Screening and selection of Sweet Sorghum Varieties from India and Nigeria for Bioethanol production potential

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Abstract-The reducing sugar content of 14 Sweet Sorghum varieties at 5, 7 and 9 weeks of age were investigated using Dinitro Salicylic acid (DNS) method. Eight sweet sorghum varieties which comprise 3 Indian: E 36-1, ICSR 93034, SPV 422; 3 Nigerian: SWSV 2006-3, SWSV 2006-5-2, SWSV 2007-1; and 2 Indian crosses: ICSA 89002 X SPV 422 and ICSA X NTJ -2 were selected out of the fourteen based on the order of increasing reducing sugar content. At ninth week, ICSA 89002 X SPV 422 had the reducing sugar value of 149.38 ± 1.53mg/ml which was significantly higher than the other varieties at $p<0.05$. Two sweet sorghum varieties: SWSV 2006-3 and SPV 422 were subsequently selected from the eight based on field performance, susceptibility to hydrolysis, % bagasse yield, cellulose, hemicelluloses and lignin content.

Keywords: Bioethanol, lignocellulosic biomass, reducing sugar, renewable energy source, Sweet sorghum, Nigeria.

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INTRODUCTION

In view of the declining fossil reserve, non-renewable nature of fossil fuels, increasingly constant demand for fossil fuel and increasing fossil fuel price, research is being intensified in the area of bioethanol production from sugar-containing energy crops and lignocellulosic feedstocks/biomass as an alternative renewable source of fuel. Combustion of fossil fuel leads to emission of various types of gases into the atmosphere. These gaseous emissions from fossil fuel combustion have negative impact on the environment. The gases such as carbon IV oxide, carbon monoxide, methane, nitrous oxide e.t.c leads to atmospheric air pollution and global warming with carbon IV oxide being the major contributor to the global warming.

Bioethanol will serve as a renewable and alternative energy for automobile and other energy consumption purposes. It has a better combustion quality with high octane rating when compared to fossil fuel (14). Utilization of bioethanol as an alternative energy will help in the reduction of green house gas emission, hence reduction in atmospheric air pollution, eventual reduction of global warming and global climate change (15). The renewable and abundant nature of biomass material (raw material), will guarantee renewable and sustainable bioethanol production.

Bioethanol production from Biomass has received considerable global attention in recent times. Brazil and the United States of America (US) are currently producing and consuming bioethanol from sugarcane and corn respectively (7). United States of America has about 29 states that produce bioethanol with over one hundred and eighty plants (3). Majority of these plants run their production using purified enzymes.

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In Canada, Iogen incorporation is currently running a commercial production of bioethanol using commercial enzymes. In other parts of the world, bioethanol is being produced in laboratory, pilot and commercial scale from wheat, sugar beets, wastes from sugar refineries, wheat-starch, potato waste, cheese whey, milo (a species of grain sorghum), sweet sorghum juice, and beverage waste (10; 6).The sugar and starch in the feedstock are used. Current researches are focusing on how to efficiently utilize the rich and abundant carbon resources in the cell wall polysaccharides of agro wastes for bioethanol production.

The short life span/generation time of Sweet sorghum plant and relatively low production cost is also considered an advantage in bioethanol production because, turnover will be high.
The nature of plant cell wall polysaccharide has posed great difficulty in utilization of this feedstock (Sweet sorghum plant) for ethanol production, thus optimization of bagasse 'chaff' (Sweet sorghum) pretreatment conditions then becomes imperative. Integration of the sugars from sweet sorghum bagasse (cellulosic residue after extraction of sweet sorghum stem juice) with the sugar derived from the Sweet sorghum stem juice will further increase bioethanol yield. Utilisation of the bagasse from sweet sorghum for bioethanol production could lead to product yield maximization. Commercial production of bioethanol is gradually gaining attention in Nigeria, however, most trials have been on sugar cane. Apart from sugar cane, Sorghum, could be an additional source for sustainability in production. Sweet sorghum is the common name for an erect and corn-like grass that is a native of Africa and Asia (Plate 1). Sweet sorghum grows very favourably in Northern Nigeria. Thus, the production of the plant in Nigeria will have a high comparative advantage. Nevertheless, the bioethanol output from the plant could be influenced by the variety, percentage composition of cellulose, hemicelluloses and

lignin of the varieties at harvest; and the percentage bagasse yield at harvest.

Cellulose and hemicellulose are sources of sugar for bioethanol production, knowing the percentage composition of these individual components will give an insight of expected yield of ethanol. However, there are factors that could militate against getting the expected yield, such as the nature of the interaction of these molecules (cellulose, hemicellulose and lignin), level of crystalinity of cellulose, and lignin composition (the quantity or the relative abundance of the components) (5; 13; 8).

Knowledge of the percentage bagasse is also very necessary because if the yield is big it will encourage the producer as an indication that it would not require much labour would not be needed to get the required quantity of raw material.

Plate1. Sweet Sorghum at 7 weeks old

The objectives of this study therefore are to:

1. screen 14 sweet sorghum varieties for reducing sugar at 5, 7 and 9 weeks.
2. determine the percentage composition of cellulose, hemicelluloses and lignin of eight selected sweet sorghum varieties at harvest.
3. determine the percentage bagasse yield at harvest.
4. determine the glucose and reducing sugar yield of 8 Sweet Sorghum varieties pretreated with 1% Concentration of pretreatment solutions HCl and H_2O_2.

MATERIALS AND METHODS

Sample Collection and Identification

Mature plant of *Sorghum bicolor L. moench* (Sweet sorghum) already identified at Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University (ABU) were harvested at periodic intervals of two weeks (5, 7 and 9weeks) from the experimental farm of the Department of Plant Science, Ahmadu Bello University located in Shika Zaria, Nigeria.

List of Sweet sorghum varieties used and their origin

Nigerian Varieties	Indian Varieties	Indian varieties crosses in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	

ICSV 93046
ENT64 DTN

Chemicals and Reagents

The chemicals and reagents used for the study were Dinitrosalicylic acid (Sigma chemicals, St Louis U.S.A), Glucose oxidase kit (Teco diagnostics), Sodium Potassium Tartrate (BDH, Poole,England). Sodium Chlorite, Acetic acid, Acetone, Potassium hydroxide, Industrial spirit, Glacial acetic acid, diethyl ether, Concentrated Sulphuric acid.

Instruments

The instruments used for the study were: Weighing balance, colorimeter (Jenway, UK), laboratory mill model 4 (Thomas wiley), shaker, water bath, oven, incubator, Aluminum pans, and autoclave. Desiccators containing desiccants.

