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Shelf life determination of two palm wine varieties and its effect on biochemical properties and enzyme production.

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Abstract

In palm wine, the available complex microbiota plays very vital roles in the general attributes of the beverage as well as the significance of the various metabolites generated. This research focused on evaluating the biochemical properties of the palm wine obtained from two different varieties of oil palm (*Elaeis guineensis*) and its potential enzyme activity, as well as assessing the effect of the changes that occur during the keeping time. The biochemical contents (alcohol, total titratable acid, reducing sugar, and pH) were evaluated every 2 h over a period of 144 h. The palm wine varieties were also analyzed for cellulase and amylase production. There was an increase in ethanol for both varieties from 7.29±0.02% (1 h) to 14.82±0.02 (144 h) for variety 1, and from 8.35±0.01% (1 h) to 16.32±0.03% (144 h) for variety 2; the TTA increased with keeping time from 2.25±0.03% (1 h) to 4.42±0.03% (144 h) for variety 1, and from 2.35±0.02% (1h) to 5.01±0.04 (144 h) for variety 2. Reducing sugar decreased from 476.58±0.04 mg/L (1 h) to 42.84±0.75 mg/L (144 h) for variety 1, while variety 2 decreased from 238.18±0.05 mg/L (1 h) to 43.73±0.63 mg/L (144 h). The pH of the palm wine also experienced a decrease for variety 1 from 5.2±0.06 at 1 h to 2.8±0.17 at 144 h, while the variety 2 decreased from 4.8±0.17 at 1 h to 2.8±0.1 at 144 h. There was a reduction in the sugar content with the accumulation of alcohol, which occurred in both varieties, and a drop in the pH due to an increase in organic acids. The palm wine varieties were screened for cellulase and amylase activity, with the highest amylase yield for varieties 1 and 2 at 0.62 U/ml and 0.21 U/ml, respectively on the first day, while that of cellulase was observed at 48 h (0.34 U/ml) for V1 and at 1 h (0.2 U/ml) for V2. The result of this current study proves the importance of the microbial consortium in fresh palm wine as well as its potential as a source of enzyme production, and also shows that these parameters are affected by the keeping time.

Keywords: Palm wine; Fermentation; Biochemical analysis; Storage; Ethanol; Enzyme

1. Introduction

Palm wine is an alcoholic beverage generated via the natural fermentation of the sweet sap acquired from tropical plants belonging to the family of palmae, like the oil palm (*Elaeis guineensis*), date palm (*Phoenix dactylifera*), coconut palm (*Cocus nucifera*), nipa palm (*Nypa fruticans*), ron palm (*Borassus aethiopum*), kithul palm (*Caryota urens*) and raffia palm (*Raphia hookeri*) [1, 2].

This beverage is known, generated, and devoured in various parts of Africa under different names, where it plays very significant functions in various cultures and embodies economic importance [3].

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The unfermented sap, which is sugary and provides a highly nutritious substrate for microbial growth, undergoes a spontaneous lactic-alcoholic-acetic fermentation that supports the buildup of bacteria and yeasts for the purpose of converting the sugary substrate into a number of metabolites [4, 5, 6, 7]. These three microbial categories, namely yeast, lactic acid bacteria, and acetic acid bacteria, are believed to play a very essential part in the production process of palm wine [7]. The fermentation process involves early lactic acid fermentation, an intermediate alcoholic fermentation and an ending acetic fermentation [8, 5]. In West Africa, the collection of palm wine sap is practiced in two ways: inflorescent tapping or stem tapping, which is a non-destructive method; and the felling of the palm trees and incision of the meristematic region, which is a destructive method [9, 10]. The microbial diversity of palm wine includes both microbiota and invading microorganisms due to the nutrient-rich medium of the sap; these microorganisms produce several metabolites as end products of fermentation, which can affect the sensory and biochemical properties [5, 6, 10].

The growth and invertase activity of the yeast cells is most likely enhanced by the increase in total acidity and reduction in pH which is a result of the organic acids produced, while acetic acid bacteria use the ethanol generated by the yeast as a substrate for the production of acetic acid [8, 11, 5]. A fresh palm wine sap has a near-neutral pH that drops after tapping, with a sugar content of just about 10-18% w/v [12, 5, 3, 4, 13]. A reduction in the pH of palm wine in the course of fermentation has been observed to be due to organic acids produced mostly by the lactic acid bacteria, followed by acetic acid, which is seen as part of the palm wine aroma [5, 4]. Also, Karamoko et al. [3] reported the presence of other organic acids like oxalic, ascorbic, fumaric, tartaric, malic, and citric in various concentrations.

