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Compositional Analyses of *Solanum Lycopersicum* Stalk and Its Hydrolysis Using *Aspergillus Niger* Isolated From Maize Grains for Reducing Sugar Production

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Abstract
Fermentable sugars are important prerequisite for ethanol production. This study is aimed at establishing the compositional contents of tomato stalk and the production of fermentable sugar by pretreatment and subsequent hydrolysis using *Aspergillus niger* isolated from maize grains using standard procedures. The compositional analyses of the lignocellulosic raw materials revealed a 71.90%, 7.2%, 1.62%, 13.93% and 5.35% for the hemicelluloses, cellulose, lignin, ash and extractives respectively. Hydrolysis using *Aspergillus niger* reveals that the highest reducing sugar produced was found to be 2.46 mg/cm^3 at day two. The study revealed the potentials of tomato stalk is a rich substrate for reducing sugar production and by extension a potential of bioethanol production

Keywords: *Solanum lycopersicum*, *Aspergillus niger*, Compositional Analyses, Reducing Sugar.

1. Introduction

The importance of reducing sugar cannot be overemphasized because it is an essential raw material for the production of ethanol, a preferable alternative transportation fuel in this generation. Ethanol is beneficial to mankind; it can be used as solvent, germicide, anti-freeze and intermediate for other organic chemicals (Otulugbu, 2012). It is a renewable biofuel that could be produced from plant biomass and burn effectively in automobile engines without emission of hazardous gases to the environment (Adelekan, 2010). The search for alternative energy has become paramount due to anthropogenic emission of greenhouse gases which originate from combustion of fossil fuel as coal, oil and natural gas (Oyedepo, 2012).
Biomass has been recognized as a major source of renewable energy and hence, great emphasis has been placed on lignocellulosic biomass for the second generation bioethanol production since they are relatively inexpensive and abundant (Sukumaran and Pandey, 2009; Wyman, 1999). Lignocellulosic biomass is a complex matrix of cellulose, hemicelluloses, lignins, pectin, extractives and other components (Chandra *et al., 2007*; Kumar *et al*., 2009). They have to be physicochemically broken down to increase the fraction of amorphous cellulose vulnerable to attack by saccharifying and fermenting organisms which ultimately enhance the release of carbohydrate from the biomass for easy conversion to reducing sugar by hydrolysis (Lucas, 2012; Malherbe and Cloete , 2003). Biological treatment using various types of fungi is safe and environmentally friendly since it does not require high energy for lignin removal from the lignocellulosic biomass although the rate of hydrolysis is usually very low and requires long residence time (Sun and Cheng, 2002).
Solanum lycopersicum is a perennial grass about 1-3 meters high with a weak stem that often sprawl over the ground. The stalks are rich in cellulose and hemicelluloses and hence could be exploited for reducing sugar production. This research is aimed at exploiting the potentials of the stalks of *Solanum lycopersicum* for commercially reducing sugar production

2. MATERIALS AND METHODOLOGY

2.1 Sample Collection and Authentication

Fresh stalks of *Solanum lycopersicum* plants were collected at Dundaye village of Wamakko Local Government Area of Sokoto State, North-Western Nigeria in the month of June, 2017. They were identified and authenticated at the Bayero University, Kano herbarium where an Accession Number: BUKHAN 0367 was issued. They were cut into small pieces and sun dried for 4 days and later dried in a hot air oven at 70^{o}C for 6 hours. They were grounded into fine powder using a milling machine

(Electric grain milling machine). The powdered sample was stored at room temperature in a air tight glass container prior to its use.

Figure 1: *Solanum lycopersicum* stalk after collection

2.2 Chemicals

Acetone (JS England), NaOH, $MgSO_4$. $7H_2O$, $(NH_4)_2SO_4$, KH_2PO_4, $FeSO_4.7H_2O$, $ZnSO_4$, $MnSO_4$, sodium tartarate and $CaCl_2$ were all procured from BDH, England.

2.3 Compositional Analysis of the Raw Lignocellulosic Material

The dried samples were analyzed at the Industrial and Environmental Technology Laboratory, National Research Institute for Chemical Technology, Zaria. The percentage extractives, cellulose, hemicelluloses, lignin and ash were determined using the methods described below

2.3.1 Extractives

2.50 g dried biomass were loaded into the cellulose thimble. 150 cm^3 of acetone was used with the soxhlet extractor set up for its complete extraction. Residence times for the boiling and rising stages were carefully adjusted to 70^{o}C and 25 min respectively on the heating mantle for 4 hours run period. After extraction, the sample was air dried at room temperature for few minutes. Constant weight of the extracted material was achieved in an oven set at a temperature of 105^{o}C. The percentage weight of the extractives content was evaluated using equation 2.1 (Blasi *et al.,* 1999; Li *et al*., 2004; Lin *et al.,* 2010).

