

alcohol. It is also used more broadly to refer to the bulk growth of microorganisms on a growth medium.

Fermentation has been used by humans for the production of food and beverages; it is employed for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers and yogurt, as well as for producing alcoholic beverages such as wine and beer.<sup>13</sup>

Fermentation could be divided into two types:

Ethanol fermentation: also known as alcoholic fermentation is the production of ethanol and carbondioxide.

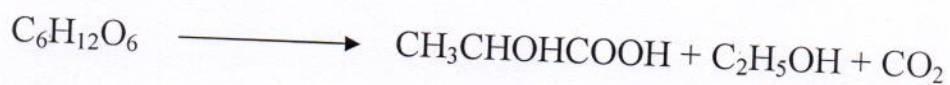


Lactic acid fermentation: refers to two means of producing lactic acid:

Homolactic fermentation is the production of lactic acid exclusively.



Heterolactic fermentation is the production of lactic acid as well as other acids and alcohols



### 1.3.1 ETHANOL FERMENTATION

In this fermentation process, one glucose molecule is converted into two ethanol molecules and two carbon dioxide molecules:



$\text{C}_2\text{H}_5\text{OH}$  is the chemical formula for ethanol. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. Alcoholic fermentation occurs in the production of alcoholic beverages, ethanol fuel and in the rising of bread dough.<sup>13</sup>

Yeast (*Saccharomyces cerevisiae*) is a member of the fungi family, often referred to as plants but strictly they are neither plant nor animal. To be specific yeast is a eukaryotic micro-organism. Not all yeasts are suitable for brewing. In brewing we use the sugar fungi form of yeast. Yeasts bring about fermentation by secreting certain enzymes (like zymase or invertase). These yeast cells gain energy from the conversion of the sugar into carbon dioxide and alcohol. The carbon dioxide by-product bubbles through the liquid and dissipates into the air.

The main product obtained (ethanol) has the following properties; molar mass  $46\text{g mol}^{-1}$ , density  $0.789\text{g/cm}^3$ , melting point  $-114^\circ\text{C}$  and boiling point of  $78.37^\circ\text{C}$ .<sup>14</sup>

## CHAPTER TWO EXPERIMENTAL

### 2.1 APPARATUS

The materials required for fermentation process include:

1. Beaker.
2. Parafilm or saran wrap to cover beaker.
3. Round-bottom flask.
4. Thermometer.
5. Soxhlet apparatus.
6. Pipette
7. Conical flasks.
8. Yeast.
9. Electric stove.
10. Sweet potato.

The following conditions are also required for fermentation and some varied for the purpose of the project research:

1. Carbon sources (Sweet potatoes)
2. Temperature (varied from 25°C to 45°C at interval of 5°C)
3. Time (varied from 24 hours to 96 hours at interval of 24 hours)

## **2.2 FERMENTATION PROCEDURES**

The following methods are carefully carried out:

### **2.2.1 COOKING OF SWEET POTATOES**

The sweet potato was cleaned with water to remove soil and other foreign materials. It was cut into small pieces and put inside the cooking vessel. Water was added at the rate of 2:1 w/v of sweet potato and it was cooked well with the electrical stove.

Cooking was accomplished by heating and stirring the mixture of sweet potato and water to slow boil for 30-60 minutes. Generally, the mash was sufficiently cooked when it was soft.

### **2.2.2 COOLING AND DILUTION OF MASH**

After cooking, the content was allowed to cool in the same container. This was followed by dilution with water of 1:2 w/v. Dilution is necessary to prevent death of yeast (microbes) at under high alcohol concentration. Dilution also facilitates easy stirring and processing of the starchy material.

Yeast was then added to the diluted mash. The quantity of yeast added varied on the different mass ratio of yeast to potato for the experiment i.e. 1:100, 1:50 and 1:10.

The temperature of the diluted mash was regulated by placing in the oven at the specific temperature i.e. 25 °C, 30 °C, 35 °C, 40 °C and 45 °C, and duration of 24, 48, 72 and 96 hours.

