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Production of Bio ethanol from Sweet Sorghum (*Sorghum bicolor*) Juice

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Abstract **– The use of fossil fuels contributes to global warming and there is a consequent need to resort to clean and renewable fuels. The production of biofuel from plant is becoming an attractive alternative to non-renewable fossil energy sources. Sweet sorghum is a very good alternative for bio-ethanol production to meet the energy needs of the country. Sweet sorghum is drought resistant, water logging resistant and saline–alkaline tolerant. Growing sweet sorghum for ethanol production is relatively easy and economical and ethanol produced from sweet sorghum is eco-friendly. The objective of this study was determine characterize of sweet sorghum juice, ethanol production from sweet sorghum juice at optimized fermentation conditions and the effect of pH, yeast concentration (saccharomyces cerevisiae) and the time of fermentation on the ethanol yield and ethanol concentration. Standard methods were used for analysis sucrose, glucose and fructose content by HPLC method. The total titratable acidity was determined by titration against 0.1N NaOH to a phenolphthalein end point, the pH using a pH meter, while moisture, protein and ash content by AOAC methods. GC methods with external standards were used to qualitatively and quantitatively determine ethanol concentrations in final bio-ethanol for three samples 1, 2 and 3. The fructose, glucose and sucrose concentration averaged 37.84, 39.5 and 120.16g/l respectively, moisture content is 81.6%, ash content is 0.53%, protein content is 1.6%, total titratable acidity is 0.25% as malic acid and pH is 5.13. The initial pH of the fermented juice decrease with increase in time from 5.13 to 3.9 at the end fermentation. Ethanol yield increase with increase in time from 5.9% to 8.5% for 24 to 72 hours. Ethanol concentration for three sample 1,2 and 3 was 44%, 38.2% and 39.2% respectively. Obtained high ethanol yield and high final bio-ethanol concentration for bio- ethanol yield was 8.5% and 44% respectively produce at (pH 5, 2% yeast concentration and 30 ± 1°C for 72 hours).**

Keywords **– Sweet Sorghum, Fermentation, Saccharomyces Cerevisiae, Bio-ethanol.**

I. INTRODUCTION

1.1 *Backround Study*

The energy system of the future must be renewable, sustainable, cost-effective, convenient and safe. Given the present demand for fossil fuel, the depletion of the world's petroleum resources is inevitable. In the last 30 years, worldwide efforts toward identifying, developing and commercializing technology for alternative transportation fuel has gained significant momentum [1]. Burning petroleum has become the major mechanism of global climate change primarily due to the emission of carbon dioxide to the atmosphere [2].