$$\% \text{ Extractives} = \frac{(\text{weight of crucible} + \text{extractives}) - (\text{weight of crucible})}{\text{Oven Dry weight (ODW) sample}} \times 100 \quad (2.1)$$

2.3.2 Hemicellulose

1.0 g of the extracted dried biomass was transferred into a 250 cm^3 Erlenmeyer flask. 150 cm^3 of 0.5 M NaOH was added. The mixture was boiled for 3.5 hrs with distilled water, filtered after cooling through vacuum filtration and washed until neutral pH was obtained. The residue was dried to a constant weight at 105^{o}C in an oven. The hemicelluloses content (%w/w) of the dry biomass was evaluated using equation 2.2 by difference between the sample weight before and after the treatment (Ayeni *et al.,* 2013, 2014a and 2014b).

$$\text{Hemicellulose} = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots (2.2)$$

Where W_1= Initial weight of sample; W_2= Final weight of sample

2.3.3 Lignin

0.3 g of the dried extracted raw biomass was weighed into test tubes containing 3 cm^3 72% H_2SO_4. The sample was kept at room temperature for 2 hrs with careful shaking at 30 min intervals to allow for complete hydrolysis. After the initial hydrolysis, 84 cm^3 of distilled water was added. The second step of hydrolysis was made to occur in an autoclave for 1 hr at 121 °C with the slurry cooled at room temperature. The hydrolysate was filtered through vacuum using a filtering crucible. The acid insoluble lignins was determined by drying the residues at 105^{o}C and accounting for ash by incinerating the hydrolyzed samples at temperature of 575^{o}C in a muffle furnace and were calculated using equation 2.3. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm. The lignin content was calculated using equation 2.4 by summation of acid insoluble lignin and acid soluble lignin (Sluiter *et al.,* 2008).

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \ldots\ldots\ldots (2.3)$$

W1= Weight of empty crucible
W2 = weight of sample + crucible before ashing
W3 = Weight of ash + crucible

$$\% \text{ Lignin} = \% \text{ ASL} + \% \text{ AIL} \ldots (2.4)$$

Where ASL = Acid Soluble Lignin; AIL = Acid Insoluble Lignin

2.3.4 Cellulose

The cellulose content (%w/w) was calculated by difference using equation (2.5) by assuming that extractives, hemicellulose, lignin, ash, and cellulose were the only components of the entire biomass (Blasi *et al.,* 1999; Li *et al*., 2004; Lin *et al.,* 2010).

$$\text{Cellulose} = 100 - (\% \text{ ash} + \% \text{ extractives} + \% \text{ hemicelluloses} + \% \text{ lignin}) \ldots. (2.5)$$

2.4 Isolation and Identification of *Aspergillus niger*

Aspergillus niger were isolated from maize grains using direct plating technique. 20 maize grains were picked randomly and surface sterilized by soaking for 1 minute in sodium hypochlorite (2.5%), and rinsed with sterile distilled water. The grains were blotted with sterile filter paper and plated on potato dextrose agar containing 7.5%

sodium chloride and 1.0 g of streptomycin sulphate in 1 litre of media (Diba *et al.*, 2007). The plates were incubated at 25^{o}C and monitored for fungal growth daily for seven days. The resulting cultures were identified based on cultural and morphological characteristics using taxonomic keys (Klich, 2002; Matasyoh *et al*., 2009). Target moulds were sub-cultured to obtain pure single-spore cultures. A small amount of the growth colonies were taken and smeared on a glass slide, covered with a slip and heated slightly to remove air bubbles before being viewed under microscope. The organism identified was compared with the standard structure of *Aspergillus niger* as described by Robert and Ellen (1988).

2.5 Enzymatic Hydrolysis

This was carried out according to Oyeleke *et al*. (2012); Nitesh *et al*. (2013). 10 g of the dried tomato stalk was weighed separately into 250 cm^3 conical flasks. $MgSO_4.7H_2O$ (0.06 g), $(NH_4)_2SO_4$ (0.28 g), KH_2PO_4 (0.4 g), $FeSO_4.7H_2O$ (1.0 g), $ZnSO_4$ (0.28 g), $MnSO_4$ (0.32 g) and $CaCl_2$ (0.06 g) were added as nutrient. Sterile distilled water was added to the 250 cm^3 mark. All flasks were covered with sterile cotton wool wrapped in aluminum foil to avoid contamination. The mixtures were autoclaved at 121^{o}C for 15 minutes, the flask was then cooled and sterile distilled water was aseptically added to make up to the mark. 10 cm^3 of freshly harvested *Aspergillus niger* cells were inoculated into each flask. The flasks was covered and then incubated at room temperature (28^{o}C) for six days. The flask was shaken at intervals to produce a homogenous solution and even distribution of the organisms in the substrate mixture. The mixture was separately filtered using Whatman No.1 filter paper. The clear supernatant of the hydrolysate at different time interval of 2, 4, 6, 8, 10 and 12 hrs were taken for estimation of reducing sugar.

