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Utilization of sponge gourd (*Luffa acutangula)* as a starting material in the production of bioethanol

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Abstract
This study was aimed at producing ethanol from Sponge gourd (*Luffa acutangula*) which is an agricultural waste. Ethanol was obtained from the sample by simple laboratory distillation. The physicochemical properties of the bio-ethanol obtained were determined using standard analytical methods and the results were compared to commercial ethanol. Sponge gourd gave 24.6% ethanol yield after 15 days of wild fermentation. Results also showed that sponge gourd high sugar contents but these sugars could not be fermented by yeast to produce high amount of ethanol because of the presence of high lignin content in the plant which acts as a barrier to microbial attack. However, higher yields of ethanol can be obtained from sponge gourd by pre-treating the sample first to remove the lignin before fermentation. The results of chemical test and FTIR spectra of ethanol obtained from the analysis were similar to commercial standards. These results suggest that agricultural wastes may serve as an alternative source of raw material for the production of industrial chemical such as ethanol.

Keywords: utilization, sponge gourd (*Luffa acutangula),* production, bioethanol

Introduction

Ethanol is a versatile solvent, miscible with water and many other organic solvents such as acetone, benzene, toluene and others (Windholz, 1976) [9]. Ethanol is a very polar molecule due to its hydroxyl (OH) group, with the high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules. It therefore attracts polar and ionic molecules. The ethyl (C_2H_5) group in ethanol is non-polar. Ethanol therefore attracts non-polar molecules. Thus, ethanol can dissolve both polar and non-polar substances; these properties make ethanol a very useful solvent used in several applications. Bio-ethanol can be obtained from a variety of feedstocks using cellulosic, starchy and sugar sources. These feedstocks include sugar beet, corn, sugar cane, bagasse, sorghum, switch-grass, barley, hemp, potatoes, wheat, wood, paper, straw, cotton and other biomasses. When ethanol fuel is produced from lignocellulosic materials such as wood, herbaceous plants, wild tubers, agricultural and forestry wastes, its use provides new markets for depressed farm economies. Although each source of biomass represents a technological challenge, the diversity of raw materials will allow the decentralization of fuel production with geopolitical, economic and social benefits (Peterson *et al*., 2009) [8]. A lot of agricultural raw materials rich in fermentable carbohydrates are being used worldwide for bio-ethanol production, but the costs of these raw materials have become a limiting factor for large scale production. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feedstock such as lignocellulosic biomass and agro-food wastes, are being considered to make bio-ethanol competitive in the open market (Peterson *et al*., 2009) [8]. In addition, the use of food materials will put pressure on the cost with attendant food scarcity. Therefore, there is the need for sourcing of ethanol from non-food materials. These lignocelluloses biomass and agric-food wastes can be used as potential feed stock for bio-ethanol production and could also be an attractive alternative for disposal of the polluting residues (Mosier *et al.*, 2005) [7]. In Nigeria, large amount of wastes is generated yearly from domestic and industrial processing of agricultural produce. Most of these wastes are used as animal feed or burnt. However, such wastes are usually rich in sugars, minerals and proteins, and can therefore be starting materials in the production of useful chemicals. In a bid to maintain sustainability, production of chemicals from renewable sources is required. Biopolymers, which include plant biomass, forestry residues and agricultural residues, are potential feedstock for production of chemicals. Biopolymeric fibres such as lignocelluloses, offer several environmental benefits owing to their renewable nature, abundance, low cost, low energy consumption in production, etc. Although, bioconversion of lignocellulosic residues has received much attention because of their important application such as efficient conversion of biomass to cellulose, most plant biomasses have not been fully exploited. Hence, in this research, an organic chemical (ethanol) shall be produced from sponge gourd in order to reduce dependence on fossil fuels as well as decrease the nuisance caused by wrong disposal of these wastes in the environment.

Materials and Method

Sample Collection and Treatment

Sponge gourd (*Luffa acutangula* fruit) was collected from a dumpsite at Akpan Andem Market, Uyo, Akwa Ibom State. The samples were washed, ground and filtered. Fresh samples (200g) were ground to obtain a paste. The paste was filtered and the filtrate poured into a standard flask and made up to 1000 cm^3 with distilled water. The liquid was allowed to stand for 5 days at room temperature to aid wild fermentation. The fermented liquid was then transferred into a round bottom flask and fixed to a simple distillation setup. Distillation was carried out at 75°C for 1 hour. Ethanol was collected in a clean conical flask. The procedure was repeated for fermentation periods of 10, 15 and 20 days in order to determine the best duration for high yield.

