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Effects of Starch Fermentation on the Shelf-Life of Cassava Starch Based Adhesive

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Author's contribution

The author, KMO personally designed the study and closely supervised the conduct of the experiments, the statistical analysis, as well as the results and discussion. He also managed the literature review. Those who helped in one way or the other in the conduct of the experiments are duly acknowledged in the acknowledgement section.

Research Article

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ABSTRACT

The effects of natural fermentation on the stability or shelf life of adhesives produced from starch of two cassava varieties (*Manihot utilissima* and *Manihot palmate*) were studied. It was observed that the HCN content of the *Manihot utilissima* reduced from 35.74±0.11 mg/kg for unfermented starch sample to 6.93±0.25 mg/kg after 5 days of fermentation (*p = 0.037*), while the sample's pH increased from 4.60±0.05 to 6.89±00 respectively. However, the reduction in HCN concentration and corresponding increase in pH for manihot palmate were only marginal, as the concentration of HCN in the starch reduced from 2.49±0.12 to 0.89±0.26 while the pH increased from 6.02±0.04 to 6.35±0.05 after five days of fermentation. The viscosity test conducted at room temperature for a period of 30 days to determine the stability or shelf of the produced adhesives show that adhesives produced from unfermented *Manihot utilissima* starch sample as well as samples of that cassava variety fermented for only 1 day have longer shelf-life as exemplified by the relative stable low viscosity of the adhesive over the 30 days test period with a highly significant effect, (*p = 0.018)*. The drying time of *Manihot utilissima* was significantly high at 7.1±0.01 minutes *(p = 0.041*) and remained relatively stable for the 30 days storage time. The results therefore show that the higher the cassava starch acidity the lower its viscosity, the higher its drying time and the longer its shelf-life.

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1. INTRODUCTION

Cassava (*Manihot esculenta*, crantz) is a tropical crop that daily supplies between 200 and 1000 calories to some 700 million people worldwide [1, 2]. Nigeria ranked first among major producers of cassava in the world, producing about 33 million metric tonnes of cassava in 1993 alone [3, 4]). Over 95% of cassava produced in Nigeria is used as food for the country's teeming population, being one of the country's staple foods [4]. The main form in which cassava is eaten in Nigeria and in the West African sub-region is a roasted granular product produced from peeled, grated and fermented cassava roots, known as gari [5, 6]. Other forms in which cassava tubers are also eaten includes boiling of the fresh cassava and soaking for between 2–7 days for the production of chickwangue, a favourite delicacy among the Ibo speaking tribes of Nigeria [6]; fufu notably in the Western part of the country [4], as well as starch (a secondary product of cassava) prepared in trace palm oil usually eaten with oil soup (ohwo) or banga soup by the people of Nigeria's Niger Delta region. The preparation of starch with palm oil in the later case is understandable since palm oil is known to remove cyanide completely from cassava [4]. Two varieties of cassava- sweet (manihot palmate) and bitter (*Manihot utilissima*) are widely cultivated in the country. However, the bitter cassava is cultivated more as a result of its high yield and economy [7]. The bitter variety has high concentration of cyanogenic glycoside (100 – 500 mg/kg fresh weight) which is distributed throughout the tuber while the sweet variety has low concentration of cyanogenic glycoside (less than 100 mg/kg) confined mostly to the peels [8]. In most cases, the cyanogens in fresh cassava roots are detoxified during processing into edible products [1, 9].

Starch is one of the major derivatives of fresh cassava roots, a by-product of gari processing. It accounts for 30 – 35% of fresh cassava roots [10]. The isolation of starch from fresh cassava roots is normally carried out in aqueous medium [11]. One of the industrial applications of cassava starch is in the manufacture of natural adhesives [7]. Natural adhesive is a substance capable of binding materials together by surface attachment with the ability to sustain the designed load requirement without failure. Natural adhesive include animal glues, casein glues and vegetable glues made from starches and dextrin. Starch has several advantages as a raw material in the production of adhesive. These advantages include its renewability, biodegradability abundance, cheapness and stability in price [12, 13, 14]. As a polymeric poly-hydroxy compound starch give adhesives an excellent affinity to polar substrates, including cellulose [15]. Starch-based adhesive are categorised either as hot-setting or cold-setting, with cold-setting adhesives containing higher solid contents than hot-setting adhesives [16]. The effectiveness of an adhesive is determined by its bonding capability i.e. its resistance to load shear which implies creep static or time independent deformation under sustained load. Other desired requirements are ease of application, reasonable setting time, and resistance to moisture, aging, heat and fungal attack [12].