Methods

Preparation of Samples

Stage 1

Periodic reducing sugar determination

Fourteen different Sweet sorghum plants varieties were harvested at 5, 7 and 9weeks. One stem per variety (Sweet sorghum) was randomly cut from the base. The bark of the stem were peeled off to expose the pith and one gram (1g) wet weight of the pith were pounded in a laboratory mortar to a fine paste, extracted with 20mls of distilled water in a shaker for three (3) hours and filtered with Whatman No. 1 filter paper. The reducing sugar content of the filtrate was determined according to the Dinitrosalicylic acid (DNS) method of (12). The reducing sugar determination procedure is presented below.

Eight sweet sorghum varieties were selected at the end of the periodic sampling based on the increasing order of reducing sugar content.

Stage 2

Bagasse preparation

Eight selected Sweet Sorghum varieties were harvested at the ninth week. Their barks were peeled off to expose the pith. The pith was crushed, extracted with water at the ratio of 1:20 [stem (g): water (ml)] in a shaker for 24hr, and filtered with Whatman No. 1 filter paper. The residues were washed with water several times to produce bagasse. The resulting bagasse was dried to a constant weight at 45 ± 3^{o}C according to the method of (9). The dried bagasse were ground with a laboratory mill (Wiley) of a mesh size of 0.5mm to form bagasse powder (ground bagasse) and stored in an air tight container at +4^{o}C. The percentage bagasse powder yield was calculated as follows:

$$\% \text{ Bagasse powder yield} = \frac{\text{Weight of ground Sweet sorghum bagasse}}{\text{Weight of unpeeled Sweet sorghum stem}} \times 100$$

Drying of bagasse for compositional analysis and pretreatment study

This method was described by (9). The bagasse of eight Sweet sorghum varieties were first air-dried before been oven-dried. Air drying method is suitable for the preparation of large quantities (>20 g) of field-collected samples into a form appropriate for compositional analysis. This method is suitable for drying materials where ambient humidity allows the sample to air-dry to moisture content below 10% as measured using LAP "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples" of (16). The bagasse of eight Sweet sorghum varieties were thinly spread out on a suitable surface and allowed to air-dry. They were turned at least once per two hour to avoid microbial growth and ensure even drying of the samples. The % moisture content of the bagasse of eight Sweet sorghum varieties was determined once every 24 hours using the formular below.

$$\% \text{ Moisture content} = 100 - \left[\frac{\text{Weight dry bagasse}}{\text{Weight wet bagasse}} \times 100\right]$$

The bagasse was considered dried when the moisture content was less than 10% by weight and the change in weight is less than 1% in 24h.

The bagasses were also repeatedly oven dried at 45± 3°C for intervals of 1h and placed in desiccators to cool to room temperature. An aluminum containers were dried for 3h at 45± 3°C and dessicated. The weight of dried container (W1), dried container plus bagasse (W2) and dried container plus dried bagasse (W3) to the nearest 0.1g were noted. Drying of Bagasse repeated until the change in the mass of the biomass is less than 1% in one hour.

$$\% \text{ Moisture content} = 100 - \left[\frac{W3 - W1}{W2 - W1} \times 100\right]$$

Determination of Moisture content of fourteen Sweet sorghum varieties

This was carried out according to the method of (16). Aluminum dishes were pre-dried by placing them in a drying oven set at 105 ± 3ºC for a minimum of four hours. They were cooled in desiccators and weighed (W1) to the nearest 0.1 mg.

One (1.0)g of Sweet sorghum pith were each weighed into the Aluminum dishes (W2) to the nearest 0.1 mg and dried overnight to a constant weight in an oven set at 105 ± 3^{o}C.

The samples were removed from the oven and allowed to cool to room temperature in desiccators. The dishes containing the oven-dried samples were weighed to the nearest 0.1mg and the weight recorded. This procedure was repeated for a minimum of four hours and re-weighed until a constant weight (W3) is achieved. Constant weight is defined as ± 0.1% change in the weight percent solids upon one hour of re-heating the sample.

$$\% \text{ Moisture content} = 100 - \frac{[\text{Weight}_{\text{dry pan plus dry sample}} - \text{Weight}_{\text{drypan}} \text{ X } 100]}{\text{Weight}_{\text{sample as received}}}$$

$$\% \text{ Moisture content} = 100 - \frac{[W3 - W1 \text{ X} 100]}{W2 - W1}$$

Determination of cellulose, hemicellulose and lignin content of the stem bagasse of eight Sweet sorghum varieties

The processed bagasse powders were subjected to compositional analysis to determine non-soluble carbohydrate content. The cellulose, hemicellulose and lignin content of the eight selected sweet sorghum stem were qualitatively determined according to the method outlined in Chemical Analysis of Ecological Materials (1).

-Determination of Cellulose content of the bagasse

One (1)g of the bagasse powder of eight Sweet sorghum varieties were weighed into a conical flask. Thirty (30) ml of water, 2ml of 10% acetic acid and 0.6g Sodium chlorite were added into the flask, mixed and incubated at 75^{o}C in water bath. Ten (10) % acetic acid and Sodium chlorite was added at hourly intervals and swirled intermittently. The flasks were removed after 4hours and cooled immediately in ice cold water. The contents were weighed through No 2 Pyrex sintered crucibles. The residues were washed about 10 times with ice cold water and washed 3 or 4 times with acetone and finally once with ether. The ether was allowed to evaporate in an oven at 105^{o}C for 30minutes, cooled in a desiccator and weighed. The weighed samples represent the holocellulose.

The ash and Nitrogen (N) contents (multiplied by 6.25 to give a correction for crude protein) on sub-samples of holocellulose were determined and these values subtracted before calculating the % holocellulose.

$$\text{holocellulose (\%)} = \frac{\text{corrected holocellulose(g) x } 10^2}{\text{Sample weight}}$$

The holocellulose were weighed into a 50ml conical flask. Twenty (20) ml of 24%KOH was added into the each flask, stopper with rubber bung and placed in water bath at 20^{o}C for 2hours. The flasks were swirled at intervals. They were later filtered through a weighed No. 2 glass sintered crucibles, washed with water until washings were free of alkali. Five (5) ml of 5% acetic acid each was added to the residues and swirled around in crucible. They were washed again with water, acetone and finally ether. The ether was allowed to evaporate. The residues were dried in an oven at 105^{o}C for 30minutes, cooled in desiccators and weighed as α-cellulose.

$$\text{Cellulose (\%)} = \frac{\text{Corrected α-cellulose (g) x total uncorrected holocellulose (g) x } 10^2}{\text{Holocellulose sub-sample (g)}}$$

-Determination of hemicellulose content of the bagasse

The procedure for α-cellulose determination is followed until the filtering stage is reached. The bagasse samples were filtered through No. 2 pyrex sinter crucibles into a reservoirs containing 8ml of glacial acetic acid. The residues were washed with both water to remove all alkali and 5ml of 5% acetic acid. They were again washed with water, acetone and finally ether. The pH of filtrates was adjusted to 4.0 with 5% acetic acid and industrial spirit was added to about 3.5 times the volume of the filtrates and left to stand overnight. They were filtered through a weighed No. 2 sinter crucible, washed with industrial spirit, acetone and ether. The ether was allowed to evaporate. The residues were dried in the oven at 105^{o}C for 30minutes, cooled in desiccators and weighed.

$$\text{Hemicellulose(\%)} = \frac{\text{Weight of hemicelluloses (g) X total uncorrected holocellulose (g) X } 10^2}{\text{Holocellulose sub-sample (g) X sample weight}}$$

-Determination of lignin content of the bagasse

One (1)g of dried bagasse powder from eight Sweet sorghum varieties were weighed into glass fibre papers and tied into a bundle with terylene thread. Ether extraction was carried out in a soxhlet for about 6hours. The ether was allowed to evaporate and the bundle was dried for 30minutes at 105^{o}C in an oven. They were cooled and weighed after removing the thread.