The ability of enzymes to combine their catalytic functions, specificity, and activity under mild conditions, makes them successful as catalysts in various industrial applications. These enzymes can be obtained from animals, plants, and microbes, but so many industrial processes depend more on the microbial enzymes produced singly, either as extra or intra cellular compounds because they are capable of enduring adverse conditions, high productivity, low cost and availability [14, 15]. However, some microorganisms also possess the ability to produce an array of enzymes in multicomplexes [16, 17]. Enzymes such as amylase and cellulase have valuable potential applications in various industrial sectors, especially using microbial sources, due to their ability to secrete extracellular enzyme with cost-effective components. The aim is to establish the biochemical properties and extracellular multi-enzyme production prospects of two palm wine varieties and the effect of storage on these parameters.

2. Materials and Methods

2.1. Materials and sample collection

Fresh palm wine was obtained from Amansea in Awka town of Anambra state, Nigeria. The fresh palm wine samples were tapped by means of a sterilized 100 ml sample bottles from two distinct varieties of the oil palm. The samples were transported to the microbiology laboratory in a container bearing packs of frozen ice cubes for microbial analysis within 1h of sample collection.

2.2. Biochemical analysis

Ethanol: The amount of ethanol present in the palm wine varieties was evaluated with the method explained by Sumbhate et al. [18]. To 1ml of the palm wine, 1 mL of acetate buffer (pH 4.3), 1 mL of sodium dichromate solution and 5 mL of 1N sulfuric acid was added to a test tube. The blend was lightly stirred for 1 min and incubated for 120 mins at room temperature which lead to development of a green color by the reaction product. The absorbance was read at 578 nm using an Axiom 752 spectrophotometer. The amount of ethanol was measured with standard ethanol (100mg/ml).

Reducing Sugar: The palm wine was evaluated for the presence of reducing sugar using the DinitroSalicyclic acid method explained by Osilo et al. [19]. The palm wine (3ml) was added to 1ml of DNS in a test tube and boiled for 5mins and then the temperature reduced under running tap water. The absorbance was noted at 540nm with a spectrophotometer (Axiom 752). The activity of the reducing sugar was determined with glucose standard.

Evaluation of Titratable Acidity and pH: Total titratable acid was estimated as reported by Afolabi and Owoola [20]. Samples of the palm wine varieties were titrated against 0.1M Sodium Hydroxide (NaOH), using 1% phenolphthalein as an indicator. The titratable acid was measured as percentage of lactic acid. The pH of the varieties of palm wine was obtained directly with the aid of a pH meter after calibrating using a standard buffers.

2.3. Enzyme Analysis

Alpha Amylase Activity: The α-amylase activity was estimated with the use of dinitrosalicylic acid as explained by Nwagu et al. [15] with slight modifications. A 1 mL sample of the palm wine varieties as crude and 1 mL of a solution of the

substrate containing 1% soluble starch substrate suspended in 0.2M sodium phosphate buffer (pH 6.9) was incubated at 25 ºC for 10 min. The enzyme reaction was ended by adding 2.0 mL of dinitro salicylic acid (DNS) and then boiled for 5 min. The mixture was diluted with water and absorbance was obtained at 540 nm with a spectrophotometer (Axiom 752, USA). A unit of enzyme activity (U) is the quantity of enzyme that can release one mmol of glucose per minute. The reducing sugar expressed as glucose was quantified from standard curve prepared.

Cellulase activity: The cellulase enzyme activity was assayed according to the method depicted by Osilo et al. [19] this was done by inserting a folded strip of filter paper (1 × 6 cm) into a test tube containing 1 mL of palmwine and 2.0 mL of 50 mM citrate buffer (pH 4.8). The test tube was incubated for 1 h at 50°C and the reaction was halted by adding 3 mL of DNS reagent, then the blend was boiled for 5 min and diluted by adding 20 mL of water. The quantity of glucose released was determined at 540 nm with the aid of a spectrophotometer and calculated using glucose standard curve.

2.4. Statistical analysis

The data obtained were statistically analyzed by means of analysis of variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) and the mean differences determined by Duncan's tests at a significance level ($P < 0.05$).