Among all the raw materials used for the manufacture of natural adhesives, cassava starch has distinct advantages over others (i.e. animal glues, vegetables and other root crops). This is because cassava starch-based adhesives are made from by-products that are normally discarded during traditional processing of gari, hence making its production cost effective. The fine, smooth texture, non-staining and non-poisonous nature of cassava starch based adhesive makes it a ready choice for domestic and most non-structural utilisation [16]. In addition, cassava-based adhesives are tackier, more viscous and their joints exhibit higher

tensile strength than those from cereal and other tuber crops. Another advantage of cassava-based adhesive is its lower gelatinisation temperature which ranges between 49ºC and 70ºC when compared to 62ºC-73ºC for cereals such as corn [15]. Thus less heating is required to gelatinise cassava starch leading to greater energy savings. However, the stability of cassava starch based adhesive over time limits its application. In the present study, the effects of natural fermentation on the stability or shelf life of adhesives produced from starch obtained from two varieties of cassava roots (*Manihot utilissima* and *Manihot palmate*) are investigated.

2. MATERIALS AND METHODS

2.1Starch Preparation

Ten medium sized tubers each of *Manihot utilissima* (bitter variety) and manihot palmate (sweet variety) were harvested from the author's garden, peeled and grated using a hand grater. The paste was then diluted with sufficient de-ionised water before sieving the solution with the aid of 5µm filter. The filtrate was allowed to stay for 6 hours and water discarded leaving the starch at the base of the container. Ten beakers labelled U1 – U5 and P1 – P5 were thoroughly washed with de–ionised water. 25g each of wet starch from the bitter variety were weighed into the beakers labelled U1 – U5 and 25g each of wet starch from the sweet variety were weighed into the beakers labelled P1 – P5. Each beaker was then half filled with de-ionised water and allowed to stand for between 1 and 5 days according to the labels for fermentation process. Another 25g each were taken from both species and dried without fermentation and the samples marked U0 and P0 for bitter and sweet varieties respectively. After 24 hours, the starch in beakers U1 and P1 were taken and dried followed by U2 and P2 after 2 days and so on. This was in preparation for adhesive production. Prior to the production of adhesive, the chemical properties of both fermented and unfermented starch for the two cassava species were determined. Analyses were conducted in triplicate in order to determine the significance of parameters such as pH, total acidity and the hydrogen cyanide content of the samples.

2.2 Chemical Analysis of Starch

2.2.1 pH determination

The pH of the starch samples was determined using a calibrated 211 microprocessor pH meter. 1.5g of each of the dry starch sample was dissolved in distilled water to make up 15mL of starch solution. The solution was allowed to stand for 30 minutes. The meter electrode was then immersed into the solution and agitated. The thoroughly mixed solution was allowed to stand until a steady reading was obtained. The method was repeated thrice for each sample in order to obtain a triplicate reading.

2.2.2 Determination of the total titratable acidity

The method of Sadler and Murphy [17] as reproduced by Onwuka and Ogbogu [7] was used. 5g each of dry starch sample were weighed into a beaker. 50mL distilled water was then added to form a uniform starch solution. 3 drops of phenolphthalein indicator was then added and titrated with 0.1N NaOH to a phenolphthalein end-point. The volume of the titrant as well as the weight of the sample and normality of the base was used to calculate the titratable acidity expressed as lactic acid according to the following equation:

$$\%\ acid\left(\%_{wt}\right) = 100\left(\frac{N\,x\,V\,x\,Eq.wt}{W\,x\,1000}\right)$$

Where

N = Normality of titrant (mEq/mL)
V = Volume of titrant (mL)
Eq. wt = Equivalent weight of predominant acid (mg/mEq)
1000 = Factor relating milligrams to grams (mg/g)