About 0.8g from ether extracted bagasse was weighed into a 600ml tall pyrex beakers and about 400ml of water was added. The contents were boiled gently for 3hours and the volume maintained at 400ml. Twenty two (22) ml of 10% Sulphuric acid was added and boiled for extra one hour. The contents were allowed to settle. The supernatant liquid was removed with No. 2 sintered filter sticks leaving the bagasse as dry as possible. Fifteen (15)ml of 72% Sulphuric acid was added to the bagasse residues at 15^{o}C and stirred with the filter sticks. The beakers were left in a water bath for 2hours at 20^{o}C and stirred continuously. The acid strength was reduced to 3% by addition of 560ml of water. The sinter sticks were washed into the beakers and removed. The contents were boiled gently for 4hours and were topped when necessary. The contents were allowed to settle and filtered immediately through a weighed No.2 sintered glass crucible and the residues was washed until it is acid free with hot water. The sinters and contents were dried for 3 hours at 150^{o}C, cooled and weighed.

Ash and N-content were determined as in cellulose determinations which were subtracted before calculating for % lignin.

$$\text{Lignin (\%)} = \frac{\text{Corrected lignin (g) X total ether extracted sample (g) X } 10^2}{\text{Weight taken for water extraction (g) X sample weight (g)}}$$

Susceptibility of the bagasse to pretreatment solutions HCl and H_2O_2

The bagasses powder of eight Sweet sorghum varieties were each treated with 1% V/V concentrations of HCl and H_2O_2 respectively according to the acid and peroxide pre-treatment methods outlined below. Two sweet sorghum varieties; one each from Nigerian and Indian varieties were selected from the eight for further studies based on the glucose yield after this initial pre-treatment. Other factors considered are: stem size; % bagasse powder yield; overall carbohydrate content (cellulose and hemicelluloses content) and lignin content; yield in glucose after 1.0% HCl and 1.0%H_2O_2; availability and level of pest infestation.

Peroxide Pretreatment

Alkaline peroxide pre-treatment was carried out according to the method of (17) with some modifications. One fifth (0.2)g each of the bagasse powders of the sweet sorghum varieties were treated with 10ml Hydrogen peroxide in 250 ml conical flask for 15 minutes at autoclave temperature of 121^{o}C. The insoluble residues in the flasks were filtered, and the filtrates were used for glucose and reducing sugar determination.

Acid (1.0% HCl) pretreatment

Bagasse powders were pre-treated with acid according to the method of (2) with a little modification. Ten (10) ml of dilute Hydrochloric acid (of known concentrations) were dispensed into 250 ml conical flasks that contained 0.2g of each sweet sorghum variety (bagasse powder) and autoclaved at 121^{o}C for 15 minutes. The insoluble residues in the flasks were filtered. The filtrates were used for glucose and reducing sugar determination.

Reducing Sugar (RS) Determination

Quantitative determination of reducing sugar of the filtrates from the sweet sorghum varieties used for periodic RS determination and filtrates from bagasse hydrolysates was carried out using dinitrosalicylic acid (DNS) method (12). DNS reagent was added to the filtrates at the ratio of 3:1(ml) in a test tube and placed in a boiling water bath for 5 minutes. The samples were allowed to cool under running water bath. The colour intensity produced from the reduction of 3, 5-dinitrosalicylic acid (DNS) to 3-amino, 5-nitrosalicylic acid was determined by taking absorbance at 540 nm. Glucose was used as a standard.

Glucose determination

Glucose concentrations of the filtrates from pre-treated sweet sorghum samples were determined using glucose oxidase reagent kit. Two (2.0)mls of the reagent were placed in a test tube and pre-incubated in a water bath at 37^{o}C for 5minutes. Ten microlitre (10µL or 0.01ml) of filtrate from the hydrolysate were added into the respective tubes, mixed and incubated at 37^{o}C for 10minute. Absorbance reading was taken in a spectrophotometer at 520 nm.

Concentration of glucose was expressed as

$$= \frac{\text{Absorbance of the unknown}}{\text{Absorbance of the standard}} \times \text{concentration of glucose standard (mg/ml)}$$

Yield calculations

Percentage glucose (G) and reducing sugar (RS) yield

This was determined as follows:

$$\text{\% saccarification or \% Glucose yield} = \frac{\text{Weight of Glucose after pretreatment X 100}}{\text{Weight of raw sample}}$$

$$\text{\% reducing sugar yield} = \frac{\text{Weight of Reducing sugar after pretreatment X 100}}{\text{Weight of raw sample}}$$

Statistical Analysis

Experiments were performed in triplicates and duplicates. Analysis of Variance (ANOVA) was used to compare mean of each treatment. The results were presented as mean ± standard deviation of glucose/reducing sugar concentration for each treatment. The difference between the mean of treatments were tested using the Duncan Multiple Range test at $p<0.05$.

RESULTS

Periodic assessment for reducing sugar concentration and moisture content of Fourteen Sweet sorghum varieties

Figure 1 Shows results of percentage (%) moisture content of fourteen sweet sorghum (S.S.) varieties at week 5, 7 and 9. Results revealed that the percentage moisture content varied among the weeks tested. A continuous decrease in % moisture content from week 5 through 7 to 9 weeks was noticed in exactly 50% of the total S.S varieties with 80% of Nigerian varieties exhibiting that trend. However, 42% of the sweet sorghum varieties showed an increase from week 5 to 7 but decreased at week 9. Approximately 67% of Indian varieties showed that trend. The only S.S. variety that showed fluctuation in pattern of decrease from week 5 to 7 and increase in its percentage moisture content at week 9 was SWSV 2007-1.

Results on reducing sugar determination of the fourteen Sweet sorghum varieties at week 5 of plant age are presented in Figure 2. The Nigerian variety, SWSV 2006-3 had the highest overall concentration of 83.08 ± 5.05mg/g which was not significantly different ($p>0.05$) from the reducing sugar value observed for ICSA 95 X NTJ -2 (82.24 ± 1.65mg/g). The Sweet sorghum variety with the lowest value of reducing sugar at week 5 was ICSV 700 with reducing sugar concentration of 27.83 ± 0.91mg/g. This value was significantly ($p<0.05$) lower than the Nigerian variety SWSV 2007-3. The Indian cross-varieties: ICSA 89002 X SPV 422 and ICSA 95 X NTJ-2, Nigerian varieties: SWSV2006-3, SWSV2006-5-2 SWSV2007-1, and the Indian varieties: SPV422, E 36-1, ICSR 93034, ENT 64 DTN were the best in reducing sugar yielding capability.

