

Comparative Evaluation of the Methods of Palm Sap Fermentation for Bio-ethanol Production

Ubong David Offiong, Godwin Edem Akpan

Department of Agricultural Engineering, Akwa Ibom State University, Ikot Akpaden, Nigeria

Email address:

offiongubong42@gmail.com (U. D. Offiong)

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Abstract: This paper evaluates the methods of fermenting palm sap for bio ethanol production and compares the yield. A survey was carried out in three local government in Akwa Ibom State, Nigeria; Essien Udim, Ikot Abasi and Uruan local government area. Personal interviews were conducted with local palm wine dealers and questionnaires distributed. Three types of palm trees were found dominant in these areas; oil, raphia and nypa palm tree. The sap from these three palm trees were used in investigation. Two methods were investigated; natural and yeasted fermentation using *saccharomyces cerevisiae*. 60% respondents of the questionnaires agreed on using additives to speed up fermentation. Experiments were carried out in which the result shows that yeasted fermentation yields more alcohol than natural fermentation and also reduce the period of fermentation.

Keywords: Bio Ethanol, Evaluation, Fermentation, Methods, Palm Sap, Production

1. Introduction

Ethanol is volatile and highly inflammable which makes it an attractive prospect for alternative fuel. It is vast becoming an essential component in the world today due to its advantage over fossil fuel. Bio-ethanol is produce mainly from sugary and starchy materials. Sugar in the form of molasses and starchy materials in corn and cassava contain high level of fructose. Glucose and sucrose are the easiest to convert to ethanol [12].

Fermentation of palm sap begins immediately after tapping as the sap is stored in an air tight container. It takes five to six days to ferment it naturally, but to speed up the rate of fermentation, some catalyst are added. This catalyst varies from places to places in the local setting. The additives may include cultured yeast, granulated sugar, etc. Mostly used are the cultured yeast which contain enzyme called *invertase* which convert sucrose in sugar to glucose and fructose. The glucose and fructose react with another enzyme called *zymase* also in the yeast to produce ethanol and carbon-dioxide. The fermentation process takes three days to complete and is favourable at temperature between 25^o to 30°C. Below and above these ranges of temperature the enzymes are rendered inactive or slow rate of fermentation is

noticed. The ethanol produce from the fermentation process contain some water which is removed through fractional distillation process [3].

[11] investigated the growth of yeast, lactic and acetic acid bacteria in palm sap during tapping, and he noticed that yeast and lactic acid bacteria were responsible for alcoholic fermentation and souring of the sap, while acetic acid is responsible for the development of vinegary taste in palm wine. Microbiological and biochemical content of the palm sap were determined during felling of palm trees and during storage. The yeast growth discovered to dominate the sap was *saccharomyces cerevisiae*, while *lactobacillus platrium* and *lacoristoic mensenteroides* were the lactic acid bacteria present. The pH, lactic acid and acetic acid bacteria concentration during tapping were; 3.5-4%, 0.1-0.3% and 0.2-0.4% respectively. [11] investigation reveals that palm sap fermented overnight has an alcoholic content between 3.24to 4.75% while palm sap held for 24 hours result in an increase in alcoholic content up to 7%, remain high for the next three days and then drop.

Several other studies show that the unfermented palm sap contains about 10-20 % sugar dominated by sucrose, whereas upon fermentation sucrose is first broken down to glucose and fructose, which are then converted to ethanol, lactic acid and other products via fermentation [6, 8, and 9]

According to [7], the concentration of alcohol in palm sap collected during tapping were found to be low and depend on many factors including the nature and system of fermentation. Sap collected within 8 hours of tapping does not increase more than 3% of alcohol. The sap was collected in the morning and evening, but sap collected in the morning, would have accumulated throughout the night. Such sap contains higher concentration of alcohol ranging from 3% to 6.5%. This is in agreement with the alcoholic concentration reported by [5]. [11] describe fermentation of palm sap in three stages;

- (1) The first stage occurs in the receptacle cut of the palm tree. It occurs as continuous culture fermentation.
- (2) The second stage occurs as palm sap accumulates in the container place under the tree. The alcoholic build up in the container is faster than in the trunk because of the loss of the product from the chamber, although there is a continuous dilution of the content with the fermenting juice trickling in.
- (3) The third stage of fermentation occurs as a batch process under more anaerobic condition which favours alcoholic fermentation by the yeast. *Saccharomyces cerevisiae* has been confirmed the dominating yeast specie responsible for fermentation of palm sap. [10] isolated *s. Cerevisiae* and *k. Apiculata* from palm sap, while [4] reported the presence of other yeast including *s. Cerevisiae*, *candida*, *endomicopia* and *hendiselula* in sap from oil palm, while *s. Cerevisiae*, *saccharomycopia* and *schizosaccharomyces* and *endomyopia* were identified in raphia palm. According to [2] the method of tapping and collection of sap influences the microbial content of the sap.

