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Research Article

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Batch fermentation of pawpaw juice into wine using palm wine yeast

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Abstract Pawpaw fruit is a very abundant fruit in Nigeria. Considering its easy perishability, a study on the production of wine from the pawpaw juice was carried out in this work. The pawpaw juice was extracted from the pawpaw pulp with the aid of a slow freezingmethod by allowing the pulp to remain frozen for 3days, after which it was allowed to thaw and the juice was expressed from it in a mechanical screw press. This was followed by batch fermentation of the pawpaw juice to wine using pure yeast strain (Saccharomyces Cerevisiae), isolated and cultured from locally tapped palm wine following standard microbiology procedures. The primary fermentation process lasted for 4days in a laboratory scale anaerobic batch fermenter, into which 99ml of the yeast broth with a microbial count of 9.1×10^6 CFU/ml was inoculated andsamples were withdrawn atintervals of 24 hours to analyse the changes in microbial load, glucose concentration (%), specific gravity, and ethanol concentration (%v/v).

The yeast growth was observed to follow an exponential growth phase and tended towards the stationary growth phase between the 3-4th day. The specific gravity of the wine decreased from 1.0374 to 1.0204 during primary fermentation and 1.0201 at the end of secondary fermentation. The glucose concentration decreased from an initial 31.25% to 4.54% at the end of primary fermentation while the ethanol concentration of the wine increased from 0.3% to 12.5% at the end of primary fermentation which was immediately followed by a secondary fermentation of the pawpaw wine in a clean bottle placed in a refrigerator at a temperature of about 5-10^{o}C for 2 weeks. At the end of secondary fermentation, the alcohol content of the wine was 12.7(%v/v). The wine produced from pawpaw juice meets the characteristics of a good table wine in terms of its flavour, colour, clarity and alcohol content.

Keywords Pawpaw, Fermentation, Yeast, Wine, Extraction, Slow Freezing.

Introduction

In the world and particularly in Nigeria, more ways and investments are being made to develop the agricultural sector, and this calls for a corresponding need for the food processing industries to add value to the products and methods available to process these agricultural products to preserved forms that are still rich in their nutritional values is preferable.

Wine is produced by fermentation of juice of ripe grapes using Saccharomyces cerevisae. Fermentation is a microbial metabolic process during which carbohydrates and other nutrients are oxidized partially to a variety of products such as alcohol, acids and other metabolites and antibiotics and a small amount of energy by microorganism [1].

Virtually all alcoholic beverages are produced using different species of *Saccharomyces*. *Saccharomyces* spp. are generally used because they are comparatively efficient in alcohol production and can tolerate higher levels of ethanol than other fungi. They also produce compounds that are believed to influence the final flavour of the fermented liquid [2].

Within the globalization of the food industry, the demand for quality juice and juice types to be fermented into wine has markedly expanded. Traditionally, only a handful of fruit juices have served this market as large multinational companies or their affiliates, have captured the majority of national and international juice trade. Juices such as orange, grape, pineapple, tomato and blends are well established in developed countries. Fruits juices of apples, berries and blackcurrants are also fermented. These however are referred to as fruits or country wine [3]. Now, minor juices, tropical fruit juices are attracting new attention.

Nigeria being in the tropical belt is blessed with an abundance of exotic fruits and vegetables. One of them is the large, oval shaped, green on the outside, and pinkish orange on the inside, fruit generally referred to as pawpaw. Fresh pawpaw fruits are very perishable, thereby making their export and storage problematic. Large quantities of pawpaw are disposed of yearly due to lack of/or poor storage facilities. This results in loss of the vital nutrients (vitamins) contained in the fruits and revenues obtainable from their sale. However, these losses can be reduced as pawpaw can be made available all year round by utilizing the fruits for other purposes such as wine production.