Results on comparison of reducing sugar concentration of eight Sweet Sorghum varieties at week five (5), seven (7) and nine (9) are presented in Figure 3. The reducing sugar concentrations of the Sweet sorghum varieties increased progressively from week 5 through 7 to 9 in about 87.5% of the Sweet sorghum varieties used. The only variety that fluctuated in pattern was E 36-1.

The reducing sugar concentration observed at week 9 is significantly ($p<0.05$) higher than values observed at weeks 5 and 7.

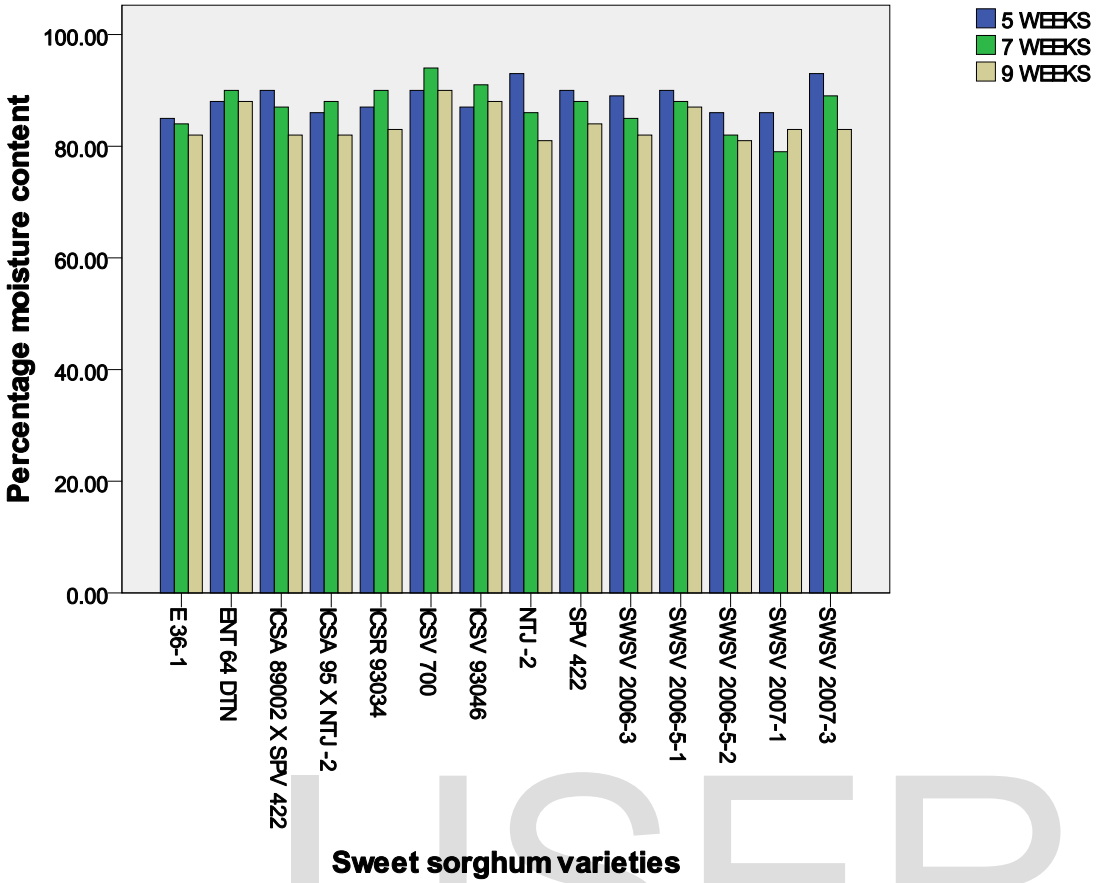

Figure 1: Percentage moisture content of bagasse from the stalks of fourteen sweet sorghum varieties