2.2.3 Determination of hydrogen cyanide content

The hydrogen cyanide contents of both the fermented and unfermented starch samples were determined using the alkaline picrate method [18, 19]. The alkaline picrate was prepared by mixing 40 mL of 2% NaOH with 20 mL of picric acid: $Na_2CO_3.H_2O$ (1:5:200 v/w/v) in a conical flask. 2g each of the starch samples was made into a paste in 20 mL distilled water. 1 mL of the paste was introduced into a test tube and 4 mL of the prepared alkaline picrate was added. The mixture was filtered with a new white cotton cloth (that had not been used before) to avoid presence of impurities that may affect the accuracy of the UV-spectrophotometer. The properly sieved solution was then incubated in a water bath at 90ºC for 5 minutes. After colour development, the absorbance value of each sample was determined at 490nm using spectron 70 UV-spectrophotometer. The cyanide content was then extrapolated from a cyanide standard curve prepared from cyanide solutions containing 50-200 µg NaCN/mL. Prior to the measurement of the absorbance of the samples, the spectrophotometer was standardised with a blank reagent prepared by mixing 4 mL of alkaline picrate with 1 mL of distilled water

2.3 Production of Adhesive and Viscosity Determination

10g of the unfermented dry starch from *Manihot utilissima* was dissolved in 100 mL of 0.01M hydrochloric acid solution and heated on a hot plate to a temperature of 100ºC with stirring. The cooked starch was then allowed to cool to 75ºC before adding 4g of polyvinyl alcohol piecewise followed with the addition of 5g of sodium tetra borate with stirring to stabilise the produced adhesive. The same procedure was repeated for same weight of dry starch from unfermented manihot palmate as well as for equal weight of dry starch from both species fermented for 1, 2, 3, 4 and 5 days respectively. The viscosities of the produced adhesives were then measured at room temperature (38ºC) every 5 days for 30 days using Brookfield VISCOlab 4000 digital viscometer to estimate the shelf or pot life of the products.

2.4 Determination of Drying Time/Bond Strength of the Produced Adhesives

The bond strength/drying time of the produced adhesives were determined by binding paper to a piece of ply-board with 0.5 mm film of the adhesive. The time taken for the bond to dry was estimate using a stopwatch. After drying, the gummed paper was carefully peeled off and the tensile strength determined using a tensiometer that operates by the principle of Hook's law. The tests were carried out for adhesives produced from unfermented starch stored for 0 days; 10 days, 20 days and 30 days for manihot palmate and *Manihot utilissima* respectively.

2.5 Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare three replications of data obtained from both the starch and adhesive analyses while the Ducan's multiple range tests was applied in separating the mean values at a level of significance established at the 5% probability level. The analysis was implemented using Microsoft office excel 2007.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the physico-chemical properties of *Manihot utilissima* and manihot palmate starches are presented in Tables 3.1 and 3.2 while the viscosities of adhesives produced from *Manihot utilissima* and manihot palmate starches are presented in Tables 3.3 and 3.4 respectively.

Table 3.1. Physico-chemical properties of *Manihot utilissima* starch

Fermentation days	Samples label	pH	Lactic acid content	HCN content (mg/kg)
0	U0	4.60 ± 0.05^{b}	0.47 ± 0.00^{ab}	35.74 ± 0.11^{a}
1	U1	5.02 ± 0.02^{b}	$0,44\pm0.02^{b}$	20.05 ± 0.62^{ab}
2	U2	5.39 ± 0.02^{bc}	0.44 ± 0.02^{a}	17.40 ± 0.12^{b}
3	U3	5.74 ± 0.03^{b}	0.38 ± 0.00^{b}	15.59 ± 0.09^{c}
4	U4	6.28 ± 0.08^{a}	0.41 ± 0.00^{b}	10.95 ± 0.31^{bc}
5	U5	6.89 ± 0.00^{ab}	0.32 ± 0.02^{b}	6.93 ± 0.25^{a}

Mean with different superscript within the same column are significantly different ($p< 0.05$)