Sweet sorghum also called (Sorgo) is an African plant belonging to the family *Sorghum bicolor* (L) Moench [3]. The plant's stalks are used for bio-fuel production, while the grain can be utilized for food or livestock feed. This crop is also not in high demand in the global food market, and thus has little impact on food prices and food security. "We consider sweet sorghum an ideal 'smart crop' because it produces food as well as fuel [4]. Sweet Sorghum refers to a type of sorghum that store carbohydrates in form of simple sugars (Sucrose, glucose and fructose) in the stalk, with sugar concentrations of 8-20% [5]. Sweet sorghum is considered a versatile and potentially ideal high-energy crop as it offers numerous characterize [6]. Sweet sorghum, which can be grown under widely differing climatic conditions, has been identified as a promising crop with the potential to provide for a wide spectrum of energy uses. It has low sulphur content, high calorific value, and a CO_2 balance close to zero hence its importance as a non-polluting source of energy [7]. The chemical composition of the sweet sorghum juice include, sugars namely, sucrose, glucose and fructose, others include, xylose, ribose, arabinose, sorbose, galactose, mannose, and poly glucose. Variety, temperature, time of the day, and maturity stage exerts an influence on the concentration of these sugars. Sucrose concentration ranges from 6.94 to 16.1 %, fructose and glucose varies from 0.18 to 4.2% and total sugar content varies from 9.19 to 23.33 % according to [8] and [9]. Sorghum has been identified as a preferred biomass crop for fermentation in to methanol and ethanol fuel [10] Bio-ethanol produced from agricultural crops or plants is a clean burning renewable fuel that is increasingly being used as a substitute fuel for road transport applications. In 2009, worldwide ethanol fuel production reached approximately 73.9 billion litres. The market for bio-ethanol road fuel is growing so rapidly that demand is starting to exceed supply [11]. Ethanol is a byproduct of fermentation, an anaerobic process where yeast convert sugar into ethanol and carbon dioxide [12]. Ethanol (ethyl alcohol) is a clear colorless volatile liquid, it is a compound containing the monovalent hydroxyl group –OH attached to carbon atom, its formula (CH_3-CH_2OH) [13]. Ethanol properties include, low toxicity, biodegradability, renewable characteristics and oxygenate resulting in reduced carbon dioxide emissions and its effects [14]. With the increasing burden of oil and natural gas prices many countries developed programmes to increase the production of alcohol by fermentation to be used in pure form or mixture (gashol), as a liquid for transportation [15]. The critical dependence of these countries on imported oil and the increased cost of imported oil have compelled them to search for fermentation alcohol. The most economic way of manufacturing ethanol from a variety of renewable sources involves fermentation by yeast [16]. Ethanol as an oxygenous biomass fuel is considered as a predominant alternative to MTBE for its biodegradable, low toxicity, persistence and regenerative characteristic [17]. Ethanol is a clean burning fuel with a high octane rating because of its low sulphates and aldehydes and existing automobile engines can be operated with gasohol (petrol blended with ethanol) without any need for engine modification [18].

1.2 *Optimization of Fermentation Process Conditions using Sweet Sorghum Juice:*

The interaction of factors such as yeasts, substrate, and environmental conditions namely, temperature, nutrients, pH and time do influence fermentation. Most of these factors govern the fermentation efficiency and resultant ethanol yield [19], the initial pH of the fermentation is most important and it is usually adjusted to 4.8 to 5.0. The lower pH increases the effectiveness of pasteurization and inhibits the growth of contaminants, particularly acid formers [20], Sugar concentration when the concentration is too high it acts adversely on the yeast, and the action of the yeast and the time of fermentation is prolonged and some of the sugar is not properly utilized. However, the use of too low concentration of sugar is uneconomic as it may lead to loss of valuable fermentation space. So, a sugar concentration of 10 to18% percent is usually satisfactory, although other concentrations are used [21], D'Amore studied the optimization of yeast concentration for ethanol production using a Saccharomyces strain. The best results for high ethanol yield were observed at a yeast concentration of 2 -3.5% w/v) [22], the Fermentation is usually completed in 50 hours or less depending on the temperature, sugar concentration, and other factors [21], the optimum temperature for the growth of certain distillers yeast is 28°C to 32°C compared with an optimum fermentation temperature of 38°C in the absence of alcohol and 32C° at alcohol concentration above 4.5% by volume [20]. The decrease in the respiratory capacity of yeast grown at the higher temperature was accompanied by decrease in yield and increase in the amount of ethanol produced [23].

1.3 *Objective*

1. Determination characterize of Sweet Sorghum juice.
2. Ethanol production from Sweet Sorghum juice.
3. Determination the optimum ethanol yield that can obtained from sweet sorghum juice under different parameters (pH, yeast concentration and Time fermentation).
4. Effect of change pH and yeast concentration on final concentration of bio-ethanol yield.

II. Materials and Methods

2.1 *Materials:*

2.1.1 *Sample Collection*

Sweet Sorghum stalks obtained from boorish Elgadarif (region of Doka), the sample was collected in the harvest season (October). Leaves were removed and stalks were tied into loose bundles and shifted to laboratory immediately.

2.2 *Methods*

2.2.1 *Sweet Sorghum Juice Extraction*

The first processing step, juice extraction involves the use of roller mills to squeezes the sugar rich juice out of the sweet sorghum stalks. The juice is further filtered using a filter cloth before use and storage at–20 °C until use.