The flow chart for the production is shown in the figure 1:

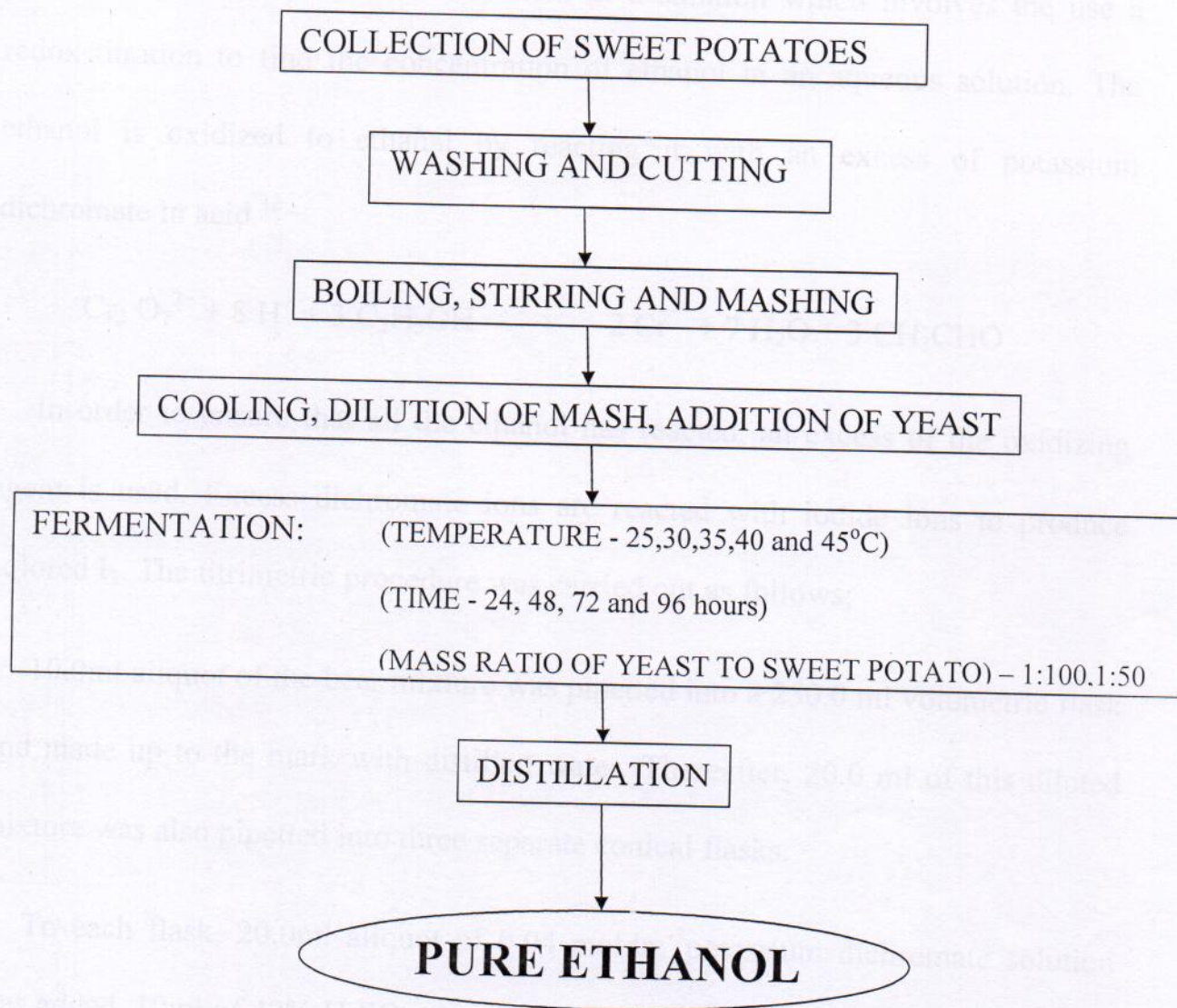


Figure 1: Flowchart for the production of ethanol from sweet potatoes

### 2.2.3 DETERMINATION OF ALCOHOLIC CONTENT BY TITRIMETRIC ANALYSIS

The alcohol content was then determined by using Bogorad and Lin (2012) method of calculating alcoholic content in a solution which involves the use a redox titration to find the concentration of ethanol in an aqueous solution. The ethanol is oxidized to ethanal by reacting it with an excess of potassium dichromate in acid.<sup>12</sup>



In order to ensure that all the ethanol has reacted, an excess of the oxidizing agent is used. Excess dichromate ions are reacted with iodide ions to produce colored  $\text{I}_2$ . The titrimetric procedure was carried out as follows;

10.0ml aliquot of the beer mixture was pipetted into a 250.0 ml volumetric flask and made up to the mark with distilled water. Thereafter, 20.0 ml of this diluted mixture was also pipetted into three separate conical flasks.