Fig 1: Fresh sponge gourd

Percentage yield of the Prepared Chemicals

The percentage yield of ethanol obtained from the analysis was calculated using the equation below

$$\text{Percentage yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \text{ X } 100$$

Where theoretical yield = the maximum amount of product a chemical reaction could create based on chemical equations.
Actual yield = the amount of the product obtained after experiment.

Characterization of the Prepared Chemicals

IR Spectroscopic Analysis

A drop of the produced ethanol was placed between two polished flat sodium chloride plates, mounted on the FTIR Spectrophotometer (PerkinElmer Co., USA) and scanned.

Density of the Chemicals

The empty density bottle was weighed and recorded as A_1. The density bottle was then filled with the prepared chemicals, reweighed and recorded as A_2. The density of the chemicals was then calculated according to equation below:

$$\text{Density} = \frac{A_2 - A_1}{volume}$$

pH of the Products

The pH of the fermentation medium and final product was measured using a pH meter.

Degree Brix

Degree Brix (°Bx) shows the amount of sugar present in an aqueous solution. This was measured in the fermentation medium using an Abbe's refractometer. The refractometer was calibrated using distilled water. The broth (1 mL) was placed onto the prism and pointed towards a light source and a reading was taken where the base of the blue color met the scale. This reading was recording as the sample's Brix.

Specific Gravity

The empty specific gravity bottle was weighed and recorded (B1). Then, the specific gravity bottle was filled with the produced chemical and reweighed (B2). The difference in weight was divided by the weight of an equal volume of water to give the specific gravity of the produced chemical as shown in the. The density of water = 0.99821 g/cm^3

$$\text{Specific Gravity} = \frac{B2 - B1}{0.99821\ g/cm^3 \times (25\ mL)}$$

Boiling Point Determination

Exactly 10 ml of the prepared ethanol was measured into a clean 100 ml beaker and a thermometer with a capillary tube was tied together and inserted. The beaker with its content was then heated using a hot plate until the first bubble was observed. The temperature at this point was recorded as the boiling point.

Jones Oxidation Test for Reducing Agents

The Jones Reagent is a solution of chromium trioxide in diluted sulfuric acid that can be used safely for oxidations of organic substrates in acetone. The distillate (10 ml) was measured into a test tube containing 1 ml acetone in 1 ml of Jones reagent. The formation of an opaque suspension with a greenish blue colour within 15 seconds upon addition of the orange-yellow reagent indicated the presence of an aldehyde and a primary or secondary alcohol.

Ethanol Estimation in distillate

This was done by specific gravity method (Borah and Mishra, 2011). The difference in specific gravity before and after fermentation is due to the conversion of sugars before fermentation into alcohol after fermentation. The concentration of ethanol was then calculated using the equation below:

$$\text{Concentration of ethanol} = \frac{SG_1 - SG_2}{0.0074}$$

Where SG_1 = initial specific gravity; SG_2 = final specific gravity

Results and Discussion

Percentage Yield of Ethanol at Different Fermentation Period

After 5 days of wild fermentation and 2 hours of distillation using 100g of the sample as the starting material, 170 ml of 21.20% ethanol was recovered from sponge gourd. This indicates that the waste can serve as cheap alternative sources of bio-fuels.

The amount of ethanol obtained from sponge gourd was relatively lower than that obtained from other agricultural wastes. This could be as a result of the low sugar content in sponge gourd which could hinder the activity of the yeast while converting the sugars to alcohol. Higher lignin content in sponge gourd (14.7%) could also be a reason for the low ethanol yield in LAF as lignin acts as a physical barrier to cellulosic enzymes (Nyong, Ita, Ita and Eka, 2020) [6]. The yield of ethanol from sponge gourd at different fermentation period is presented in Table 1. Results showed that optimum yield was obtained after 15 days.

Table 1: Percentage Yield of Ethanol Obtained from Fermentation of sponge gourd

Fermentation days	5	8	10	12	15	18	20
Ethanol yield	21.2	21.6	22.7	23.0	24.6	23.1	22.2

Effect of Fermentation time on Ethanol Yield

Ethanol yield of 21.2% was obtained from sponge gourd after 5 days of fermentation (Table 1). The yield of ethanol gradually increased until after the 15th day, the yield decreased gradually till the 20th day. The amount of fermentable sugar present in the medium must have reduced completely after the 15th day, hence the decrease in yield. The highest ethanol yield in was obtained on the 15th day. This could be as a result of the fermentation method. Wild fermentation usually take longer time since the yeast is cultured naturally (Dewes and Hunsche, 1998) [5]. When compared to other biopolymers, the ethanol yields obtained in this study are higher than 10.08% obtained after 7 days fermentation of corn cob using *Aspergilusniger* and *Saccharomyces cerevisial* co-culture (Itelima *et al.*, 2013) [4].