2.5.1 Qualitative Test for the Produced Sugar

2 cm^3 of Benedict's reagent was added to 5 cm^3 of the hydrolysates sample and the resulting mixture placed in a boiling water bath for 5min. Brown coloration indicates the presence of reducing sugar (Plummer, 1971).

2.5.2 Estimation of Reducing Sugar

This was carried out in accordance with method described by Rabah, *et al.* (2011). The reducing sugar content of the hydrolysate was assayed by adding 3 cm^3 of 3, 5-dinitrosalicylic acid reagent to 3 cm^3 of the sample. The mixture was heated in a boiling water bath for 10 minutes for the red brown color to develop. 1 cm^3 of 40% potassium sodium tartarate solution was added to stabilize the color and then cooled at room temperature under running tap water. The absorbance of the sample was measured at 575 nm using UV-VIS spectrophotometer (Miller, 1959). The concentration of the produced reducing sugar will be obtained by extrapolation from the glucose standard calibration curve.

3.0 Results and Discussion

3.1 Compositional Analyses

The results of the compositional analyses of the raw lignocellulosic material is presented in Table 1

Table 1: Compositional Analyses of Raw Lignocellulose Stalk Residues of *Solanum lycopersicum*

Analyses	%w/w
Extractives	5.35 ± 0.02
Hemicellulose	71.90 ± 0.10
Lignin	1.62 ± 0.02
Cellulose	7.20 ± 0.10
Ash	13.93 ± 0.02

These results is lower than that reported by Guntenkin *et al*. (2009) who reported values of 7.14% extractives, 4.15% lignin, and 88% holocellulose (cellulose + hemicelluloses) from tomato stalk. The result reported however indicates that the production of bioethanol from tomato stalk is technically viable.

3.2 Isolation of *Aspergillus niger*

A black to brown color was observed and the presence of conidia, metula, hyphae on the microscopic structure shown in Figure 2a confirms the presence of *Aspergillus niger*. It was compared with the standard morpho;ogical strain reported by Robert and Ellen, 1988 (Figure 2b)

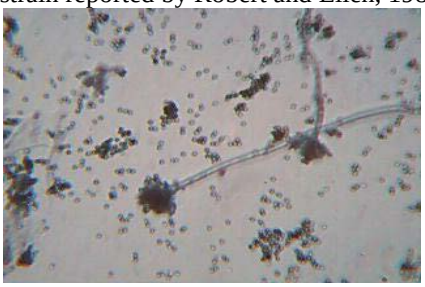

Figure 3a: Morphological structure of *Aspergillus niger* **x 250**

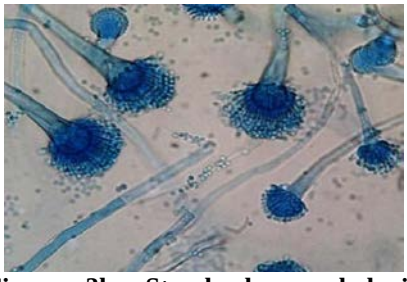

Figure 3b. Standard morphological structure of *Aspergillus niger* x 300

3.3 Reducing Sugar Yield

The result of the enzymatic hydrolysis of the *Solanum lycopersicum* stalk using *Aspergillus niger* is presented in Table 2.

Table 2. Reducing sugar yield of the *Aspergillus niger* hydrolyzed tomato stalk substrates

Sample	Yield from enzymatic hydrolysis (mg/cm^3)	Mass of Reducing sugar after enzymatic hydrolysis (g)
1	2.44 ± 0.004	0.610
2	2.46 ± 0.015	0.615
3	2.42 ± 0.013	0.605
4	2.43 ± 0.004	0.608
5	2.43 ± 0.008	0.608
6	2.44 ± 0.005	0.610

The highest sugar yield of 2.46±0.015 mg/cm^3 was observed after 48 hours of hydrolysis while the lowest reducing sugar yield of 2.42±0.013 mg/cm^3 was observed after 72 hours of hydrolysis. The use of microbes to cause hydrolysis is a slow but secure way of obtaining glucose for fermentation (Ephraim *et al.*, 2012).

3.3 Test for Reducing Sugar

After the Benedict's solution test a brick red precipitate was observed, which indicated the presence of reducing sugar

4. Conclusions

Aspergillus niger hydrolysis of *Solanum lycopersicum* stalks maximizes reaction time for optimum fermentation and hence a potential source of industrial reducing sugar production.

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