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

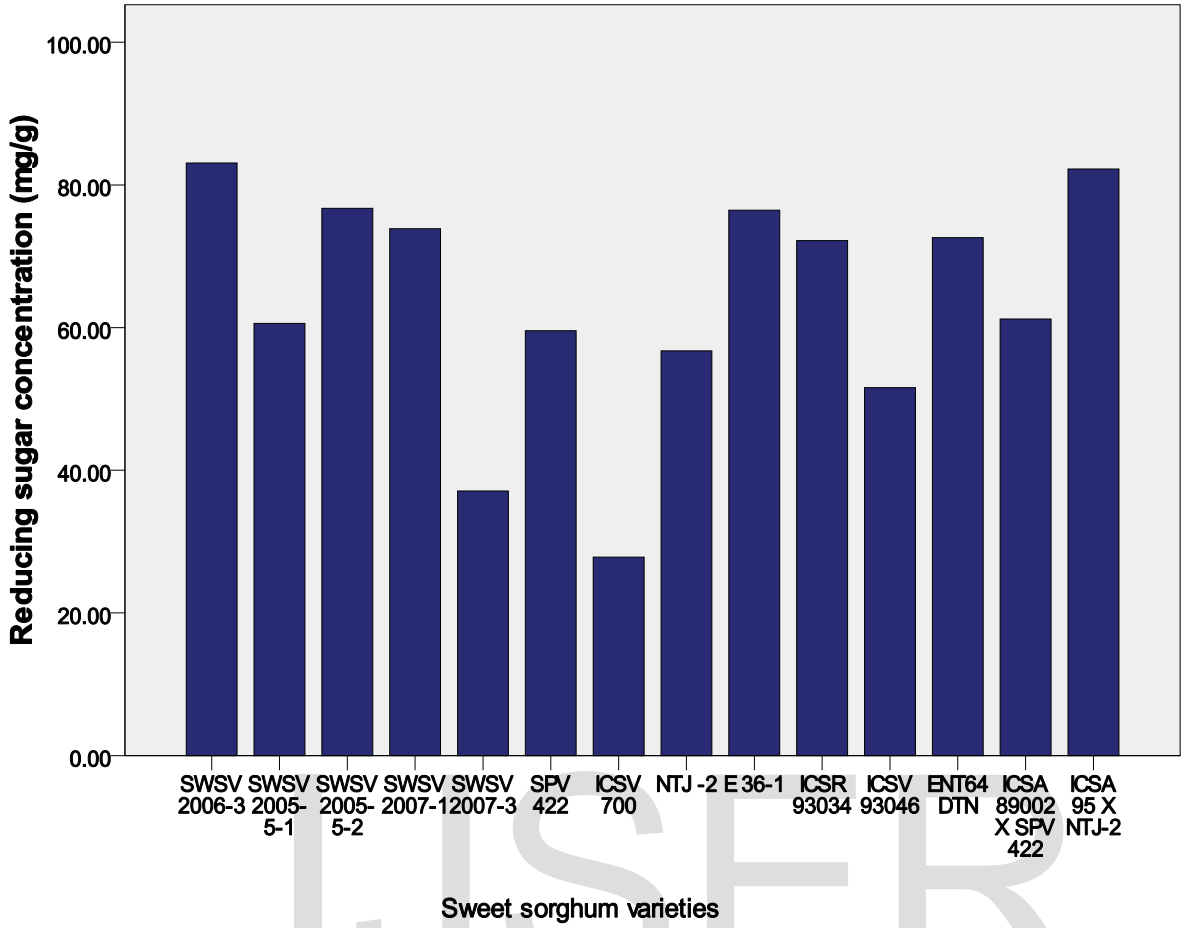

Figure 2: Reducing sugar concentration (mg/g) of the stem from growing stalks of 14 Sweet sorghum varieties at 5 weeks

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2005-5-1	ICSV 700	ICSA 95 X NTJ -2
SWSV 2006-5-2	NTJ -2	
SWSV 2007-1	E 36-1	
SWSV 2007-3.	ICSR 93034	
	ICSV 93046	
	ENT64 DTN	

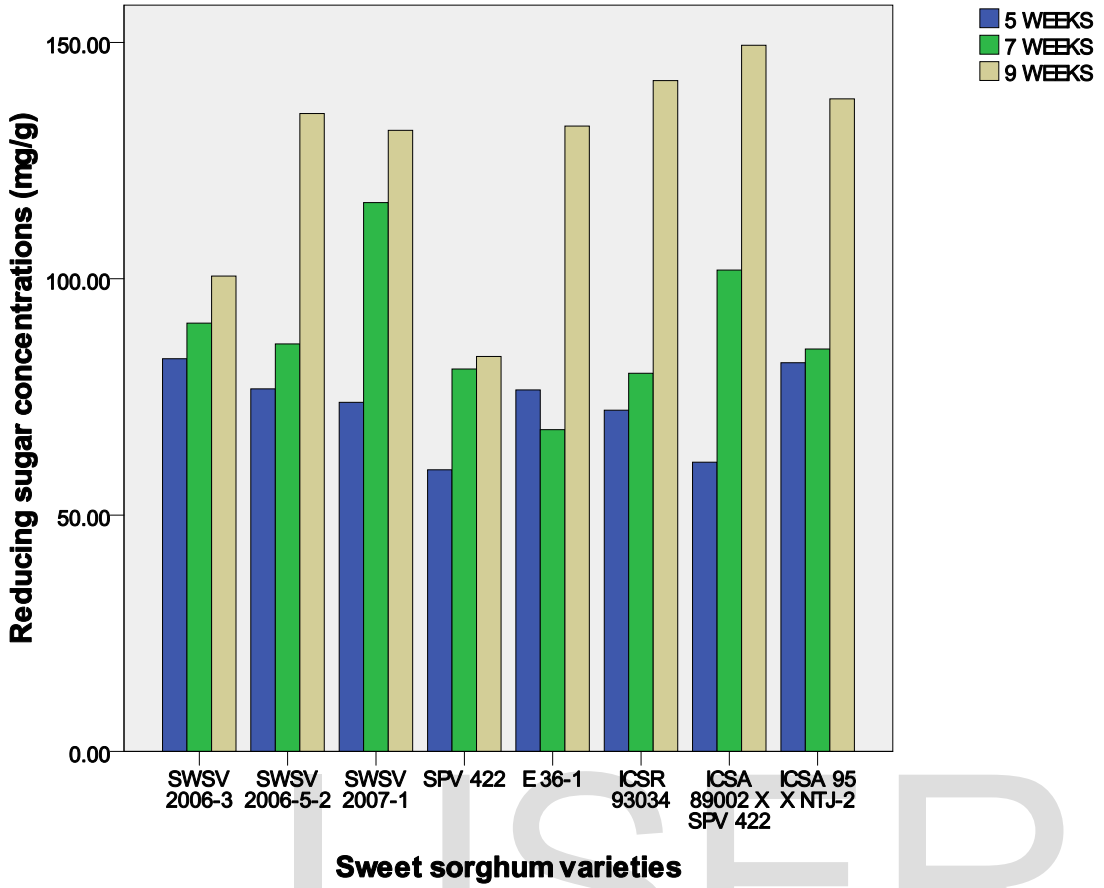

Figure 3: Reducing sugar concentrations (mg/g) of the stem from growing stalks of 8 Sweet sorghum varieties at 5, 7 and 9 weeks

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

Cellulose, Hemicellulose, lignin content and Harvest Yield

The results in Figure 4 showed a wide variation in the percentage composition of cellulose, hemicellulose and lignin of the bagasse of the three categories of the sweet sorghum varieties analysed. The Nigerian S.S. Variety; SWSV 2007-1 had the highest cellulose percentage of 36.64±0.02 while the Indian Variety, ICSA 95 X NTJ-2 was lowest in cellulose content with percentage cellulose of 30.60 ± 0.02. The percentage hemicellulose composition was also highest in SWSV 2007-1 and lowest in E 36-1 with values of 20.40 ± 0.01 and 16.74 ± 0.04 respectively. However, the highest percentage lignin content of 22.18 ± 0.02 was recorded in ICSA 89002 X SPV422, a value which was not significantly higher than 22.16 ± 0.02 for ICSA 93034 while the lowest lignin value of 18.76 ± 0.02 was recorded in S.S. variety with tags ICSA 95 X NTJ-2.

The Nigerian varieties were observed to have higher cellulose composition than the other two groups. The variations in cellulose, hemicelluose and lignin content of the Sweet sorghum are lesser among varieties in the same category; less variation was observed among sorghum varieties of Nigerian origin than between Nigerian and Indian. The hybrids of Indian varieties were much closer to the Indian varieties in composition than the Nigerian varieties.

Figure 5 shows that ICSA 89002 X SPV 422 had a better yield of 2.71% bagasse (fibre) powder after harvest, a value very close to 2.42% for S.S. variety, ICSA X NTJ -2 while SWSV 2006-5-2 recorded the lowest value of 1.48%.

The percentage sweet sorghum bagasse yields were generally very low. However, ICSR 93034 and all the Nigerian varieties had relatively lower values when compared with all the Indian and Indian hybrid varieties.