Pawpaw fruits, have being processed into fruit juice mainly for homemade consumption and not so much on a commercial scale. This can be attributed to the fact that the juice of pawpaw extracted with the conventional fruit extraction method, can be quite viscous and also with so much suspended insoluble solids. Therefore, the need for extracting the pawpaw juice using the slow freezing aided extraction method is considered a better option. Water makes up over 90 percent of the weight of most fruits. This water and other chemical substances are held within the fairly rigid cell walls, which give support structure, and texture to the fruits. [4]. Freezing fruits actually involves freezing the water contained in the plant cells. The water content of these tissues is either inside the cells (intracellular fluid) or surrounding these (extracellular fluid). Since the lowest concentration of solutes is found in the extracellular fluids, the first ice crystals are formed there during freezing [5].

The extent of cell wall rupture can be controlled by the rate at which the freezing takes place. In rapid freezing, a large number of small ice crystals are formed. These small ice crystals produce less cell wall rupture than slow freezing which produces fewer but large ice crystals.

Slow freezing of fruits is the process of allowing the fruit to get frozen at a slow rate such as in a refrigerator. During slow freezing of the pawpaw pulp, the ice crystals formed causes the cell walls to rupture and there will be time for the cell to loose water by diffusion and the water will freeze on the surface of the crystals already formed. As the cells keep losing water, the cell shrinks more and more until it collapses [6].Consequently, the texture of the pawpaw, when thawed, will be much softer than it was before it was frozen. This effect of slow freezing on the fruit texture, can be taken advantage of in the pawpaw juice extraction process as more of the pawpaw juice is easily released with increased clarity, fresh flavour and still retaining the colour of pawpaw.

The sap of *Elaesisguineansis* (palm wine) contains several bacteria and yeast flora microorganisms that bring about natural fermentation in the palm wine [7].

Palm wine yeasts have been found to possess good sedimentation properties for high product recovery. Also these palm wine yeast when compared to baker's yeast are better in producing volatile esters that improve the flavors of the wine during fermentation.[8].

In wine fermentation, sugar, glucose is converted to ethanol and carbon dioxide through a series of enzymes catalysed reactions following the glycolytic pathway. This involves the conversion of glucose first to pyruvic acid and pyruvic acid to acetaldehyde and finally to ethanol.[9]. This paper therefore shows that pawpaw juice can be used to produce effective table wine.

$C_5H_{12}O_6 \longrightarrow 2CH_3COOH + NADH_2$

Glucose Pyruvic acid

$2CH_3COOH \longrightarrow 2CO_2 + 2CH_3CHO$

Acetaldehyde

$2CH_3CHO \longrightarrow 2CH_3CH_2OH + 2NAD$;(With the removal of hydrogen from $NADH_2$)

Ethanol

The overall chemical reaction for the fermentation processwhich represents a short cut of the glycolytic pathway is given as

$C_6H_{12}O_6 + 2ADP + 2PO^{-3}_4 \longrightarrow 2C_2H_5OH + 2CO_2 + 2ATP$

Materials and Methods

Materials

- Ripe pawpaw fruits were purchased from the local market inChoba, Port Harcourt, Rivers state, Nigeria. After removal of the covering from the pulp and the pawpaw seeds, the weight of the pawpaw pulp used was measured as 3.098kg.1ml of palm wine was obtained from a palm tree in Alakahia community, Rivers state, Nigeria.
- Other materials used in the course of the isolation, culturing and propagation of the pure yeast strain (*S.Cerevisiae*) areDistilled water, lactic acid, Potato Dextrose Agar (PDA) (39g/l), ethanol (99.7% purity)
- Anthrone reagent, Glucose (98% purity) and distilled water were used in analysing the changes in glucose concentration of the pawpaw juice during the fermentation process.

Fruit Juice Extraction

The ripe pawpaw fruits were washed, their outer skin of the pawpaw fruits were peeled off and the pulp were dissected to remove the seeds.This was followed by rinsing of the pawpaw pulps with warm water and slicingthem into small sizes into a transparent plastic container and placed inside a freezer for 3days to undergo slow freezing. After 3days, the pawpaw was brought out of the freezer and allowed to thaw completely. Subsequently, the thawed pawpaw pulp was poured into a muslin cloth and the juices was pressed out using a mechanical screw press.

50cl of clean water at a temperature of 60^0C was added to the residual pulp from the press and allow to stand for about 40mins.This comminuted pulp of the pawpaw was pressed again to release the juicefrom it.The total quantity of the juice obtained from the pawpaw pulp which weighted 3.098 kg was 2.3litres.