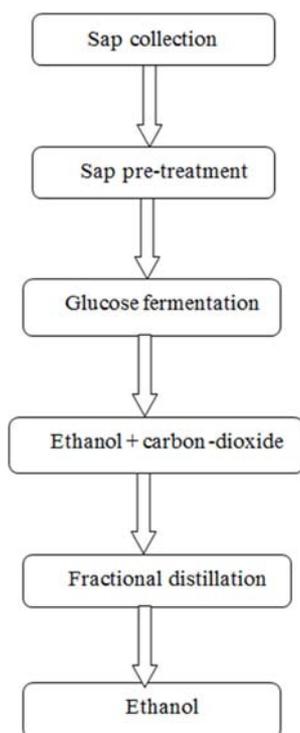


Figure 1. Flowchart of Bio-ethanol Production from Palm Sap. [7].

2. Materials and Methods

2.1. Survey

A survey was carried out from three locations in Akwa Ibom State to ascertain the local methods of ethanol production from palm sap. These locations cut across the three geo-political zones of the state. They are:

- a) Essien Udim local government area
- b) Itu local government area
- c) Ikot Abasi local government area

In each of these locality, personal interviews were granted, questionnaires distributed among palm wine dealers and methods of processing were assessed.

Palm sap sample were collected from three species of palm tree from these locations. They include: Oil palm (*Ealasis guinesis*), Raphia palm (*Raphia hookeria*) and Nypa palm (*Nypa fruticana*). There were put in a sterile plastic containers and the containers were immediately immersed in a freezing mixture of sodium chloride and ice chips and then taken to the laboratory for analysis. The freezing was to delay fermentation process from taking place rapidly.

2.2. Materials Used

The following materials were used in the investigation

- (1) 5 liters of Nypa palm sap
- (2) 5 liters of oil palm sap
- (3) 5 liters of Raphia palm sap
- (4) 10g of saccharomyces cerevisae
- (5) 100ml of distil water
- (6) Acolizer
- (7) 3 Clean rubber containers
- (8) 500ml of YPG broth
- (9) Mercury-in-glass thermometer

2.3. Experimental Method

- (1) Natural Fermentation: Three litres of each of the samples were put in a clean rubber container accurately sealed to prevent air from affecting the fermentation process. The choice of the rubber container was to prevent the effect of environmental factors from affecting the sap, as excessive temperature and lowering of temperature may affect the rate of fermentation. Room temperature of 35°C was conditioned for the fermentation process. They were allowed to ferment naturally for six days. Their alcoholic content was measured every 24 hours using an acouliser.
- (2) Catalysed Fermentation A 24 hour culture of *saccharomyces cerevisiae* was prepared in 500ml of YPG broth and the yeast cell separated by centrifugation and washed with sterile distilled water. The yeast residue was transferred into the three clean plastic containers. Unfermented sap of oil, *nypa* and *raphia* palm were sterilized by autoclaving at 121°C for 15 minute. The medium were then inoculated with *saccharomyces cerevisiae* and kept for six days. Alcoholic content was measured every 24 hours using an acouliser.

3. Results and Discussion

3.1. Survey

Table 1 shows the classification of respondents of the questionnaires sent out based on the method of fermentation of palm sap. 60% respondents accept using additives for fermentation, while 40% still sticks to natural fermentation. With the additives like granulated sugar, it is believed by the respondents that it fastens the rate of fermentation but still produces the amount of ethanol as the natural fermentation.

Table 1. Number of Respondent on Effect of Additives on Fermentation of Palm Sap.

Number of respondents	Classification of respondents	Percentage
30	Yes	18
	No	12
		40

On the assessment of local methods of processing of palm sap, the additive locally use for fermentation was granulated sugar. This may be functional, but a more cultured form of yeast should be adopted. This yeast is the *saccharomyces cerevisiae*. [11] confirms this in his investigation by recommending *saccharomyces cerevisiae* for fermentation of palm sap since it the dominating yeast in the sap. This is in line with the investigation carried out by [1] which he identified *saccharomyces cerevisiae* as the dominating yeast biota in fell palm trees. Fermentation can also be delayed in palm sap. This is made possible through the use of lime. Though this affects the taste of the sap and the colour, no report has been made on its effect on the yield of ethanol from the sap. A freezing mixture of ice chips and salt could be more appropriate.

3.2. Experimental Result

Figure 2 shows the yeild of alcohol with respect to the days of fermentation. The palm sap was allowed to ferment naturally. The graphs are found to be linear. This means that the more the days of fermentation, the more the yeild of alcohol. The volume of alcohol in the palm sap increases as the days of fermentation increase. Among the three varieties of palm sap, Nypa palm sap was observed to produce more alcohol than others.