Table 3.2. Physico-chemical properties of *Manihot palmate* starch

Fermentation days	Samples label	pH	Lactic acid content	HCN content (mg/kg)
0	P0	6.02 ± 0.04^{a}	0.48 ± 0.00^{ac}	2.49 ± 0.12^{cd}
1	P1	6.18 ± 0.02^{cd}	0.39 ± 0.00^{c}	1.92 ± 0.25^{c}
2	P2	6.21 ± 0.00^{b}	0.41 ± 0.01^{a}	1.02 ± 0.18^{a}
3	P3	6.22 ± 0.08^{a}	0.42 ± 0.02^{c}	0.93 ± 0.15^{ad}
4	P4	6.32 ± 0.02^{b}	0.44 ± 0.02^{cd}	0.90 ± 0.33^{b}
5	P5	6.35 ± 0.05^{a}	0.38 ± 0.02^{b}	0.85 ± 0.26^{bcd}

Mean with different superscript within the same column are significantly different ($p< 0.05$)

Table 3.3. Viscosities of adhesives produced from *Manihot utilissima* starch

Storage Time (days)	0	5	10	15	20	25	30
Starch fermentation Time (days)	Viscosity of adhesive produced ($Ns.m^{-2}$)						
0	5.73 ± 0.15^{ad}	5.74 ± 0.05^{cd}	5.75 ± 0.00^{cd}	5.76 ± 2.01^{cd}	5.76 ± 0.00^{cd}	5.77 ± 0.54^{cd}	5.79 ± 0.55^{cd}
1	5.50 ± 0.42^{c}	5.52 ± 0.22^{a}	5.57 ± 0.08^{a}	5.62 ± 0.04^{bd}	5.66 ± 0.47^{c}	5.67 ± 0.16^{bd}	5.76 ± 0.61^{bd}
2	4.45 ± 0.39^{bd}	4.65 ± 1.07^{a}	4.89 ± 0.22^{bd}	4.92 ± 0.08^{ad}	5.01 ± 0.22^{a}	5.07 ± 1.29^{b}	5.14 ± 0.84^{c}
3	4.42 ± 0.08^{c}	4.43 ± 0.02^{a}	4.49 ± 0.18^{a}	4.50 ± 0.29^{ad}	4.55 ± 0.02^{b}	4.63 ± 0.00^{c}	4.76 ± 0.02^{a}
4	4.20 ± 0.00^{a}	4.20 ± 0.09^{c}	4.27 ± 0.02^{ad}	4.32 ± 0.00^{a}	4.53 ± 0.09^{bd}	4.66 ± 0.07^{a}	4.82 ± 0.01^{ad}
5	4.19 ± 0.00^{ad}	4.20 ± 0.12^{bd}	4.20 ± 0.00^{a}	4.26 ± 0.03^{ad}	4.42 ± 0.18^{c}	4.44 ± 0.00^{bd}	4.72 ± 0.83^{ad}

Mean with different superscript within the same column are significantly different ($p< 0.05$)

Table 3.4. Viscosities of adhesives produced from *Manihot palmate* starch

Storage Time (days)	0	5	10	15	20	25	30
Starch fermentation Time (days)	Viscosity of adhesive produced ($Ns.m^{-2}$)						
0	4.36 ± 0.05^{ad}	4.44 ± 0.05^{ac}	4.54 ± 0.0^{ad}	4.68 ± 0.05^{ab}	4.80 ± 0.05^{ad}	4.82 ± 0.05^{a}	4.90 ± 0.05^{a}
1	4.33 ± 0.05^{ad}	4.39 ± 0.05^{cd}	4.44 ± 0.05^{ad}	4.56 ± 0.05^{a}	4.68 ± 0.05^{bd}	4.78 ± 0.05^{a}	4.88 ± 0.05^{ab}
2	4.20 ± 0.05^{a}	4.23 ± 0.0^{a}	4.39 ± 0.00^{ab}	4.55 ± 0.05^{bd}	4.60 ± 0.05^{b}	4.68 ± 0.05^{ab}	4.85 ± 0.05^{a}
3	4.21 ± 0.05^{a}	4.21 ± 0.05^{ad}	4.34 ± 0.05^{cd}	4.51 ± 0.05^{a}	4.51 ± 0.05^{b}	4.69 ± 0.05^{bd}	4.75 ± 0.05^{b}
4	4.20 ± 0.05^{a}	4.20 ± 0.05^{ab}	4.20 ± 0.0^{a}	4.30 ± 0.05^{b}	4.44 ± 0.05^{ab}	4.58 ± 0.05^{b}	4.67 ± 0.05^{a}
5	4.12 ± 0.05^{ab}	4.13 ± 0.05^{ad}	4.18 ± 0.05^{bd}	4.24 ± 0.05^{cd}	4.35 ± 0.05^{a}	4.49 ± 0.05^{a}	4.60 ± 0.05^{ad}