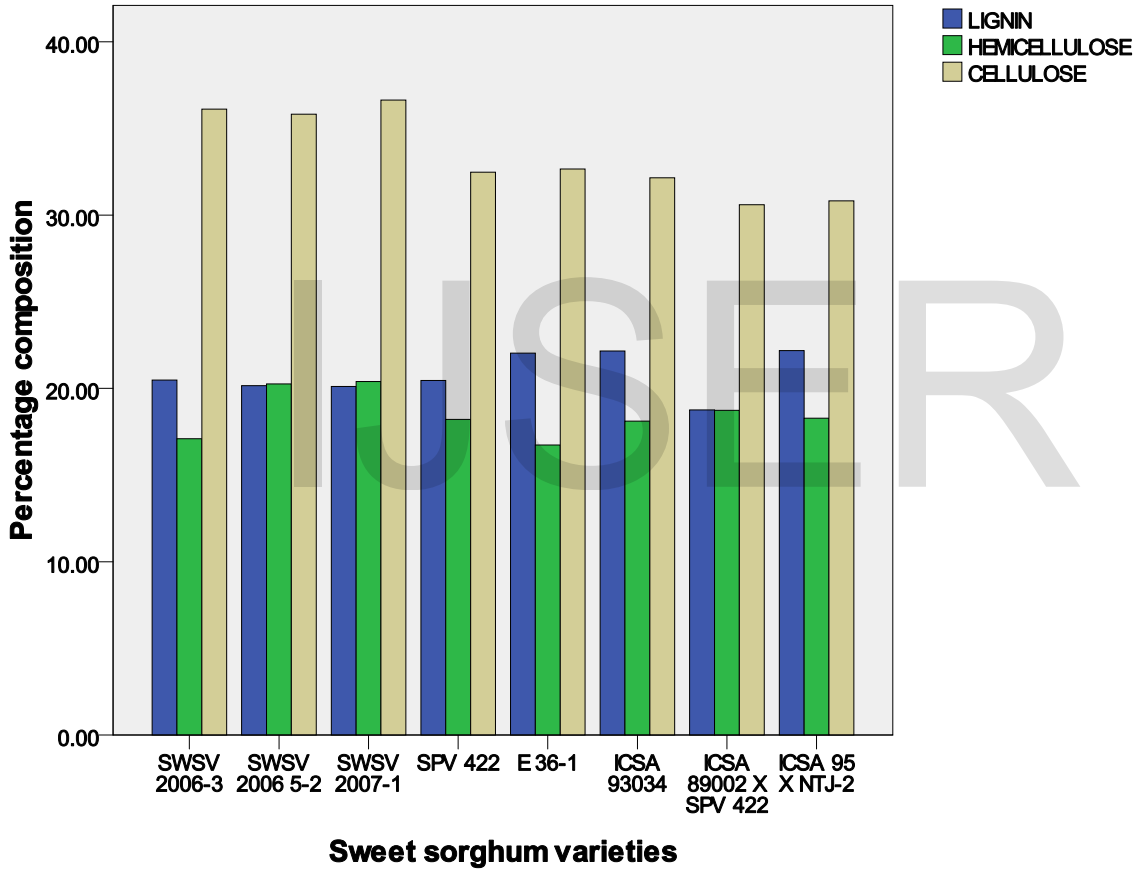

Figure 4: Cellulose, Hemicellulose and lignin content of bagasse from the stalks of eight Sweet sorghum varieties after harvest

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

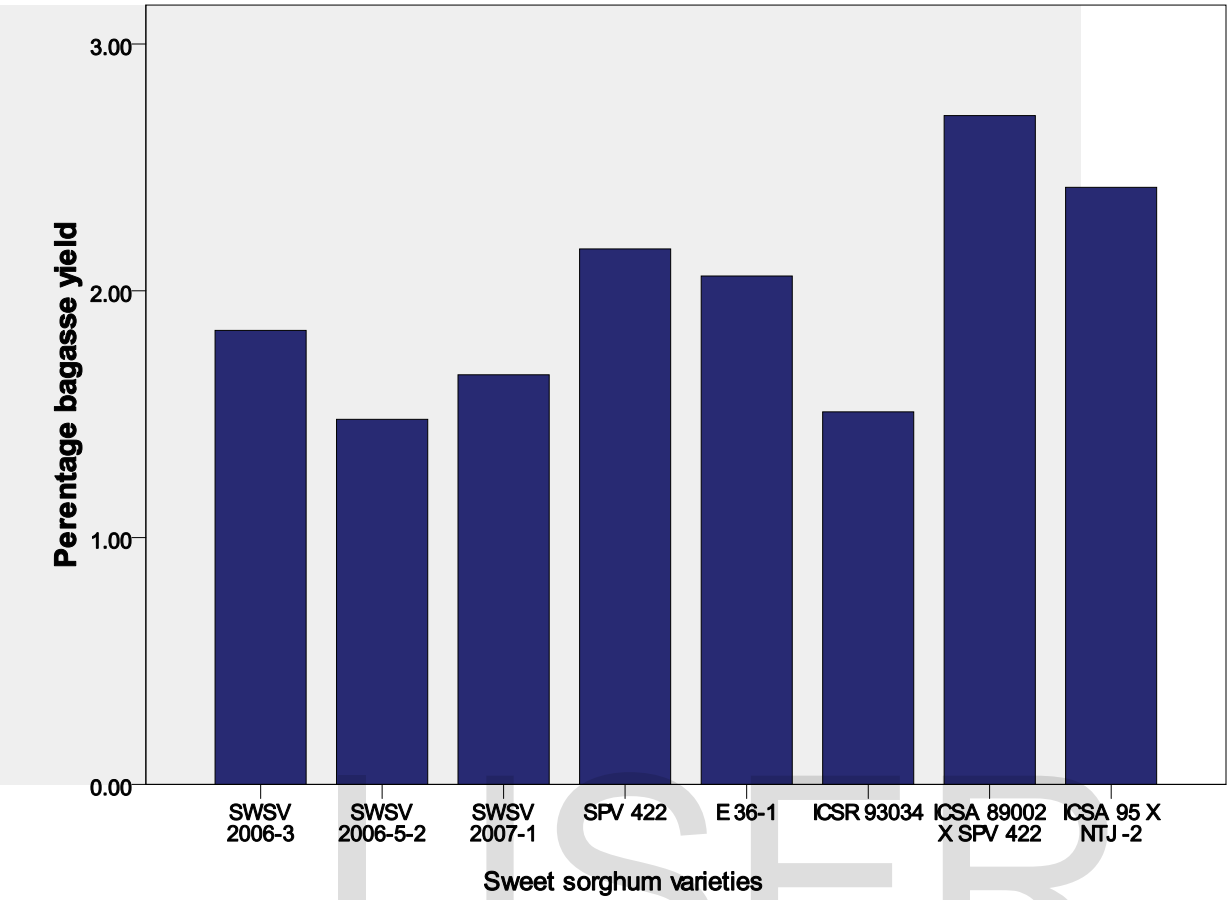

Figure 5: Percentage bagasse yield of eight Sweet sorghum varieties after harvest

Nigerian Varieties	Indian Varieties	Indian varieties crossed in IAR Zaria
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

The percentage glucose and reducing sugar yield (w/w) of eight sweet sorghum varieties

The initial 1.0%HCl and 1.0%H_2O_2 pretreatment was carried out to determine how susceptible the sweet sorghum varieties are to hydrolysis. Table 1 presents the percentage glucose yield from eight Sweet sorghum varieties after the pretreatment. Among the Nigerian varieties, SWSV 2006 5-2 was more susceptible to 1.0%HCl hydrolysis in terms of glucose yield. For the Indian S.S. varieties treated with 1.0%HCl, the value of 13.24 ± 0.00% was observed for SPV 422, a value which is significantly ($p<0.05$) higher than others. This is also 41.11% greater than the closest variety E 36-1 in the amount of glucose released. The lowest % glucose yield of 2.50 ± 0.21% was recorded in both ICSA95 X NTJ-2 and ICSR 93034. This value was not significantly different ($p>0.05$) from 2.94 ± 0.00% and 3.24 ± 0.00% for SWSV2007-1 and SWSV2006-3 respectively.