2.2.2 *Chemical Analysis*

2.2.2.1 *Determination of Moisture Content*

Moisture content was determined according to the method of association of official's analytical chemists [24]. Moisture content (M.C.) was calculated using the formula; M. C (%) = $(W_2\text{-}W_1)$ - $(W_3\text{-}W_1)$/ $(W_2\text{-}W_1) \times 100$

M.C: Moisture content W_2: weight of crucible with sample before drying.

W_1: weight of empty crucible W_3: weight of crucible and sample after drying.

2.2.2.2 *Determination of pH*

The pH of SS juice, and fermentation broth was measured using pH/ meter, (HANNA) according to [25]

2.2.2.3 *Determination of Total Titratable Acidity*

The following formula was used to calculate the total titratable acidity; % acid (wt/wt) = N×V ×Eq. wt ×100/ W×1000.

N is normality of the titrant usually NaOH W is mass of sample (g)

V is volume of titrant (ml) 1000 is the factor relating mg to g

Eq. wt is the equivalent weight of predominant acid (mg/ mEq)

2.2.2.4 *Determination of Ash Content*

The ash content of the SS juice were determined according to [24]Method .Ash content was calculated using the following equation: Ash Content % = W_2 - W_1/ W_3 × 100

W_1 is the weight of crucible, W_2 is the weight of crucible with ash, W_3 is the weight of the sample.

2.2.2.5 *Protein Content:*

Protein content was determined according to [24]. The protein content was calculated as follows: Protein% W/W =V × N × 1.4 × 6.25/W.

Where: V = volume of Hcl, N = Normality of Hcl, W = Weight of sample in grams, 6.25 = general factor of protein.

2.2.2.6 *Determination of Sucrose, Fructose and Glucose of Sweet Sorghum juice (HPLC Method)*

2.2.3 *Microbiological Methods:*

2.2.3.1 *Micro-organism:*

Commercially bought dried baker's yeast (Saccharomyces cerevisiae) was used as micro-organisms in the fermentation process.

2.2.3.2 *Activation of Yeasts (Saccharomyces cerevisiae)*

2.2.3.3 *Fermentation of Sweet Sorghum juice:*

Fermentation involved placing 400 ml of SS juice in 500 mls flask and the adding activated yeast at 2 % v/v. Fermentation conducted in incubators pre- set at 30 ± 1°C for 72 hours [26].

2.2.4 *Determination of Ethanol Concentration:*

Ethanol concentration profile were determined using gas chromatography (Shimadzu, GC-2010, Japan), with Inert cap FFAP, an auto headspace samples injection and a flame ionization detector (FID). The detection column was run at 120 °C, using N_2 as a carrier gas and H_2 as a combustion gas.

2.2.5 *Determination of Optimal Fermentation Process Conditions:*

2.2.5.1 *Effect of pH on Ethanol Production:*

To study the effect of pH on the ethanol yield was investigated by varying the pH of the fermentation broth 5, 6 and 7. Saccharomyces cerevisiae was added (2%v/v) and the fermentation was carried out in an incubator at 30°C for 24 hours. The pH of the medium was adjusted by gradually adding 2N H_2SO_4 and 2N NaOH (if required) [26].

2.2.5.2 *Effect of Yeast Concentration on Ethanol Production:*

To study the effect of yeast concentration on ethanol production, fermentation conducted at yeast concentration levels of 2, 4 and 6 %, at 30 °C and constant pH of 5. The fermentation process was run for 24 hours[26].

2.2.5.3 *Effect of Time on Ethanol Production:*

The effect of time on the ethanol yield was determined at pH 5, 30 °C and 2% yeast concentration for different fermentation time 24, 48 and 72 hours.

III. Result and Discussion

3.1 *Chemical Characteristics of Sweet Sorghum juice*

The experiment was conducted to determine the chemical composition of sweet sorghum juice and their extracted juice was used for ethanol production. The chemical characteristics of sweet sorghum juice is given in table. 1.