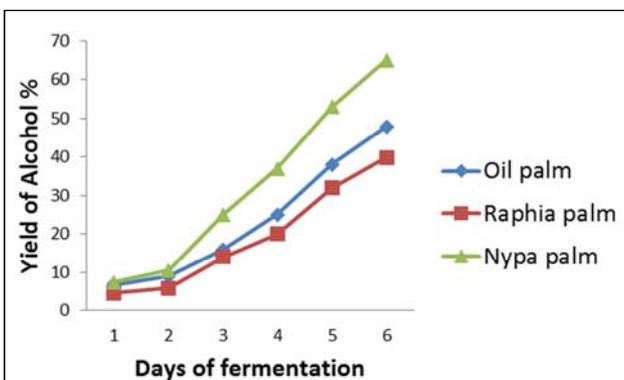


Figure 2. A Graph Showing Alcoholic Yield during Natural Fermentation of Palm Sap.

Figure 3 shows the yeild of alcohol with the use of *saccharomyces cerevisiae*. It could be observed that the yield of alcohol increases in the first four days significantly, signifying that rapid rate of fermentation occur within these days. On the fifth day, there was a reduction in the alcoholic accumulation rate, and on the sixth day, the increment is insignificant. Nypa palm sap is once again observed to produce more alcohol than others. From this observation, it can be concluded that catalysed fermentation period last for four days. As after this period, the yeild of alcohol becomes insignificant.

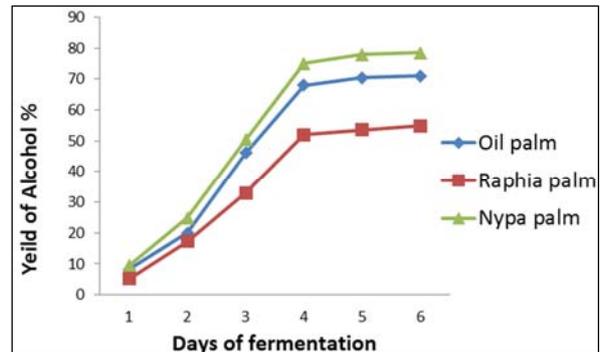


Figure 3. A Graph Showing the Alcoholic Yield during Fermentation using *Saccharomyces Cerevisiae* as Catalyst.

Figure 4 compares the yeild of alcohol in Nypa palm sap between natural and catalysed fermentation. It was observed that on the fourth day of fermentation, alcoholic yield in catalyse fermentation is twice what is obtained from natural fermentation. Also, upon fermenting the palm sap naturally for six days, the yield obtained is still less when compared to the yield obtain on the fourth day with catalysed fermentation. This observation confirms the report from [3] where he concluded that catalyze fermentation does not only reduce the period of fermentation, but also increase the yeild of alcohol.

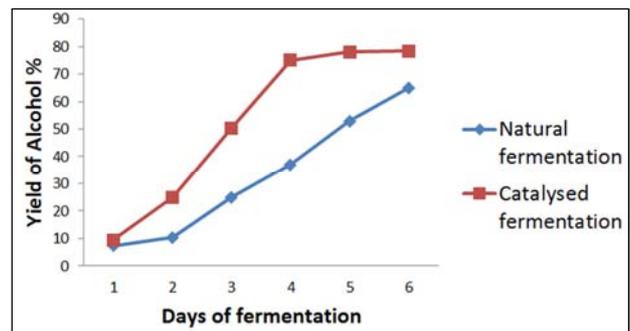


Figure 4. A Graph Showing the Comparison of the Yield of Alcohol in Nypa Palm Sap between the Two Methods of Fermentation.

Table 2. Ethanol Yield from Palm Sap during Natural Fermentation.

Days of fermentation	Oil palm sap (%)	Raphia palm sap (%)	Nypa palm sap (%)
1	6.6	4.5	7.4
2	8.9	5.8	10.5
3	16	14	25
4	25	20	37
5	38	32	53
6	48	40	65

Table 3. Ethanol Yield from Palm Sap using *Saccharomyces Cerevisiae* as Catalyst.

Days of fermentation	Oil palm sap (%)	Raphia palm sap (%)	Nypa palm sap (%)
1	8.3	5.2	9.5
2	20	17.3	25
3	46	33	50.5
4	68	52	75
5	70.5	53.5	78
6	71	55	78.5

4. Conclusion

This research was designed to compare and evaluate the different methods of fermentation of palm sap, and to investigate the possibility of scaling up the process for the production of fuel ethanol. A survey was conducted in three local government area in Akwa Ibom state Nigeria of which palm sap samples were collected from three species of palm tree found dominating in these localities. The samples were subjected to various experimental procedures and it was discovered that;

- (1) Increase in the days of fermentation leads to a corresponding increase in the yeild of bio-ethanol.
- (2) Yeasted fermentation using *saccharomyces cerevisiae* produces bio-ethanol twice the amount produced during natural fermentation.
- (3) There is a reduction in the period of fermentation with yeasted fermentation over natural fermentation.
- (4) Cultured fermentation process produce more alcohol than the natural form of fermentation

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