To each flask, 20.0ml aliquot of  $0.04\text{ mol dm}^{-3}$  potassium dichromate solution was added. 10ml of 40%  $\text{H}_2\text{SO}_4$  was also added to each flask. The flasks were then heated in a water bath at  $45\text{ }^\circ\text{C}$  for 10 minutes.

2g potassium iodide was then added to each flask and the resulting solution was titrated against  $0.01\text{ mol dm}^{-3}$  sodium thiosulphate solution.

1 ml of 1% starch solution was then added and the titration continued until blue color disappeared and the solution was turned green. The titration was carried out in triplicates and the average titre value recorded.

Average titre value for the sodium thiosulphate solution was calculated and then the concentration of the ethanol was then calculated.

In each titration, the burette is filled thrice as the volume of thiosulfate used in each titration exceeded 100ml.

#### **2.2.4 PRODUCT RECOVERY**

The mash after being fermented contains water, ethanol and carbondioxide (which is given off as by product). The ethanol produced was recovered by distillation. Pure ethanol boils at 78 °C. Distillation was repeatedly carried out to ensure high yield of ethanol.

## CHAPTER THREE

### RESULTS

#### 3.1 DETERMINATION OF ALCOHOLIC CONCENTRATION USING TITRIMETRIC ANALYSIS

Ethanol can be oxidized to ethanal (acetaldehyde,  $\text{CH}_3\text{CHO}$ ) using a suitable oxidizing agent such as acidified potassium dichromate solution ( $\text{K}_2\text{Cr}_2\text{O}_7$ ).

Redox titration method is used to find the concentration of ethanol in an aqueous solution. The ethanol in this work was oxidized to ethanal by reacting it with an excess of potassium dichromate under acidic conditions.



In order to ensure that all the ethanol has reacted, an excess of the oxidizing agent is used. Excess dichromate ions are reacted with iodide ions to produce colored  $\text{I}_2$ .

The amount of unreacted dichromate is then determined by adding potassium iodide solution which is also oxidized by the potassium dichromate forming iodine.



The iodine is then titrated with a standard solution of sodium thiosulfate and the titration results are used to calculate the ethanol content of the original solution.



Tables 3 and 4 contain the concentration ratios of ethanol of the various kind of sweet potatoes.



TEMP	25°C				30°C				35°C				40°C				45°C			
	1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10		
Mass ratio of yeast to sweet potato																				
24 HOURS	13.28	13.93	14.28	14.24	15.16	15.40	15.23	16.46	16.75	15.22	16.17	16.37	15.58	16.07	16.35					
48 HOURS	13.93	14.49	14.75	14.58	15.67	16.22	16.26	17.70	18.08	16.69	17.43	17.72	16.53	17.16	17.52					
72 HOURS	12.91	13.17	13.19	13.05	13.34	13.52	14.67	15.91	16.30	14.99	15.45	15.67	14.75	14.97	15.02					
96 HOURS	12.30	12.87	13.02	12.98	13.33	13.36	14.01	15.05	15.60	13.91	14.53	14.89	13.52	13.82	14.00					

Table 3: Alcohol content (%)calculated for Carolina Ruby sweet potatoes at different fermentation conditions.

TEMP	25°C			30°C			35°C			40°C			45°C		
	Mass ratio of yeast to sweet potato			1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10	1:100	1:50	1:10
24 HOURS	11.93	12.58	13.13	12.89	13.61	14.05	13.88	15.11	15.40	14.27	14.82	15.02	14.23	14.72	15.00
48 HOURS	12.58	13.14	13.40	13.23	14.32	14.87	14.91	16.35	16.73	15.34	16.08	16.37	15.18	15.98	16.17
72 HOURS	11.55	11.82	11.84	11.70	12.10	12.17	13.32	14.56	14.95	13.64	14.10	14.32	13.40	13.62	13.67
96 HOURS	10.95	11.52	11.67	11.63	11.88	12.01	12.66	13.83	14.25	12.56	13.19	13.34	12.26	12.57	12.65

Table 4: Alcohol content(%) calculated for O'Henry sweet potatoes at different fermentation conditions.

## CHAPTER FOUR

### 4.1 DISCUSSION OF RESULTS

#### 4.1.1 EFFECT OF TIME ON FERMENTATION EFFICIENCY

From tables 3 and 4, fermentation of sweet potato proceeded most favorably at 48 hours as this time yielded the highest percentage of alcohol.

The improved yield at 48 hours might be due to increased microbial activities upon prolonged exposure (48 hours). However, a decrease in the alcoholic content was observed at 72 and 96 hours of treatment. This is attributed to consumption of alcohol by the microbes (yeast). The microbes, thus became dormant due to lack of sufficient substrate (sugar or starch) which could be converted to alcohol.