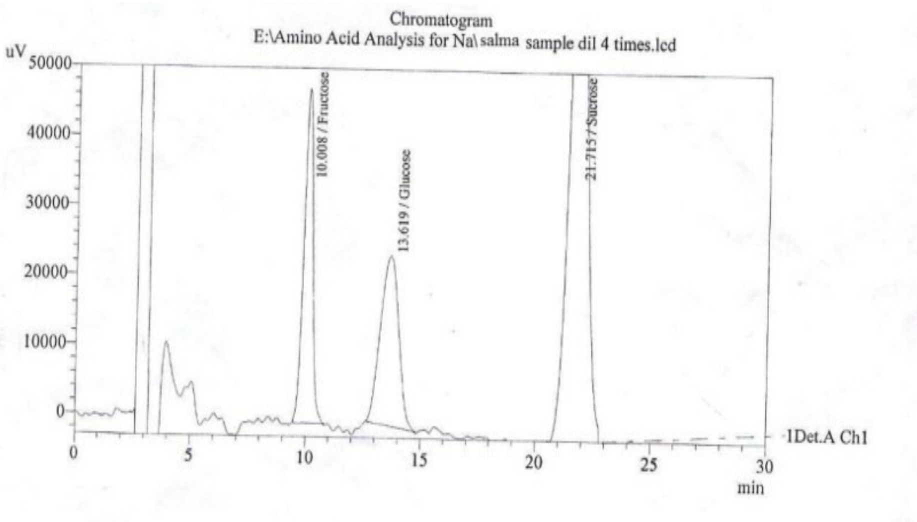
Table 1. Chemical characteristics of sweet sorghum juice.

Analysis	Result
Moisture content	81.64%
pH	5.13
Total titratable acidity	0.25%
Ash content	0.53%
Protein content	1.59%

Moisture content of SS juice showed in table 1, was 81.64%, which is similar to findings of [26] who found a moisture Range of 73.99 to 89.6% for Sweet Sorghum juice. The pH value showed in table.1, was 5.13. This pH value is similar to the findings of [27] found that the mean value of pH of 33 Sweet Sorghum genotypes grown at Shambat in milk stage ranged from 4.6 to 5.28. Total titratable acidity the sum of titratable acids in the sweet sorghum juice showed in table. 1 was 0.25% (as malic acid). While determining total titratable acidity of sweet sorghum juice found a TTA content of 0.24 % (as % malic acid) [28]. The ash content showed table 1 was 0.53%. The finding is similar to those of [29] who reported a maximum ash content of 3% for SS juice. Protein content of SS juice showed in table 1 was 1.59% which is similar the findings of [30] found the range of protein content is 1.04 to 2.16%.

3.1.2. *Sucrose, Glucose and Fructose Contents of Sweet Sorghum Juice*

The results in Fig. 1. shows the amount of sucrose in sweet sorghum juice 120.16g/l was higher compared to the other sugars (glucose and fructose) was 39.52g/l and 37.84g/l respectively. These results similar to findings of [31] who found the amount of sucrose 132.97g/l, glucose 47.76g/l and fructose 31.90g/l. and reported by [31] where sucrose was the most abundant sugar.



Quantitative Results

Detector A

ID	Name	Ret. Time	Area	Height	Conc.	Units	Conc.
1	Fructose	10.008	1305276	44187	0.473	g/50ml	37.84g/l
2	Glucose	13.619	1352975	24091	0.499	g/50ml	39.52g/l
3	Sucrose	21.715	4969154	92295	1.502	g/50ml	120.16g/l

Fig. 1. sucrose, glucose and fructose content in sweet sorghum juice.

3. 2 *Changes during Fermentation of Sweet Sorghum Juice:*

The changes in the pH and ethanol content were estimated during the fermentation process, with aim determining the time when optimum Ethanol production is generated by the fermenting yeast.