Yeast Cell Isolation And Culture

The Yeast cell, *Saccharomyces cerevisae* used for the fermentation was isolated from palm wine by carrying out a ten-fold serial dilution of the palm wine and culturing 1ml of the palm wine solution on a Yeast Potato Dextrose Agar (PDA) broth, at 25^{o}C for 24 hours, using the spread plate technique. Yeast cells were collected from the growths observed after 24hours and sub cultured on newly prepared PDA broth for another 24 hours, using the streak plate technique, so as to obtainpure strains of S.*cerevisae*.Finally a strain of the pure yeast cell was collected from the petri dish and propagated for 24 hours in a newly prepared liquid PDA broth. The microbial count was carried out on the liquid broth after the 24 hours, just before it was inoculated into the batch fermenter and the initial yeast population was obtained as 9.1×10^6 CFU/ml.

Colony forming Unit (CFU) =**Average Colony X** $\frac{1}{\text{Dil}}$ **X** $\frac{1}{\text{Vol}}$

Where Dil represents Dilution factor &Vol represents Volume of sample inoculated into nutrient broth.

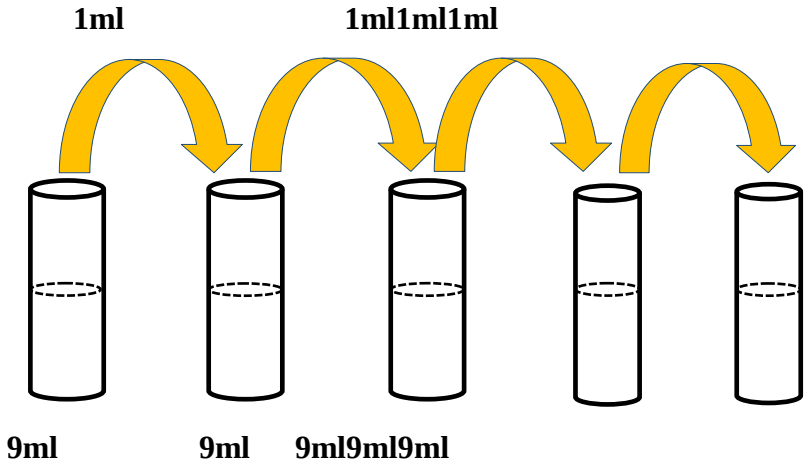

Figure 1: Schematics of the Ten –fold Serial Dilution

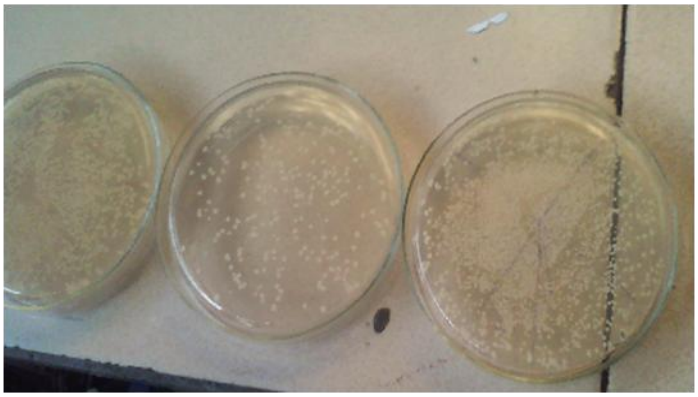

Figure 2: Yeast growth cultured by the spread

Figure 3: Yeast growth plate method for microbial countstreaking on a petri dish

Batch Fermentation of Fruit Juices

The fermentation of the pawpaw juice was carried out in a batc into whicha thermometer wasfitted for temperature measurement, corks placed in some openings to ensure

complete anaerobic condition, a sample collection point, and another cork through which a hose was connected into an air tight conical flask containing acid water for the carbon dioxide produced during fermentation to be dissolved in. Primary fermentation lasted for 4days while secondary fermentation lasted for 2 weeks.The pawpaw juice (2liters) was poured into the fermenter and the whole fermentation set up was sterilized in a pressure pot and thereafter allowed to cool. The 99ml liquid media yeast culture was introduced into the juice in the fermenter through one of the corked openings. This marked the beginning of the fermentation process.At this time t=0, 200ml was withdrawn for the analysis of the microbial concentration, ethanol concentration, and glucose concentration.The culture was maintained at a temperature of range of 25-32^0 C for 4days (96hrs).Samples were withdrawn at 24hrs.intervals for the 4days to analyse the changes in microbial concentration, ethanol concentration, and glucose concentration.