For hydrogen peroxide (1.0%H_2O_2) pretreated S.S. varieties, the highest % glucose yield of 8.38 ± 0.62% was observed for SWSV2006-3; which is not significantly different ($p>0.05$) from 7.94 ± 1.25%, 7.50 ± 0.21%, 7.35 ± 0.00% observed for ICSR93034, SWSV2007-1 and E36-1 respectively. The lowest glucose concentration of 3.97 ± 0.21% was obtained in ICSA89002 X SPV422.

Table 2 shows the percentage reducing sugar yield of eight sweet sorghum varieties.

SPV422 had the highest reducing sugar concentration of 52.72 ± 0.00% after the pretreatment with 1.0%HCl. The lowest reducing sugar value of 44.95 ± 0.69% was recorded in SWSV2007-1. The highest reducing sugar concentration of 3.69 ± 0.034% was also obtained from E36-1 for S.S. varieties treated with 1.0 %H_2O_2.

This value is not significantly different ($p>0.05$) from the value for SPV422 variety (3.67 ± 0.69%). The lowest value of 1.12 ± 0.03% was obtained in ICSR93034.

Table 1: Percentage Glucose yield (w/w) of eight sweet sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2

Sweet sorghum varieties	Pretreatment solutions		
	Control (distilled water)	1.0% HCl	1.0% H_2O_2
SWSV2006-3	0.44 ± 0.21^{ab}	3.24 ± 0.00^{ab}	8.38 ± 0.62^{d}
SWSV2006-5-2	0.59 ± 0.00^{ab}	8.38 ± 1.04^{c}	6.18 ± 0.83^{bc}
SWSV2007-1	0.74 ± 0.21^{b}	2.94 ±0.00^{ab}	7.50 ± 0.21^{cd}
SPV422	0.59 ± 0.0^{ab}	13.24 ± 0.00^{d}	5.44 ± 0.62^{ab}
E36-1	0.59 ± 0.00^{ab}	7.79 ± 0.21^{c}	7.35 ± 0.00^{cd}
ICSR93034	0.59 ± 0.00^{ab}	2.50 ± 0.21^{a}	7.94 ± 1.25^{d}
ICSA89002 X SPV422	0.29 ± 0.00^{a}	3.53 ± 0.00^{b}	3.97 ± 0.21^{a}
ICSA95 X NTJ-2	0.44 ± 0.21^{ab}	2.50 ± 0.21^{a}	6.76 ± 0.42^{bc}

Values are Mean ± SD of duplicate determinations.
Means with different superscript down the column are significantly different at P<0.05.

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

Table 2: Percentage Reducing sugar yield (w/w) released from the bagasse of eight sweet sorghum varieties pretreated with 1.0% HCl and 1.0% H_2O_2

Sweet sorghum varieties	Pretreatment solutions		
	Control (distilled water)	1.0% HCl	1.0% H_2O_2
SWSV2006-3	1.36 ± 0.10^{b}	50.05 ± 0. 34^{c}	1.32 ± 0.03^{b}
SWSV2006-5-2	3.23 ± 0.07^{e}	48.59 ± 1.72^{bc}	3.35 ± 0.03^{f}
SWSV2007-1	3.69 ±0.03^{f}	44.95 ± 0. 69^{a}	2.77 ± 0.03^{e}
SPV422	2.65 ± 0.21^{d}	52.72 ± 0.00^{d}	3.67 ± 0.07^{g}
E36-1	2.92 ± 0.10^{de}	50.05 ±0.34^{c}	3.69 ± 0.03^{g}
ICSR93034	0.78 ± 0.03^{a}	50.29 ± 0. 69^{c}	1.12 ± 0.03^{a}
ICSA89002 X SPV422	2.31 ± 0.27^{c}	47.14 ± 0.34^{b}	2.60 ± 0.07^{d}
ICSA95XNTJ-2	1.00 ± 0.07^{a}	50.29 ± 0.69^{c}	1.51 ± 0.03^{c}

Values are Mean ± SD of duplicate determinations.
Means with different superscript down the column are significantly different at P<0.05.

Nigerian Varieties	**Indian Varieties**	**Indian varieties crossed in IAR Zaria**
SWSV 2006-3	SPV 422	ICSA 89002 X SPV 422
SWSV 2006-5-2	E 36-1	ICSA 95 X NTJ -2
SWSV 2007-1	ICSR 93034	

DISCUSSION

The percentage moisture content

The variation in trend of percentage moisture content of the Sweet sorghum varieties (Figure 1) can be attributed to changes in season and microclimatic conditions surrounding each variety which is created by the nature of the variety in question. The varieties that showed an increase in percentage moisture from week 5 to 7 and a decrease in week 9 had relatively thicker stems and wider leave diameter which shaded the soil especially the rhizosphere from direct impact of heat from the sun. This resultant microclimatic condition led to reduction in the evaporation of water from the soil, thus the plants can absorb available water from the soil.

Conversely, those that experienced a progressive decrease had slender stems and relatively thinner/ narrower leaves surface area which could hardly shade the rhizosphere of the roots from direct impact of heat from the sun. Consequently, the soil had less available water for the plants to absorb as most of the soil water is lost through evaporation when the soil temperature increased and relative humidity of the micro environment became highly reduced. As dry season approaches, the microclimatic condition around the plant including the relative humidity, temperature, light intensity and soil moisture gradually changes. Evaporation of water from the soil and the rate of transpiration increases because of less water in the atmosphere. Consequently, the percentage moisture content of the plant decreases. This agrees with the findings of (4), who documented a percentage moisture content of 70% for a certain variety of sweet sorghum although this value is much lower than the ones gotten for the varieties tested in the present study. Season and period of growth could affect the amount of water (moisture) the pith contains.