3.2.1 *The pH Changes during Fermentation Process:*

The initial pH of sweet sorghum juice was 5.13, Fig. 2. showed the initial pH decrease to 4.1 after 24 hours from beginning fermentation and the pH decline to 3.9 at the end fermentation. This results are similar to the findings of [32] stated that pH of the medium gradually decreased from 4.7 at the beginning of fermentation to 4.1 at 14hours then it remained constant until the end fermentation.

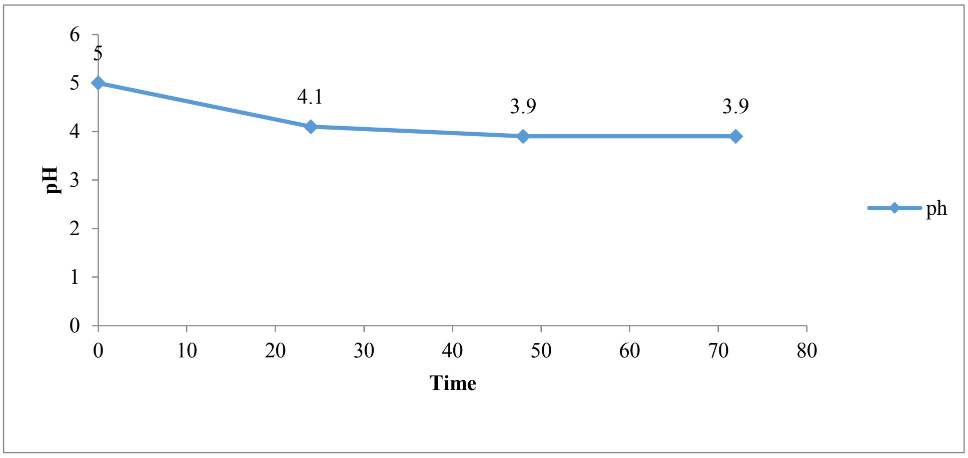


Fig. 2. pH changes during fermentation process

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3.2.2 *The Ethanol Production Changes during Fermentation Process:*

The alcohol content increased with time showed in Fig. 3. was increased from 5.9 to 8.6% after 24 to72 hours respectively, this is in agreement with [33] who reported an increase from 4.5 to 9.28 % and 1.78 to 8.05% after 48 to 72 hours respectively. Conversion efficiency of sweet sorghum juice to ethanol is related to both juice yield and the sucrose content in the juice [34].

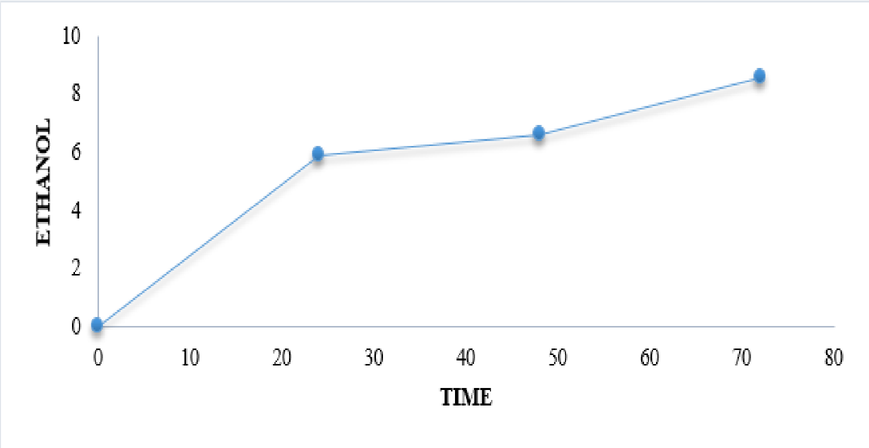
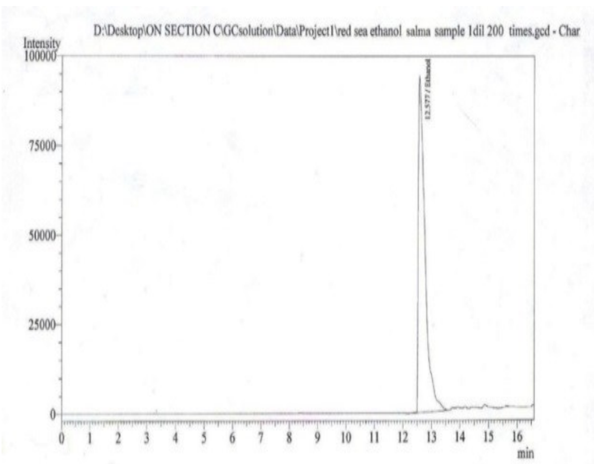


Fig. 3. Ethanol production changes during fermentation process.