Wine Analysis

During the fermentation process the wine undergoing fermentation was analysed to monitor the changes in the microbial growth, glucose concentration and alcohol concentration at every 24 hours interval from the 0hr to the 96 hr.

Glucose Concentration Analysis

The glucose concentration test was carried out at the Food Industrial Laboratory in the Department of Microbiology in the University of Port Harcourt using the Cleg Anthrone method.

- 0.1g of the wine sample was weighed out into a 25ml volumetric flask.
- 1ml of distilled water and 1.3ml of perchloric acid was added to the sample and the mixture was shaken for 20 minutes to homogenize completely.
- The flask was then filled up to the 25ml mark with distilled water and stopper.
- The solution formed was filtered through a paper filter, after which 1ml of the filtrate was collected with a pipette and diluted into 9ml of distilled water in a 10ml test tube.
- 1ml of the working solution obtained was pipetted into a clean test tube and 5ml of the anthrone reagent was added to it.
- 1ml of distilled water and 5ml of the anthrone reagent was mixed in another test tube (as blank solution). Also1ml of a diluted standard glucose solution and 5ml of the anthrone reagent was mixed in another test tube (as standard solution).
- The absorbance of the three solutions were read at 480nm in a photometer colorimeter and the value of the carbohydrate as glucose was calculated using the formula.

$$\% \text{ CHO as glucose} = \frac{25 \text{ X Absorbance of Sample}}{\text{Absorbance of standard Solution}} \text{ X } 100$$

Alcohol Concentration Analysis

The alcohol concentration test was also carried out at the Food Industrial Laboratory in the Department of Microbiology in the University of Port Harcourt following the Density bottle method.

- The 200ml sample collected is made to undergo distillation in a simple distillation set up in which the ethanol is collected as distillate and condensed in a Liebig condenser. Heat was added until about 25ml of the condensed distillate is collected in a beaker.
- The density bottle was first dried in an oven and allowed to cool to room temperature in adesiccator.
- A 25ml density bottle was weighed to get the actual weight of the bottle as 18.140g.
- The density bottle was then filled to the brim with the condensed ethanol distillate.
- The weight of the samples in the density bottle was measured with a digital weighing balance. The formula below was then used to calculate the density of the ethanol sample:

$$\text{Density} = \frac{\text{Mass of sample}}{\text{Volume of density bottle}}$$

- Standard ethanol solutions of 0%, 5%, 10%, 15% and 20% ethanol were prepared by dissolution of standard alcohol in distilled water and the steps above was repeated for each of these standard ethanol solutions and the values obtained were recorded.
- A graph of the density against alcohol percentage was plotted for the standard ethanol solutions and the plot was use toobtain the ethanol percentages in the samples by getting the values that corresponds to the density of the samples as calculated above.

Secondary Wine Fermentation/Bottling

At the end of the four days primary fermentation, the fermented wine was transferred from the fermenter into a sterile bottle. It was corked and placed in a fridge at close to 5^o C for a period of 2 weeks. At the end of the 2 weeks the, clear wine was decanted into a new sterile bottle and corked. A sample of the wine was tested for final alcohol percentage in the wine.