Effect of Temperature and pH on Ethanol Production

Temperature plays a major role in the production of ethanol since the rate of alcoholic fermentation increases with increase in temperature. The optimum temperature for ethanol production (Table 2) ranged from 28ºC to 32ºC. The highest yield of ethanol was obtained at 31ºC in sponge gourd. This agrees with the findings of Krishna and Chowdary (2000) [2, 3] who reported that as the temperature increases beyond 32ºC, enzymes begin to denature or unfold and thus become inactive. Each enzyme has a different temperature range where it becomes inactive. The pH values of the fermentation broth ranged between 5 and 6. The gradual decrease in pH recorded in this study indicates that acids were slightly accumulated in the fermentation medium during the fermentation process. This observation is similar to the findings of Itelima *et al.* (2013) [4] who reported a pH variation between 3.05 and 7.58 and an ethanol yield of 10.08% after 7 days of fermenting corn cob with *Aspergilus niger* and *Saccharomyces cerevisiae.* In general, yeast is an acidophilic organism and grows better under acidic condition (Wong and Sanggari, 2014). The optimum pH range for yeast growth may vary from pH 4.0 to 6.0, depending on the temperature, the presence of oxygen and strain of yeast. Optimum pH values are required for the activity of plasma membrane bound proteins, including enzymes and transport proteins (Narendranath and Power, 2005) [1]. During growth, it is important for the yeast to maintain a constant intercellular pH. Therefore, in this study, the highest yield of ethanol (30.8%) was obtained at 31ºC and a pH value of 6.10.

Table 2: Temperature, specific gravity and pH Values obtained during fermentation of sponge gourd

Fermentation Days	Temperature of broth ºC	Brix (%)	pH	Δ SG	Ethanol Yield (%)
5	28.2	7.1	5.98	0.158	21.1
8	30.3	6.8	5.79	0.160	21.6
10	31.1	6.6	6.10	0.168	22.7
12	31.3	6.5	6.10	0.173	23.0
15	31.3	6.4	6.29	0.785	24.6
18	32.2	5.2	6.38	0.171	23.1
20	32.2	4.8	6.32	0.164	22.2

Physicochemical Parameters of the Produced Ethanol

The density and boiling point of the ethanol produced from sponge gourd is presented in table 3. The results obtained reveal that the boiling point and specific gravity closely approximates that of commercial ethanol. The different acid treatment and fermentation period had no significant effect on the physicochemical properties of the products.

Table 3: Physicochemical parameters of Ethanol obtained from fermentation and distillation of sponge gourd extract

*SG 1	SG 2	Vol. of distillate Obtained (ml)	% ethanol in distillate	Boiling point
1.12	0.962	170	21.2	79.5

Distillation time = 120 minutes; weight of fresh sample = 200g; fermentation period = 5 days

FTIR Spectra of Ethanol Obtained from TOF and LAF

The FTIR spectra of ethanol produced from sponge gourd distillate is presented in Figure2. The spectra showed well defined peaks at 3316, 2973, 1442 and 1045cm^{-1}.

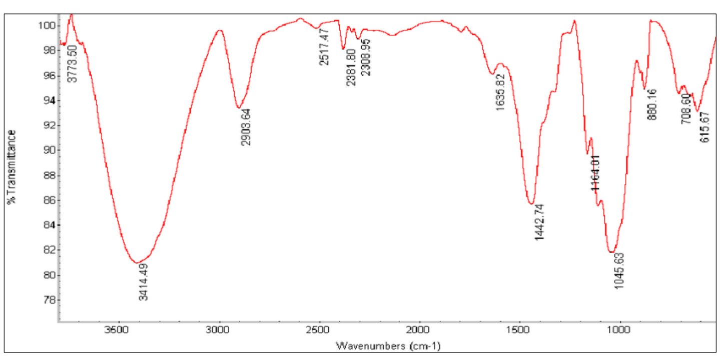

Fig 2: FTIR spectrum of ethanol from sponge gourd

Conclusion

The objective of this research was to produce ethanol from sponge gourd (*Luffa acutangula*) fruit, determine its percentage yield and compare the physicochemical properties of the produced ethanol with their commercial counterparts. From the results obtained, yields of ethanol from sponge gourd were lower than that obtained from literature; ethanol yield of 23.0% was obtained from sponge gourd after 15 days of wild fermentation. Analysis showed that sponge gourd high sugar contents but these sugars could not be fermented by yeast to produce high amount of ethanol because of the presence of high lignin content in the plant which acts as a barrier to microbial attack. However, higher yields of ethanol can be obtained from sponge gourd by pre-treating the sample first to remove the lignin before fermentation. When compared to commercial ethanol, the result obtained showed a promising resemblance. This makes sponge gourd fruit wastes useful as substitutes for the production of polymers and organic compounds.

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