3.3 *Ethanol Concentration:*

Ethanol concentration was determined for three samples (1), (2) and (3). Fig.4. (a,b and c) showed ethanol concentration for three sample 1, 2 and 3 was 44%, 38.2% and 39.2% respectively. The results indicate the sample (1) is highest ethanol concentration compared to sample (2) and (3), sample (2) is smallest ethanol concentration.

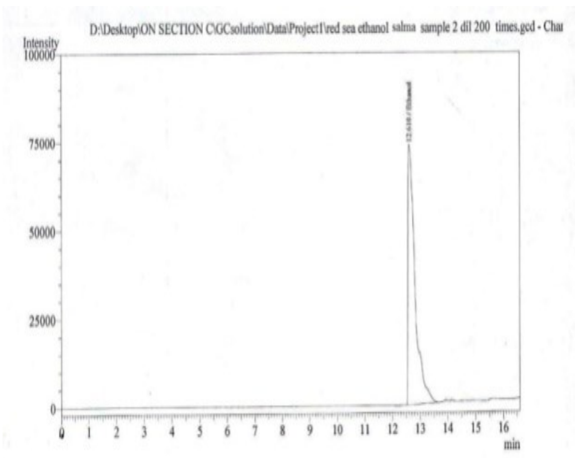
(a)



Peak Table

Peak	Ret. Time	Area	Hieght	Conc.	Units	Name
1	12.577	1529325	94207	0.220	%	Ethanol
Total		1529325	94207			

(b)

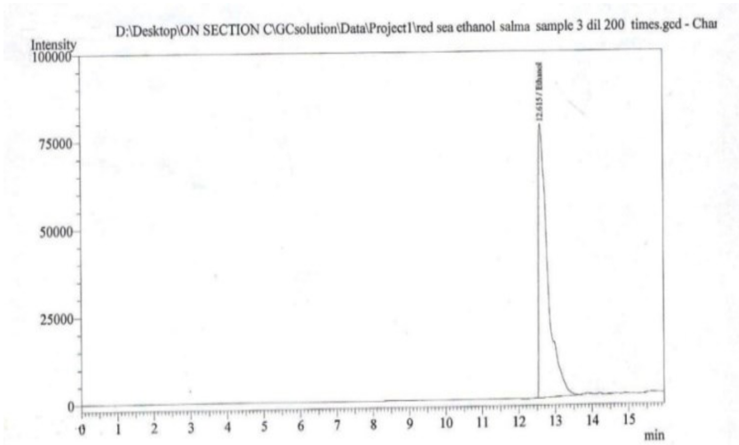


Peak Table

Peak	Ret. Time	Area	Hight	Conc.	Units	Name
1	12.610	1308370	73770	0.191	%	Ethanol
Total		1308370	73770			

International Journal of Engineering Innovation & Research
Volume 8, Issue 5, ISSN: 2277 – 5668

(c)



Peak Table

Peak	Ret. Time	Area	Height	Conc.	Unit	Name
1	12.615	1349022	78582	0.196	%	Ethanol
Total		1349022	78582			

Fig. 4. (a, b and c) ethanol concentration for three samples.

Fig. 5. show the highest ethanol concentration produced at pH 5 and 2% yeast concentration , smallest ethanol concentration produced at pH 7 and 2% yeast concentration. So the effect of increase in pH on fermentation process and concentration of bio-ethanol yield is greater than the effect of increase in yeast concentration. The three sample could be ranked in descending order for bio-ethanol yield concentration as follows: sample (1) 44%, sample (3) 39.2% and sample (2) 38.2%.