Mean with different superscript within the same column are significantly different ($p< 0.05$)

3.2 Discussion of Results

It could be observed from Tables 3.1 that the pH of starch from *Manihot utilissima* (bitter cassava variety) increases steadily as fermentation days increases. Analyses revealed that at the beginning of the fermentation process *Manihot utilissima* starch had low pH value of 4.60±0.05 when compared with 6.02±0.04 for manihot palmate, which is highly significant *(p = 0.038).* Lower pH in *Manihot utilissima* starch could probably be as a result of the presence of higher cyanohydric acid (HCN) concentration in that cassava variety. However, as the pH of the starch increases, the pH of the effluent water decreases. This is to be expected as fermentation is associated with the fermentative activities of bacteria and yeast [20]. During such fermentation, monosaccharides (glucose and fructose) coming from the breakdown of sucrose are slowly metabolised into organic acids by falcultative anaerobic microorganisms such as lactic acid bacteria, thus reducing the acidic level of the starch while increasing the acidic level of the effluent water. This leads to increase in the pH of the starch and a corresponding decrease in the pH of the effluent water. As the pH of the effluent water decreases to about 5 and below, the cyanohydric acid formed disintegrate to form HCN which is later evaporated from the fermented starch. The findings of Eze and Azubuike [21] agree with this result. The change in pH of manihot palmate is not significant *(p = 0.55)* as can be seen from the results presented in Table (3.2). The low acidic level of starch from this variety of cassava explains this trend. The pH values of cassava starch had been found to be one of the indicators for assessing the quality of adhesives and gums produced from such starch. Rattanapitigom et al. [22] showed that good quality adhesive and gum are produced from starch having a pH value ranging from 4.0 to 5.5. They asserted that, if the pH falls below 4.0 or rises above 5.5 there will be decreased gel strength due to the weakening of the granular structure of cassava starch. From the results of this study, unfermented starch from *Manihot utilissima* as well as starch from that cassava variety fermented for between one and two days meet the requirement for the production of good quality adhesive.

The titratable acidity of starch samples from the two cassava varieties is very low with highly significant level *(p < 0.001)* and it is not affected by fermentation as can be seen from Tables 3.1 and 3.2. The lactic acid content of the two cassava varieties, both in its unfermented and fermented state range between 0.48±0.00 and 0.32±0.02. High concentration of lactic acid in cassava aids its fermentation process and reduces iodine deficiency disorders in cassava products such as starch and garri when eaten [23,24]. However, the lower the lactic acid content, the higher the hydrogen cyanide, the lower the pH, the better suited the cassava starch is for adhesive production.

The hydrogen cyanide content of starch from the two cassava varieties reduces as the fermentation days increases. *Manihot utilissima* has the highest HCN concentration of 35.74±0.11 mg/kg, reducing gradually to 6.93±0.25 mg/kg after the fifth day of fermentation showing a highly significant level *(p = 0.037)* while the HCN concentration of unfermented manihot palmate was 2.49±0.12 mg/kg which reduced to 0.85±0.25 mg/kg after the fifth day of fermentation. These results agree with the submission of Eze and Azubuike [21] that soaking cassava in water for a period of 2-5 days allows a submerged fermentation to occur leading to the breakdown of cyanogenic glycosides with the release of gaseous HCN. From Table 3.2 it is evident that the reduction rate in the HCN concentration of manihot palmate starch is very small since the HCN concentration of the unfermented starch is very low due to its sweet nature. However, despite the fact that *Manihot utlissima* starch has HCN concentration of 35.74±0.11 mg/kg, it is far lower than the HCN content of fresh cassava roots for that cassava variety which ranges between 100 and 500 mg/kg [24]. This drastic

drop in HCN content may be as a result of detoxification processes undergone by the fresh cassava roots during its processing into starch. This observation agrees with the findings of earlier workers that much of the hydrogen cyanide in fresh root cassava is removed during fermentation, washing, soaking and dewatering [25]. Bokanga [26] also noted that when cassava roots are mashed or grated, the enzymes from the damaged plant cell structure can act on the cyanogenic glucoside leading to the hydrolytic release of unstable acetone cyanohydrin and 2-butanone. These unstable compounds spontaneously decompose to the corresponding ketone and HCN which is lost by volatilisation during processing into starch.