3. Results

3.1 Biochemical qualities of the fresh palm wine acquired from two separate varieties of Elaeis guineensis is shown in Table 1. The parameters obtained on the first hour of tapping showed that ethanol, TTA, RS and pH, gave 7.29±0.02%, 2.25±0.03%, 476.58±0.04 and 5.2±0.06 for the first variety; while the second variety showed 8.35±0.01%, 2.35±0.02%, 238.18±0.05 and 4.8±0.17 respectively. All through the storage period, there was a successive decline in the activity of the reducing sugar and the pH of the palm wine varieties at different rates, with a steady increase in the ethanol content and the total titratable acid. The fresh palm wine had very high sugar content in both varieties, which decreased from an initial concentration of 476.58±0.04mg/L (V1) and 238.18±0.05 mg/L (V2) on the first day to 42.84±0.75mg/L (V1) and 43.73±0.62mg/L (V2) on the last day of storage as seen in fig 4. During the storage, the percentage alcohol content range was between 7.29-14.82 for V1 and 8.35-16.32 for V2. The pH values of 5.2±0.06 (V1) and 4.8±0.17 (V2) on the first day, dropped to 3.2±0.1 (V1) and 3.5±0.4 (V2) in 48h and then subsequently to 2.8±0.17 for V1 and 2.8±0.10 for V2 on the last day. The percentage titratable acid for both varieties experienced an increase from 2.25 – 4.42 (V1) and 2.35 - 5.01 during the 6days of storage.

Table 1 Biochemical properties of two palm wine (Elaeis guineensis) varieties obtained during storage

Parameters/ Varieties		**Storage time**		
	1h	48h	96h	144h
Ethanol (%) V1	7.29^{a}±0.02	10.89^{b}±0.02	14.26^{c}±0.04	14.82^{d}±0.02
V2	8.35^{a}±0.01	13.86^{b}±0.02	14.98^{c}±0.02	16.32^{d}±0.03
TTA (%) V1	2.25^{a}±0.03	3.5^{b}±0.06	3.91^{c}±0.06	4.42^{d}±0.03
V2	2.35^{a}±0.02	4.10^{b}±0.7	4.40^{bc}±0.5	5.01^{c}±0.04
RS (mg/L) V1	476.58^{d}±0.04	234.63^{c}±0.38	161.38^{b}±0.72	42.84^{a}±0.75
V2	238.18^{d}±0.05	67.92^{c}±0.10	58.60^{b}±0.41	43.73^{a}±0.62
pH V1	5.2^{c}±0.06	3.2^{b}±0.1	3.0^{b}±0.06	2.8^{a}±0.17
V2	4.8^{c}±0.17	3.5^{b}±0.4	2.8^{a}±0.06	2.8^{a}±0.1

Key: V- Variety; TTA- Titratable acid; RS- Reducing sugar

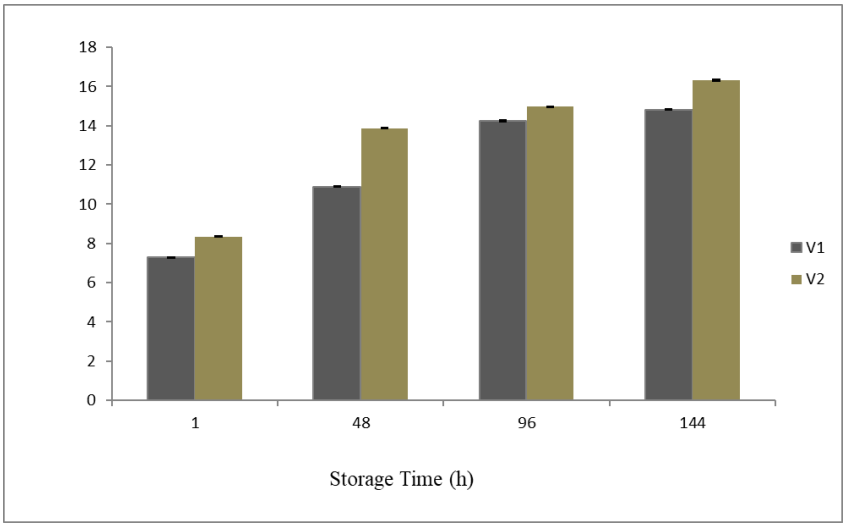

Figure 1 Ethanol content of the palm wine varieties during storage(V- Variety)