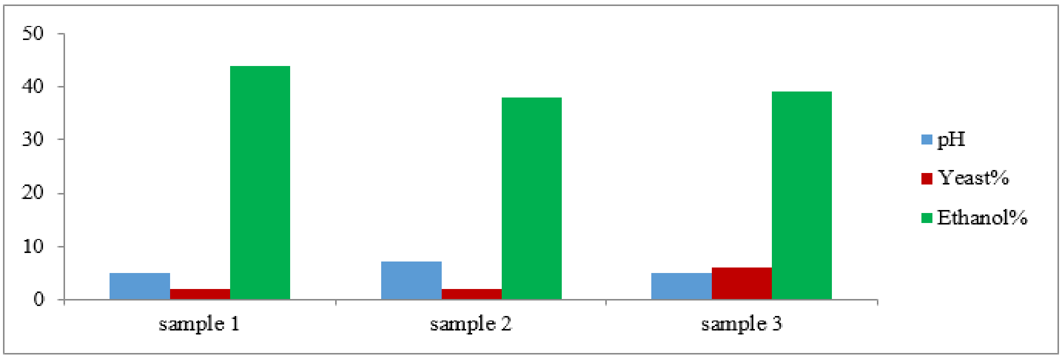


Fig. 5. Effect of pH and Yeast% on ethanol concentration.

3.4 *Optimization of the Fermentation Process Condition using Sweet Sorghum Juice:*

3.4.1 *The Effect of pH on the Ethanol Yield:*

The alcohol content obtained was 5.9, 3.4 and 2.7% for 5, 6 and 7 pH levels respectively at 24hours. Fig.6. shows that the optimum ethanol yield of 5.9% was observed at pH of 5. Increasing the pH resulted in a decreased in the ethanol production. At pH 7 the lowest ethanol production of 2.7% were obtained. This corresponds to his findings [35] in his study relationship between pH and ethanol production by S. cerevisiae note that the best ethanol production at pH 5 and pH5. 5.

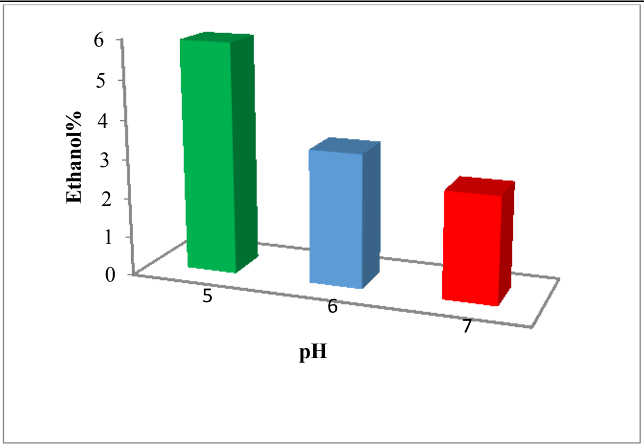


Fig. 6. Effect pH on the ethanol yield.

3.4.2 *The Effect of Yeast Concentration on the Ethanol Yield:*

The optimal yeast concentration was determined by fermenting SS juice at 2, 4 and 6% yeast concentration levels at pH 5 and temperature 30ºC at 24 hours. Fig. 7. the alcohol content obtained was 5.9%, 4.75% and 3.16% for 2, 4, and 6 % yeast concentrations respectively. Therefore, 2 % yeast concentration resulted in higher ethanol yield as compared to 4 and 6 %. The results are similar to the findings of [36], who found out that 2 % of actively growing yeast when inoculated in SS juice, rapid fermentation and optimum ethanol yield resulted. Reduced ethanol yield with increasing yeast concentration resulted from increased biosynthesis of glycerol which is non-fermentable [37].