From the results of the viscosities of the adhesives produced from both the fermented and unfermented starch for the two cassava varieties measured over a period of 30 days presented in Tables 3.3 and 3.4 respectively, it is clear that the viscosities of the adhesive increases significantly as the shelf life increases *(p = 0.028).* For the bitter cassava (*Manihot utilissima*) variety, the adhesive produced from the unfermented starch sample (U0 sample) and starch fermented for just a day (U1 sample) looks relatively stable over time as can be seen from Fig. 3.1. This may be as a result of its high HCN content and low pH values. On the other hand, the viscosities of adhesives from samples of *Manihot utilissima* fermented for 2, 3, 4 and 5 days (U2–U5 samples) as well as adhesives from fermented and unfermented samples of manihot palmate (sweet cassava; i.e. P0–P5 samples) increases steadily as the storage time increases (Fig. 3.2).

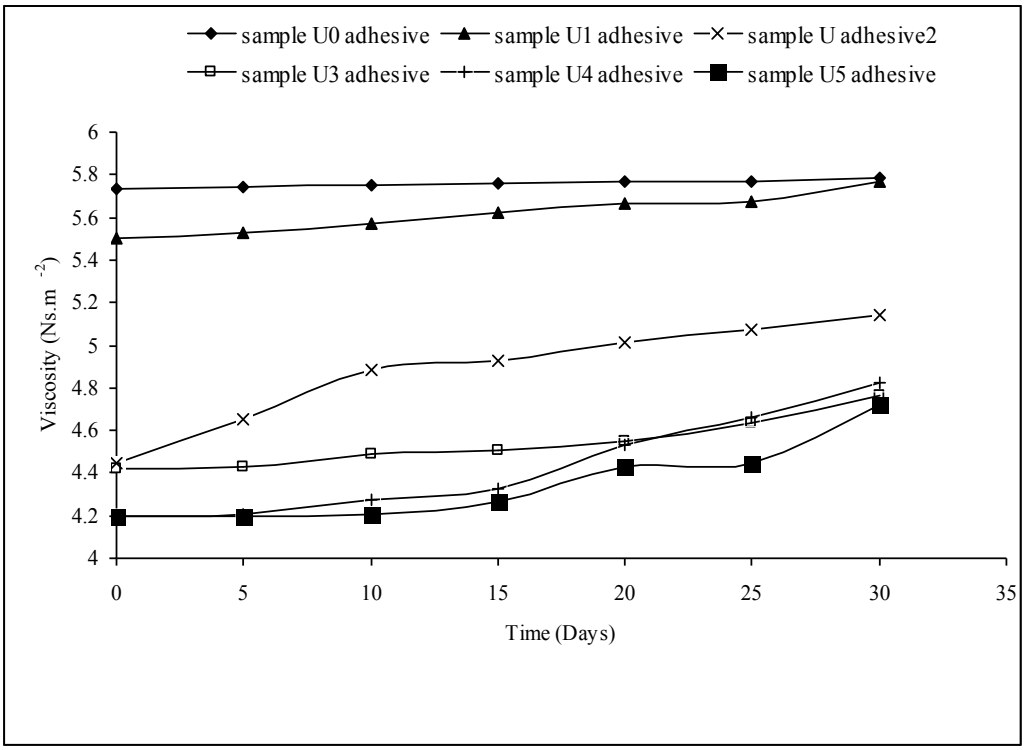

Fig. 3.1. Viscosities of adhesive produced from *Manihot utilisima* (bitter cassava) samples measured over 30 days