The result of this study agreed with previous works by Sankaranarayanan and Mukarukaka<sup>16</sup>. The study revealed that the alcohol production from potato increases up to 48 hours of fermentation, thereafter, the alcohol content decreased, though, the fermentation continued.<sup>16</sup>

#### 4.1.2 EFFECT OF MASS RATIO OF YEAST TO SWEET POTATO ON FERMENTATION EFFICIENCY

Tables 3 and 4 showed that fermentation of sweet potato proceeded most favorably a higher mass ratio of yeast to sweet potato. The improved yield at a

higher mass ratio of yeast to sweet potato might be due to increased microbial activities upon increase in the microbes (yeast).

This result of this study agreed with previous work carried by Jamie and Ari<sup>17</sup> on "Optimal Amount of Yeast per 100g of sugar for the production of alcohol". The hypothesis was tested with 100g of sugar which was fermented with different amount of yeasts. The highest amount of yeast produced the greatest amount of alcohol.<sup>17</sup>

However, yeast saturation was observed at mass ratio of yeast to sweet potato of 1:10 as this led to little increase in alcohol content produced when compared to the difference between 1:100 and 1:50 mass ratio.

#### **4.1.3 EFFECT OF TEMPERATURE ON FERMENTATION EFFICIENCY**

Temperature has an exponential effect on fermentation reaction and others such as bacteria growth. Yeasts bring about fermentation by secreting certain enzymes (like zymase or invertase) and enzymes have an optimum temperature or a temperature range which is essential for them to take effect.

Thus, from tables 3 and 4, it could be deduced that the optimum temperature required is 35°C as this temperature proceeded most favorably with a higher yield of alcohol produced. This improved yield at 35°C might be due to increased

microbial activities as the microbes (yeasts) are activated at this temperature and hence, are more active and collides more rapidly.

However, temperature above 35 °C gives lower yield. This is attributed to the dormancy of the microbes (yeasts) as a higher temperature could kill or deactivate the yeast enzymes.

In view of this, the most efficient fermentation conditions are 48 hours fermentation time, fermentation temperature of 35°C and a mass ratio of yeast to potato that would not be oversaturated on the potato mash.

#### **4.1.4 EFFECT OF SWEET POTATO VARIATION ON ALCOHOL PRODUCTION.**

It was also observed that more alcohol is produced from Carolina Ruby when compared to O'Henry variety of sweet potato. The improved yield might be due to the fact that Carolina Ruby contains much more sugar content than its counterpart. This aligns with the organoleptic test carried out on both varieties that ascertained that Carolina Ruby is sweeter than O'Henry and hence, it has more fermentable substrate (sugar) which is acted upon by the yeast to produce more alcohol.

#### **4.2.1 CONCLUSION**

The use of ethanol by human beings cannot be overemphasized as it is of great importance in barely all areas of human life.

In view of this, ethanol production must be enhanced and one of its major biological methods of production is the fermentation method. Since sweet potato has been singled out among other major food staples for its high sugar content, then it is a promising substrate for alcohol fermentation. Moreover, sweet potatoes have a short shelf life of three to five weeks after harvest, thus, in order to prevent its spoilage, its use in the production of alcohol is of great potential.

From this study, the most efficient fermentation conditions have been established i.e. 48 hours fermentation time, fermentation temperature of 35°C and a mass ratio of yeast to potato that would not be oversaturated on the potato mash.

Although the production of alcohol is not a new study in science, however it needs to be domesticated as it could serve as a source of internally generated revenue and a source of job opportunities for many.

#### **4.2.2 RECOMMENDATION**

In the face of dwindling revenue at the local and federal levels, it is advisable that sweet potatoes should be cultivated on industrial scale in order to increase alcohol

production, thus, improving its availability, which in turn reduces the cost and makes it cheaper and more accessible for effective use and the money generated, could serve as an Internally Generated Revenue (IGR).

It is also recommended that the Department of Industrial Chemistry liaises with the Faculty of Agriculture on the cultivation of sweet potatoes, which will be sold at a cheaper rate when compared to its market price. The sweet potato can then be used to produce ethanol which can be used in the department; and can also be sold to other departments, school clinics and hospitals. The findings of research work if well implemented could also serve as a source of foreign exchange for the nation; as ethanol is usually imported for its diverse use in science and technology. Furthermore, the production of ethanol from this study could also provide income and job opportunities for the nation's citizens.

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