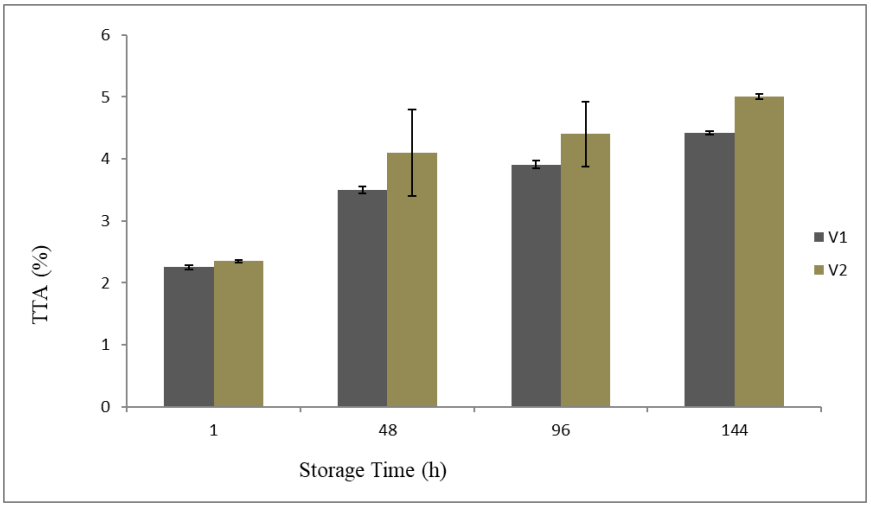

Figure 2 Titratable acid content of the palm wine varieties during storage (V- Variety, TTA- Titratable acid)

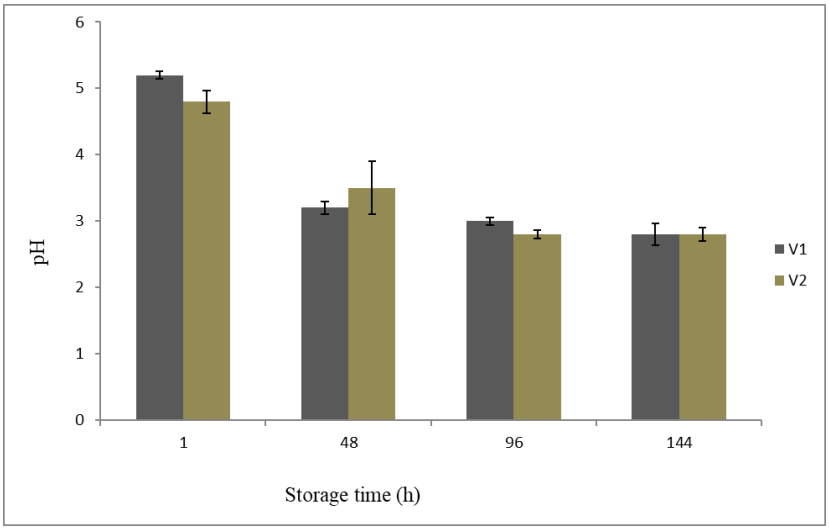

Figure 3 pH values of the palm wine varieties during storage (V- Variety)

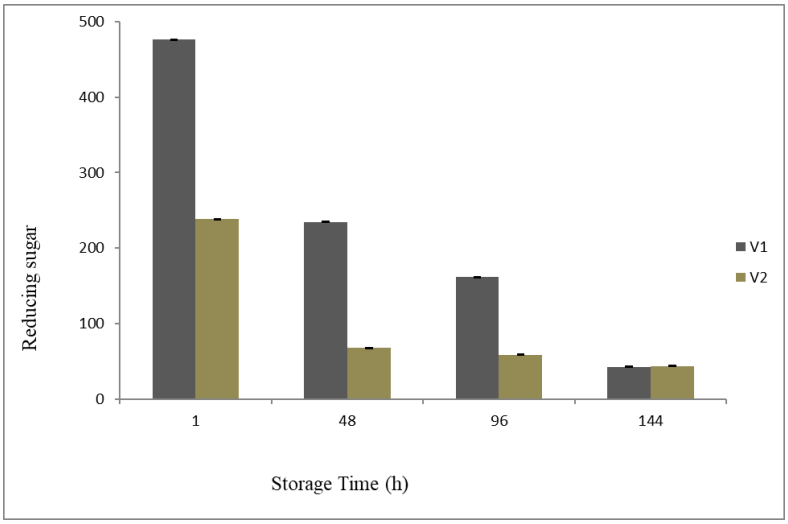

Figure 4 Reducing sugar content of the palm wine varieties during storage (V- Variety)