Figure 4: Bottled pawpaw wine at the end of secondary Fermentation

Figure 5: Primary Fermentation set up of the pawpaw juice

Figure 6: Sedimentation during secondary fermentation of the wine

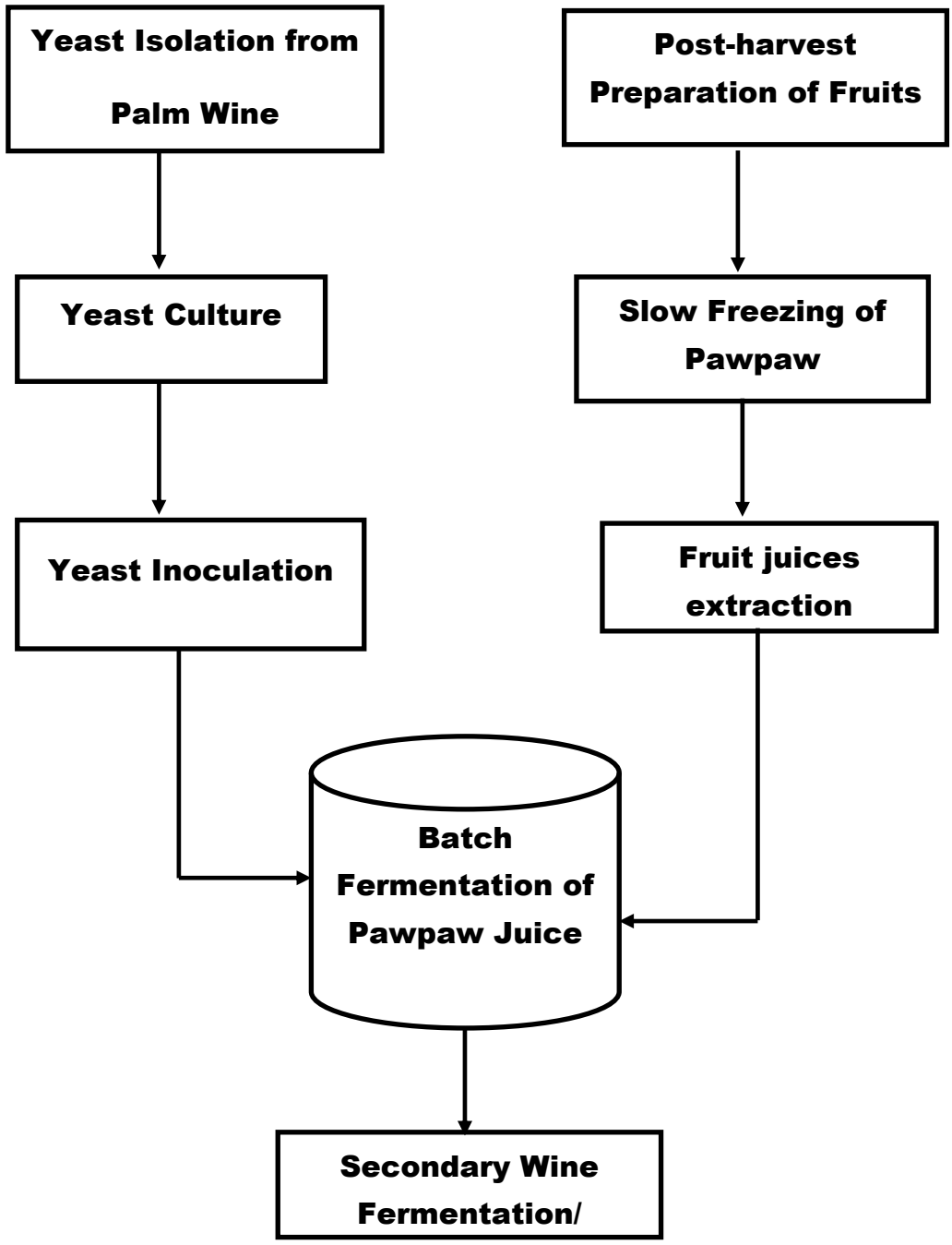

Figure 7: Process Flow Diagram of the Pawpaw Wine Production Process

Results and Discussions

Table 1: Results of Wine Analysis during Fermentation of Pawpaw Juice

Time (Hrs)	Glucose Concentration %	Density (g/cm^3)	Specific Gravity	Alcohol Concentration %	Microbial Load (CFU/ml)
0	31.25	1.03744	1.03744	0.35	2.05E+07
24	22.73	1.02968	1.02968	5.9	1.96E+08
48	15.91	1.0259	1.02968	8.7	3.19E+08
72	6.82	1.0219	1.0219	11.5	3.48E+08
96	4.54	1.0204	1.0204	12.5	3.58E+08
End of Secondary Fermentation		1.0201	1.0201	12.7	-

