/j.1365-2672.2006.03074.x.

AOAC, 1984. Official methods of analysis association of official analytical chemists. 14th Edn., Airlington, Washington, DC: AOAC.

AOAC, 1990. Official methods of analysis of the association of official analytical chemists. 15th Edn., Washington DC: Association of Official Analytical Chemists, Inc.

Barnett, J.A., R.A. Payne and D. Yarrow, 1990. Yeast characteristics and identification. 2nd Edn., Cambridge: Cambridge University Press.

Browne, C.A. and F.W. Zerban, 1948. Physical and chemical methods of sugar analysis. 3rd Edn., New York: John Wiley & Sons, Inc.

Chandrasekhar, K., S. Sreevani, P. Seshapani and J. Pramodhakumari, 2012. A review on palm wine. International Journal of Research in Biological Sciences, 2(1): 33-38.

Christensen, W., 1946. Urea compositione humani paracolon protea medium differre inter se et generum salmonella bacillus. Journal of Bacteriology, 52(4): 461-466.

Dongare, M., P. Buchade, M. Awatade and A. Shaligram, 2014. Mathematical modeling and simulation of refractive index based brix measurement system. Optik-International Journal for Light and Electron Optics, 125(3): 946-949.Available at: https://doi.org/10.1016/j.ijleo.2013.09.002.

Echeverria, E. and M.A. Ismail, 1987. Changes in sugars and acids of citrus fruit during storage. Proceedings of the Florida State Horticultural Science, 100: 50–52.

Ezeagu, I., M. Fafunso and F. Ejezie, 2003. Biochemical constituents of palmwine. Ecology of Food and Nutrition, 42(3): 213-222.Available at: https://doi.org/10.1080/03670240390226222.

Harrigan, W.F. and M.E. McCance, 1976. Laboratory methods in food and dairy microbiology. London: Academic Press.

Hodge, J.E. and B.T. Hofreiter, 1962. Methods in carbohydrate Chemistry (Eds), Whistler, R. L and Be Miller, J N). New York: Academic Press.

© 2019 Conscientia Beam. All Rights Reserved.

Karamoko, D., N. Djeni, K. N'guessan, K.-P. Bouatenin and K. Dje, 2012. The biochemical and microbiological quality of palm wine samples produced at different periods during tapping and changes which occured during their storage. Food Control, 26(2): 504-511.Available at: https://doi.org/10.1016/j.foodcont.2012.02.018.

Kiss, I., 1984. Testing methods in food microbiology. A. Textbook. New York: Elsevier.

Mendham, J., R.C. Denney, J.D. Barnes and M. Thomas, 2000. Vogel's textbook of quantitative chemical analysis. England: Pearson Education Ltd.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3): 426-428.Available at: https://doi.org/10.1021/ac60147a030.

Moreno, P. and V. Salvado, 2000. Determination of eight water- and fat-soluble vitamins in multi-vitamin pharmaceutical formulations by high-performance liquid chromatography. Journal of Chromatography A, 870(1-2): 207-215.Available at: https://doi.org/10.1016/S0021-9673(99)01021-3.

Naknean, P., M. Meenune and G. Roudaut, 2010. Characterization of palm sap harvested in Songkhla province, Southern Thailand. International Food Research Journal, 17(4): 977-986.

Nielsen, S., 2014. Food analysis. Irish Booksellers, Portland, USA: Springer Science & Business Media.

Nollet, L.M., 1992. Food analysis by HPLC. New York. Agilent Application Note 5091-3194E.

Nwachukwu, I., V. Ibekwe and B. Anyanwu, 2006. Investigation of some physicochemical and microbial succession parameters of palm wine. Journal of Food Technology, 4(4): 308-312.

Obi, C., J. Ogbulie and A. Nkwo, 2015. Assessment of microbial growth and survival in fresh rafia palmwine from Umuariaga community, Ikwuano LGA Abia State, Nigeria. International Journal of Current Microbiology and Applied Sciences, 4(1): 484-494.

Okafor, N., 1978. Microbiology and biochemistry of oil-palm wine. Advances in Applied Microbiology, 24: 237-256.Available at: https://doi.org/10.1016/s0065-2164(08)70642-7.

Okafor, N., 1987. Industrial microbiology. Nigeria: University of Ile Ife Press. pp: 205-217.

Olasupo, N.A. and O.S. Obayori, 2003. Utilization of palm wine (Elaeis Guineensis) for the improved production of Nigerian indigenous alcoholic drink-Ogogoro. Journal of Food Processing Preservation, 27(5): 365-372.Available at: https://doi.org/10.1111/j.1745-4549.2003.tb00523.x.

Ouoba, L., C. Kando, C. Parkouda, H. Sawadogo-Lingani, B. Diawara and J. Sutherland, 2012. The microbiology of Bandji, palm wine of Borassus Akeassii from Burkina Faso: Identification and genotypic diversity of yeasts, lactic acid and acetic acid bacteria. Journal of Applied Microbiology, 113(6): 1428-1441.Available at: https://doi.org/10.1111/jam.12014.

Santiago-Urbina, J.A., A.G. Verdugo-Valdez and F. Ruiz-Terán, 2013. Physicochemical and microbiological changes during tapping of palm sap to produce an alcoholic beverage called "Taberna", which is produced in the south east of Mexico. Food Control, 33(1): 58-62.Available at: https://doi.org/10.1016/j.foodcont.2013.02.010.

Staroverov, V., V. Deineka, A. Grigor'ev, E. Prokhoda, M. Pokrovskii and V. Ivanov, 2004. HPLC analysis of water-soluble vitamins in the multivitamin syrup Oligovit. Pharmaceutical Chemistry Journal, 38(3): 172-174.Available at: https://doi.org/10.1023/b:phac.0000034310.75374.48.

Stringini, M., F. Comitini, M. Taccari and M. Ciani, 2009. Yeast diversity during tapping and fermentation of palm wine from Cameroon. Food Microbiology, 26(4): 415-420.Available at: https://doi.org/10.1016/j.fm.2009.02.006.

Talapaiboon, S., 2004. Effect of high pressure and heat treatment on quality of palm sap. M.Sc. Thesis, Prince of Songkla University. Thailand.

Zoecklein, B.W., K.C. Fugelsang, B.H. Gump and F.S. Nury, 1990. Influence of pH in Winemaking in: Production wine analysis. New York: Van Noststrand Reinhold VI.

Views and opinions expressed in this article are the views and opinions of the author(s), The International Journal of Biotechnology shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/arising out of the use of the content.

© 2019 Conscientia Beam. All Rights Reserved.