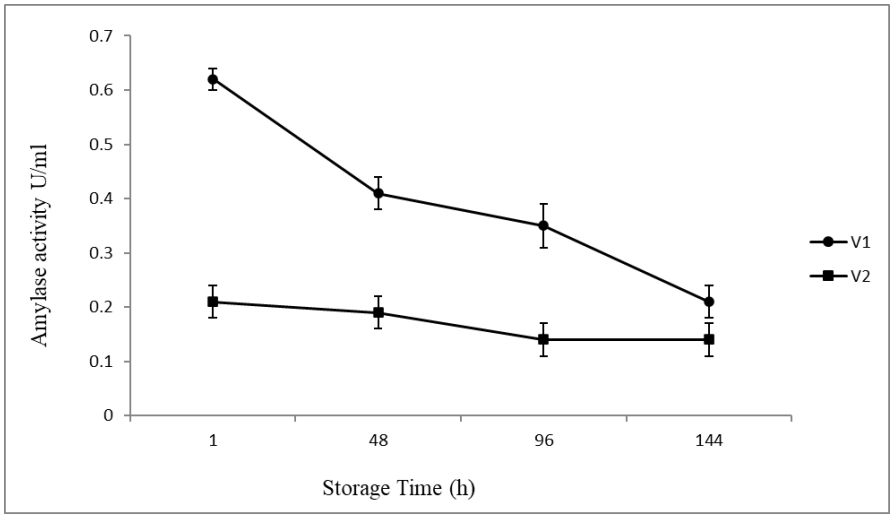

Figure 5 Amylase activity of the palm wine varieties during storage (V- Variety)

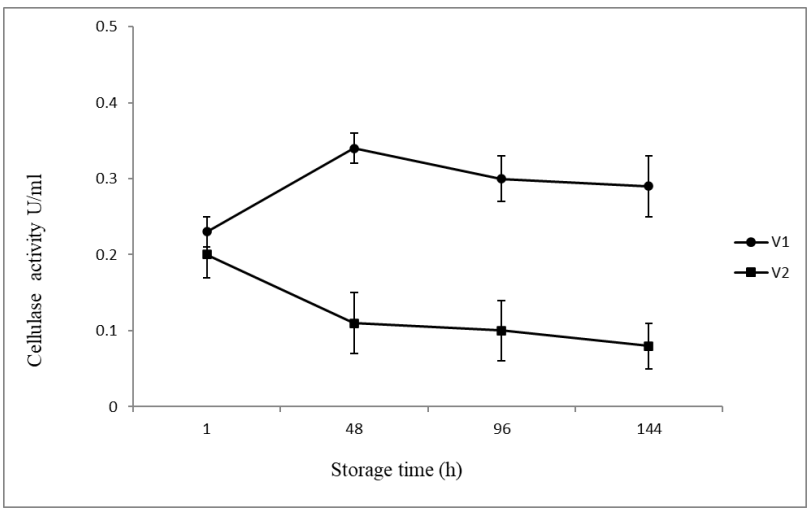

Figure 6 Cellulase activity of the palm wine varieties during storage (V- Variety)

3.2 The enzyme activity profile of the palm wine varieties were determines and recorded as seen in fig 5 and fig 6 for amylase and cellulase respectively. Interestingly, the two varieties of palm wine used showed the presence of cellulase and amylase in different quantities. At the initial stage, the cellulase present gave 0.23±0.02 U/ml and 0.20±0.03 U/ml for V1 and V2 respectively while amylase showed 0.62±0.02 U/ml (V1) and 0.21±0.03 U/ml (V2). There was a consistent decrease in the amylase activity of variety 1 during storage but the cellulase activity experienced an increase after 48h followed by a decrease. In the second variety, the cellulase activity decreased steadily while the amylase activity remained constant at 96h and 144h following a decrease.

The last day of storage recorded an enzyme profile of 0.21±0.03 U/ml (v1) and 0.14±0.03 U/ml (v2) for amylase; for cellulase, variety 1 and 2 gave 0.29±0.04 U/ml and 0.08±0.03 U/ml respectively. The highest amylase activity for variety 1 and 2 was seen on the first day at 0.62±0.02 U/ml and 0.21±0.03 U/ml respectively while that of cellulase was observed at 48h (0.34±0.02 U/ml) for v1 and at 1h (0.2±0.03 U/ml) for v2.