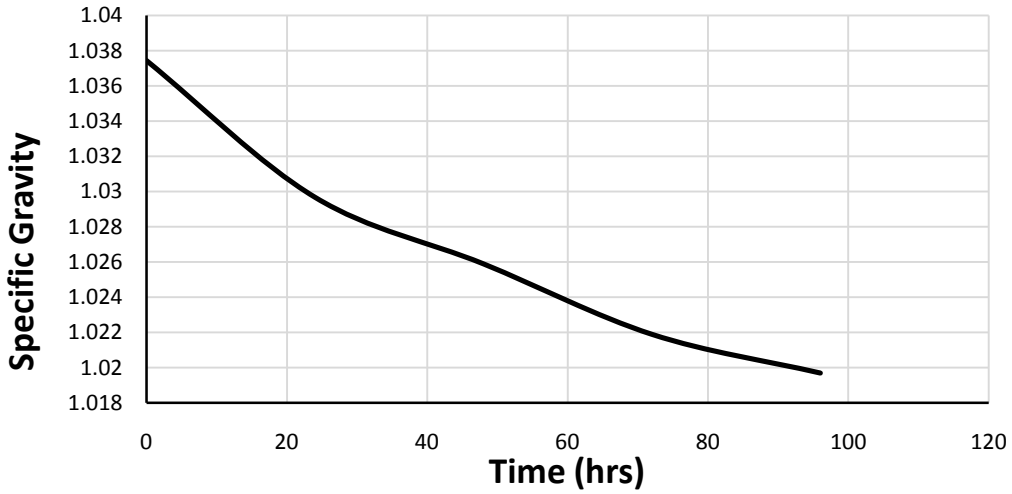

Figure 8: Graph of Specific Gravity of wine samples against Time (hrs)