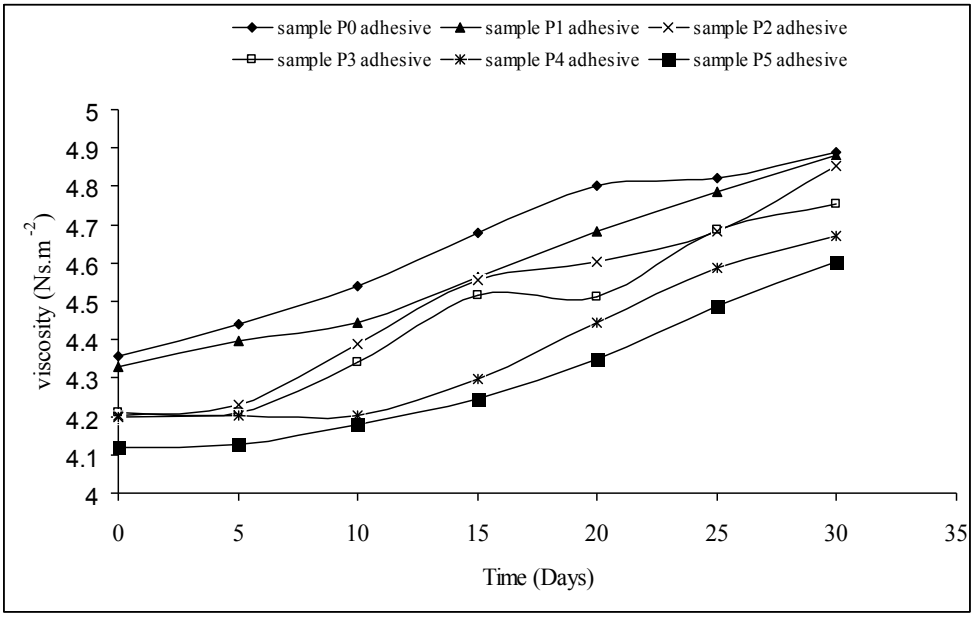

Fig. 3.2. Viscosities of adhesive produced from *Manihot palmate* (sweet cassava) samples measured over 30 days

This trend may be as a result of the extent of retrogradation of the adhesives produced from samples U2–U5 and P0 – P5 respectively. Retrogradation leads to increased rigidity of the starch gel as a result of re-association of the starch granules upon cooling which results in the release of water called syneresis [27,28] resulting in the short shelf life of these adhesives. On the other hand, the viscosity of samples U0 and U1 remained relatively stable for the 30 days period considered with a highly significant effect *(p = 0.018)*. As we can see from the results of the starch analyses, samples U0 and U1 have high HCN content and low pH. It is therefore suggested that the low viscosity and relative stability (over the 30 days test period) of the adhesive produced from these samples is as a result of the high acidity or high HCN content of these samples. The low viscosity and the stability in viscosity of these adhesives may be due to the hydrolysing action of HCN resulting in the formation of radical fissures on the starch granules which keeps the viscosity of the adhesive relatively stable for a reasonable period of time. This observation seems to agree with the results of earlier works [29,30]. Sajeev et al. [30], in their study particularly noted that hydrochloric acid added to starch paste reduces the paste viscosity far lower than when acetic acid was added to the paste. They attributed the lowering of the viscosity to the high acidity of hydrochloric acid.

The result of the drying time/bond strength analyses indicate that the drying time for adhesives produced from unfermented *Manihot utilissima* has a high significant *(p = 0.041*) drying time of 7.1±0.01 minutes when compared to the drying time of 4.4±0.12 for unfermented manihot palmate starch-based adhesive. The drying time for *Manihot utilissima* remained relatively stable over the 30 days storage period while that of *Manihot utilissima* reduced gradually to 2.1±0.02 minutes after 30 days of storage. This shows that as the viscosity of the adhesive increases the drying time decreases. The drying time of adhesives is one indicator of its bonding capacity as shown by the results of the tensiometer. The

higher the drying time the better the bonding strength of the adhesive. This result agrees with that of Akpa [12]

4. CONCLUSION

The effect of starch fermentation on the shelf life of cassava based adhesive was studied. The result of the study shows that cassava starch with high HCN content and low pH produces high quality and stable adhesive. Such adhesive, however, will only be suitable for industrial purposes due to its toxic nature. Nevertheless, if adhesive for domestic application is desired, low HCN starch can be used with salts and non-toxic oxidising agents such as ferrous sulphate, aluminium chloride, sodium hypochlorite and acetic acid added to increase the shelf life.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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