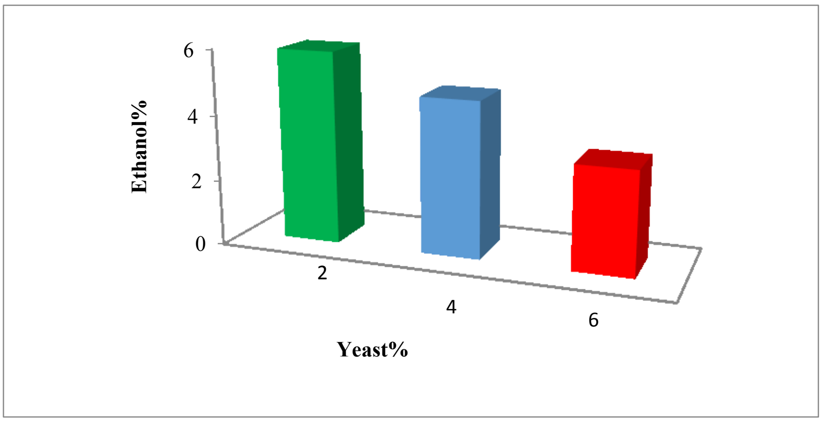


Fig. 7. Effect of yeast concentration on alcohol production.

3.4.3 *The Effect of Time on Ethanol Production:*

The time is one of the important factors that influence alcohol production. The optimal time, determined by fermenting SS juice at 24, 48 and 72 hours at pH 5, yeast 2% and 30°C. Fig. 8. show alcohol content increased with time. The alcohol was 5.9, 6.6 and 8.5% for 24, 48 and 72 hours respectively. Therefore 72 hours produced higher alcohol content than 24 and 48 hours. Similar findings were reported by Food and Agricultural Organization [8] it was stated that alcohol degree varied from 4.5% to 9.28% for good varieties of sweet sorghum after 48 and 72 hours.

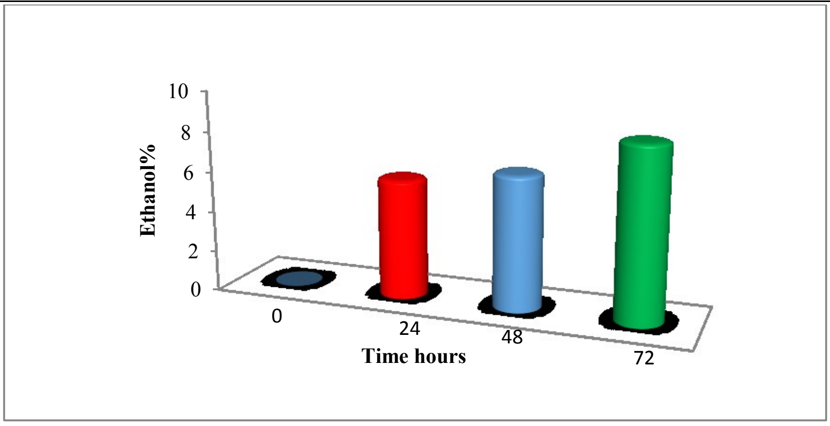


Fig. 8. Effect of fermentation time on alcohol production.

IV. Conclusion

The results obtained from this study have demonstrated Sweet sorghum juice can an effective alternate raw material for ethanol production, the maximum ethanol yield and ethanol concentration was achieved at pH5, 2% yeast Concentration and 30±1°C for 72 hours therefor optimal fermentation process condition pH 5, 2% yeast Concentration and 30±1°C for 72 hours and the effect of pH on fermentation process greater than the effect of yeast concentration. .

V. Recommendation

There is an urgent to support and commercialize the production of bio-fuel in order to get rid of the energy and to strengthen the economy of the country, the production of bio-ethanol will support sustainable development of renewable resources., The use of bio-ethanol with conventional vehicle fuels to provide a clean and cost effective environment., Bio-ethanol can be operated with gashol (petrol blended with ethanol) without any need for engine modification [18], Increase the biomass (sweet sorghum) and benefit from vast untapped land in the cultivation of sweet sorghum because of its good properties to combat desertification and the production of bio-fuels.

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