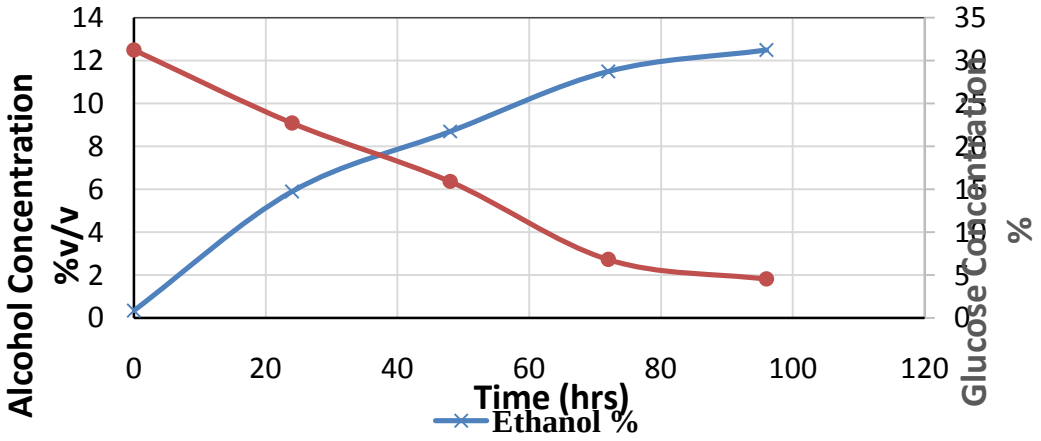

Figure 9: Graph of Alcohol (%) and Glucose (%) in wine against Time

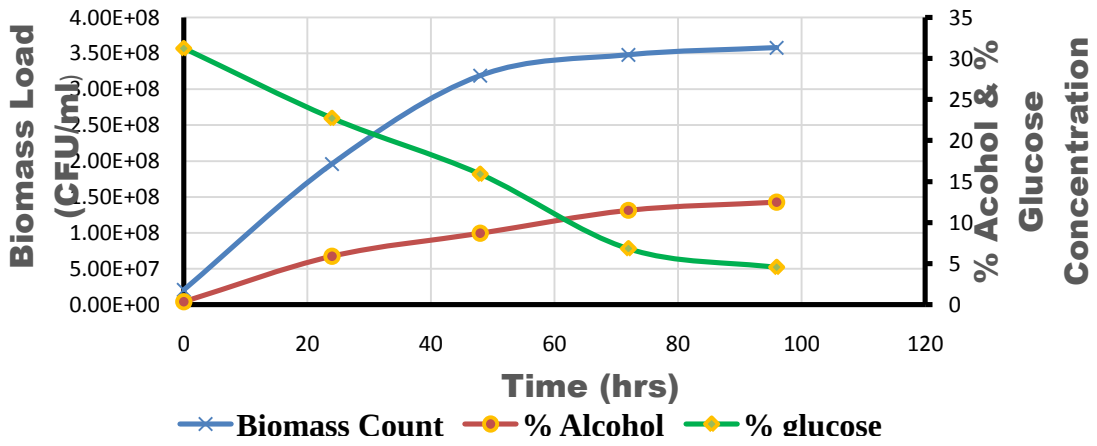

Figure 10: Graph of Alcohol (%), Glucose (%) and microbial load against Time

Rate of glucose consumption

Fig.9 shows that the glucose concentration of the pawpaw juice decreased with time during thefermentation process. This is attributed to the metabolism of the yeast cells which involves the utilization of the glucose for their growth. The high decrease in sugar between 0-24hr brought about a corresponding increase in the ethanol (0.3% to 5.9%) in the wine as the initial glucose percentage of the pawpaw juice (31.25%), was rapidly consumed by the yeast to 22.73%.

Rate of Ethanol Production

Fig.9 shows that ethanol concentration (%) increased with time, during the fermentation of the pawpaw juice. The highest change in the ethanol concentration was observed between 0-24hrs. This can be attributed to the rapid growth of the yeast cells during this period, as a result of the initial glucose concentration in the pawpaw juice. A decrease in the rate of alcohol production observed as fermentation time increased especially between 72-96th hours and also during secondary fermentation was as a result of the low glucose in the wine and it can also be attributed to inhibition of yeast growth by the accumulated ethanol in the fermentation broth. The final alcohol content of the wine (12.7%) after secondary fermentation ranks it among good table wines. According to Michael (2000) [3], a good table wine must have alcohol content between 8 and 14%.

Changes in specific gravity of wine

The drop in the specific gravity of the wine, as shown in fig.8, from 1.03744 to 1.0204as primary fermentation proceeded corresponds to the increase in ethanol content of the wine since as the alcohol concentration increases the density of the wine decreases. The specific gravity is also an indication of the decrease in turbidity and increase in the clarity of the wine, brought about by settling offlocs of dissolved solids at the bottom of the fermenter, during fermentation.

Growth rate of Yeast cells

Figure 10 shows the trend of the microbial load in the pawpaw juice during the fermentation process. The curve indicates the exponential growth of the yeast cells (from 0 to 48hrs) after which the rate of the cells growth decreased gradually tending towards the stationary growth phase**.** Another major observation was that the high initial concentration of the yeast cells (2.05×10^7 CFU/ml) brought about the fast rate of fermentation of the pawpaw juice. There was also a high sedimentation rate of the dead yeast cells leading to clarification in the wine during secondary fermentation. This explains theconditioning function of secondaryfermentation on the wine, since at this stage, all the sugar had been consumed and the alcohol in the wine has become toxic to the yeast cells, leading to their death.

Conclusion

The slow freezing method employed in this work for the pawpaw juice extraction has proven to an efficient fruit juice extraction method especially for fruits like banana, pineapple and mango etc. As observed in the juice yield (2.3litres from 3.098g of pawpaw pulp), the flavor, colour and clarity of the juice were all commendable. Yeast isolate from palm wine for use in the production of alcoholic beverages like wine is better when compared to the commercial bakers' yeast in terms of the flavour of the wine produced and the alcohol tolerance of the yeast.

The initial total sugar concentration of the fruit juice determines the extent of fermentation and the alcohol concentration at the end of fermentation.

Finally, the pawpaw fruit wine produced in this study is acceptable in terms of its alcohol percentage (12.7%), clarity, flavour and colour. Therefore, pawpaw just like many other tropical fruits can be used to produce good table wines.

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