4. Discussion

This research was targeted towards the effect of the keeping time on the biochemical properties and the multi-enzymatic (amylase and cellulase) potential of palm wine. The alcohol content experienced an increase throughout the entire period (144h) of storage (Fig 1.), with the first day showing the lowest (7.29^{a}±0.02) for V1, probably as a result of the high sugar present in the palm wine sap (476.58^{d}±0.04) with a probable low population of yeast present after 1h of collection [3]. The last day of storage gave the highest alcohol content V1 (14.82^{d}±0.02) and V2 (16.32^{d}±0.03), this could be as a result of the complete breakdown of sugar into alcohol by the yeast present. Although, according to research, lactic acid bacteria can also synthesize ethanol, but in very little amounts [21, 22]. Also, the palm wine obtained at the break of day showed very high alcohol activity possibly due to its accumulation all through the night [5].

Breakdown of the sugar by these microorganisms during storage produces not just alcohol but several organic acids as well [3]; which is observed by the steady increase in the total titratable acid content in this research seen in the range of 2.25 – 4.42 % for V1 and 2.35 – 5.01% for V2 . These high concentrations recorded can be said to be due to buildup of organic acids during the storage period.

The V1 contained more sugar (476.58^{d}±0.04) than the V2 (238.18^{d}±0.05) at the early stage and decreased significantly during storage as seen in fig 4. The variation might be due to the different varieties of palm wine trees used, method of tapping employed as well as the time of collecting the palm wine sap [7]. The decrease in the sugar content during storage shows that a large portion of the sugar was fermented which is indicative of the high alcohol observed as well as the increased acidity. This shows that acid production occurs concurrently with alcohol production.

There was a reduction in the pH and reducing sugar of the palm wine varieties during storage. The lactic acid bacteria, which is normally discovered in palm wine is known to ferment sugars producing lactic acid which can lead to a reduction in pH. The initial pH value of the V1 palm wine gave 5.2^{c}±0.06, which is higher than V2 with 4.8^{c}±0.17; these outcomes are in line with the work conducted by Karamoko et al. [22], where the initial pH gave 5.23 ± 0.18 for the Dura and 4.64 ± 0.2 for the Tenera specie of the oil palm.

A complex microbial development is experienced throughout the spontaneous process of palm wine fermentation due to the fact that the sap is a highly nutritious medium that can sustain the development of a variety of microbes like yeast, lactic acid bacteria etc [3]. These microorganisms has shown to be great potential producers of various industrial enzymes

The presence of these enzymes in the palm wine varieties may be due to the array of microorganisms involved in the fermentation by breaking down the sugars present and releasing extracellular enzymes. Amylase production was observed by the palm wine varieties and the highest amylase activity was seen at 1h for both V1 (0.62±) and V2 (0.21±). A number of organisms isolated from palm wine have shown great potential in producing amylase enzyme [23, 24].

A research carried out by Lakshmi et al. [23], shows that Bacillus sp isolated from palm wine was able to produce high amylase activity of 164.126.17 ±0.2 U/mL and 113.4 ±0.26 U/mL when acid treated pineapple and lotus stem residues were used as substrate respectively in submerged fermentation.

The amylase activity detected was observed to decrease during storage for both varieties, although there was a higher rate of decrease in the amylase activity observed in V1 when compared to the equivalent values for V2 palm wine during storage (Fig 5). This reduction could be as a result of the decrease in the reducing sugars.

Cellulase enzyme was also present but in low amounts, with the highest activity observed at 48h for V1 with 0.34±0.02 U/mL and 0.20±0.03 U/mL for V2 at 1h, this shows the presence of cellulolytic microorganisms in palm wine. This was also observed in the study conducted by Omojasola & Jilani, [25], where *Saccharomyces cerevisae* isolated from palm wine gave a cellulase profile of 1.11 U/mL. Amaeze et al. [26], similarly reported *Saccharomyces cerevisae* gotten from palm wine to be an adequate cellulase producer with an enzyme activity of 0.269 mg/ml. Cellulase producing ability have been reported mostly by fungi but a number of studies have observed cellulase yield from bacteria [27].

5. Conclusion

During storage, fermentation of palm wine by the microbial consortium lead to a decline in the sugar content as a result of its breakdown and invariably an increase in alcohol content; giving way to reduction in pH due to an acid build up which eventually makes the palm wine unacceptable. This further emphasizes the significance of these microbes in palm wine fermentation. Palm wine is capable of serving as a very good source of various industrial enzymes due to the varieties of microorganisms involved in its fermentation. These microorganisms have the potential of generating diverse enzymes and releasing them extracellularly in to their environment, which can be optimized and scaled up for commercial production to satisfy the high demand in various industrial sectors.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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