The reducing sugar concentration (Figure 2 and 3)

Sweet sorghum just like any other plant synthesizes and accumulates sugar, the product of photosynthesis, in the form of starch in the grain and juice together with sucrose, fructose glucose and others sugars. Sweet sorghum as a C-4 plant has the extra capability to efficiently utilize carbon dioxide (CO_2) for carbohydrate synthesis. The reducing sugar concentration of sweet sorghum could drop after heading because much sugar will be diverted for storage as starch in the seeds. This implies that for optimum bioethanol production, the industry can harvest at this stage except if grain is desired otherwise, fibre (bagasse) and foliage can be gotten from such harvest.

Cellulose, hemicelluloses and lignin content of bagasse from eight sweet sorghum varieties

The percentage of cellulose was higher than that of hemicelluloses and lignin in the eight Sweet sorghum varieties (Figure 4). This indicates that Sweet sorghum is a good raw material for bioethanol production. The percentages of cellulose

in the three selected Nigerian varieties were relatively higher (Figure 4) as they were well adapted to the environment. The fact that the crossbred varieties had the lowest percentages of cellulose can be attributed to the fact that the purpose of crossing was not to increase their percentage cellulose yield. Though the Nigerian varieties had higher cellulose percentage when compared with others, most of them had small stem diameter, relatively low survival rate and higher rate of pest infestation. The wide variation among all the sweet sorghum varieties could be as a result of origin and genetic differences. This also shows that similar environments, though in different regions of the globe can support growth of a species in as much as the environmental requirements for the survival of such species are intact. This agrees with (11) who reported the % composition of a sweet sorghum variety YSS-9 which had % cellulose, % hemicellulose and %lignin closer to that of Nigerian varieties.

Percentage bagasse yield

The low yield observed in ICSR 93034 and all the Nigerian varieties in figure 5 could be attributed to some factors which could affect their usefulness as resources for bioethanol production. These factors are; infestation of pest (larva of a pest possibly stem borer) early in the plant life and secondly the nature of the stem (some of the varieties had a rather slender thin stems). Attack of pests on the pith leads to early breakage and also affected the pith of the survived ones in such a way that they became useless. Secondly some sweet sorghum varieties among those that had low bagasse yield also had slender and thin stems. The highest percentage yield observed in the hybrid variety ICSA 89002 X SPV 422 (Figure 5) can be attributed to the fact that they have bigger stem diameter, fewer rots and taller stem than other varieties. Some of these characteristics could be the reason for crossing different Indian varieties to develop them.

Susceptibility of the pith bagasse to hydrolysis

The association between cellulose, hemicelluloses and lignin in the Indian variety, SPV 422 was in such a way that allowed much glucose to be released after bagasse pretreatment with HCl (Table 1 and 2). The same reason can be attributed to the highest yield obtained from SWSV 2006-3 when treated with H_2O_2.

Selection of two sweet sorghum varieties used for subsequent studies

Among the Nigerian sweet sorghum varieties tested, SWSV 2006-5-2 had the highest stem juice reducing sugar concentration at week 9 as shown in Figure 3 and is more susceptible to 1.0%HCl hydrolysis (Table 1) than the other two Nigerian varieties, a quality which will be highly desirable in the bioethanol industry. However, this variety (SWSV 2006 5-2) had slender tall stem that are prone to pest (maggot-like larva of a pest possibly stem borer) infestation. The leaves were also attacked. The pest attacked and destroyed majority of the stems such that it drastically reduced the percentage bagasse/fibre (powdered) yield at the time of harvest. SWSV 2006 5-2 variety had the overall lowest percentage bagasse yield. Similarly, SWSV 2007-1 is characterised also by tiny slender stems though a little bigger than SWSV 2006 5-2. The leaves were attacked by the same type of pest which destroyed the pith and also affected the quality of the pith of the survived ones. This led to a relatively poor bagasse yield. Although a high stem juice reducing sugar concentration was observed in this variety, its least susceptibility to 1.0%HCl hydrolysis is also a disadvantage. This implies that both SWSV 2006 5-2 and SWSV 2007-1 may not serve as a good variety for bioethanol production except if genetic modification of these promising line (high reducing sugar-yielding varieties) could introduce enhancement in stem size and a pest resistance factor. However, SWSV 2006-3 had better stem characteristics than the other two varieties in terms of size and height with the stem being moderately attacked by the pest. The bagasse yield was also low but better than the other two. It had the highest % glucose yield when treated with 1.0%H_2O_2, highest reducing sugar with 1.0%HCl and lowest reducing sugar concentration with 1.0%H_2O_2 (Tables 1 and 2 respectively). For this reason, SWSV 2006-3 was selected among the three Nigerian varieties screened. For the Indian and the hybrid varieties, SPV 422 was more susceptible to 1.0%HCl as presented in table 1 with E 36-1 ranking second while ICSR 93034 was more susceptible to 1.0% H_2O_2 than E 36-1, ICSA 95 X NTJ-2 and SPV422 which ranked second, third and fourth respectively in the % glucose yield released after hydrolysis. E 36-1 had value that is not statistically different from the value observed for ICSR 93034 while value SPV 422 was also not significantly different from that of ICSA 95 X NTJ-2. Treating the bagasse with 1.0%HCl gave the highest reducing sugar concentration with SPV 422 which is not significantly different from the value obtained from E 36-1. E 36-1 had the highest concentration of reducing sugar released after treatment with 1.0%H_2O_2 value which was not significantly different from that of SPV 422. It was observed from tables 1 and 2 that SPV 422 is generally more susceptible to HCl than E 36-1 while in table 1, E 36-1 and ICSR 93034 are more susceptible to H_2O_2 hydrolysis but in table 2 only E36-1 was more susceptible with value not significantly different from that of SPV 422.

Though ICSR 93034 in Figure 3 showed a carbohydrate (Cellulose + Hemicellulose) content comparable to the value recorded for SPV422, its lignin concentration is significantly higher than that of SPV 422. However, the stems of ICSR 93034 were also attacked by pest during growth and this also affected its % bagasse yield at the time of harvest.

E 36-1 had a mixture of slender and medium sized stem. E 36-1 had equal or close to equal likelihood to be selected based on the facts discussed above but other factors such as the composition of overall carbohydrate in the fibre was considered. E 36-1 had much lower carbohydrate content than SPV 422, higher lignin content and also lower % bagasse yield than SPV 422. It also had a mixture of medium and slender stems unlike SPV 422 which had relatively bigger stems with about 100% seed survival rate. This was why SPV 422 was preferentially chosen for further studies.

CONCLUSION

The initial field reducing sugar sampling provided information on the promising sweet sorghum varieties based on the amount of reducing sugar they can accumulate and the following: ICSA 89002 X SPV 422, ICSR 93034, ICSA X NTJ -2, E 36-1, SPV422, SWSV 2006-5-2, SWSV 2006-3, SWSV 2007-1 were selected. After the final screening with HCl and H_2O_2 pretreatment chemicals, two sweet sorghum varieties were selected to be very promising for bioethanol production.

However, the conditions for optimal bioethanol production using these selected varieties need to be studied.

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