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Reducing sugars from agro-waste biomass suitable for industrial applications

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ABSTRACT

Determination of reducing sugars present in the juices of Calabash (Crescentia cujete) and Fluted Pumpkin (Telfairia occidentalis) pulps was investigated. The dinitrosalcylic acid (DNS) method was used. A calibration curve was plotted and the sugar concentration extrapolated. The reducing sugar was reported as 3.22 mg/ml for Calabash and 3.70 mg/ml for Fluted Pumpkin; representing 0.322% and 0.37% yield, respectively. Qualitative test was previously carried out on the juices using Benedict's solution method. Calabash and Fluted Pumpkin pulps have high moisture contents, which is evident in the volume of juice recovered per fruit, hence their use in industrial applications could be economically feasible.

Keywords: Agro-wastes, biomass, reducing sugars, and industrial application.

INTRODUCTION

Agricultural wastes are abundant in our environment, and if not properly handled may constitute health hazards. When they are dumped at sites, they form compounds that may leach into water bodies and could suffocate fish and other aquatic organisms, as well as affect other beneficial microorganisms [1]. The chemical composition of these agricultural wastes are mostly lignocelluloses; comprising of cellulose, hemicelluloses and lignin. Cellulose and hemicellulose components are made up of long chains of sugars, mainly glucose.

These sugars can be converted to useful products, thereby generating wealth as well as waste management [1]. Hydrolysis of these lignocellulosic components could be done by physical, chemical or biological treatments. The resulting sugars are mostly reducing sugars such as glucose, hexoses and pentoses which can be easily metabolized by several genera of microorganisms to industrial products such as biofuels [1].

However, the methods to make these sugars available are faced with a lot of technological challenges, especially in our own society here. This thought led us to research into getting the parts of Agro wastes that are rid of lignocelluloses, hence, having readily available sugars for industrial purposes.

This study is aimed at analyzing the juices from pulp wastes of Calabash (*Crescentia cujete*) and Fluted Pumpkin (*Telfaria occidentalis*) fruits for available reducing sugars and comparing their yield.

MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

The Calabash fruit (*Crescentia cujete*) was sourced from homes in Mbaise, Imo State, where they serve as hedge, and are underutilized. The Fluted Pumkin pods were purchased from three major markets in Mbaise: Afor Enyiogugu, Afor Ogbe and Nkwo Mbaise.

The calabash pod was cut open and the pulp collected,the juice squeezed out using muslin cloth and sieve of diameter 21.5cm x 38cm L x 10cm H [2;3].The fluted pumpkin seeds were removed and the pulp also squeezed to get the juice. The juices were then analysed for reducing sugars using dinitrosalicylic acid (DNS) method [4].

2.1 Analysis

2.1.1 Production Yield: This was determined by material balancing [5].

2.1.2 Determination of Reducing Sugar Content: This was done by the dinitrosalicylic acid (DNS) method[4].

1) Preparation of Standard glucose solution

Anhydrous glucose, 0.1g was dissolved in distilled water and the volume was made up to 100 ml in a volumetric flask.

2) Preparation of Dinitrosalicylic acid Reagent (DNS)

a) Sodium potassium tartarate, 300g was dissolved in about 500 ml distilled water.
b) 3, 5-dinitrosalicylic acid, 10g was dissolved in 200 ml of 2N NaOH
c) DNS Reagent was prepared by mixing solutions in (a) and (b) above and raising the final volume to 1 liter with distilled water.

Procedure:

i) A series of clean test tubes were labeled tubes 1 – 6 respectively and volumes of standard stock glucose added: 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml

Table 1.0 Data for Plotting Glucose Standard Curve

Tube No	Standard glucose (ml)	Water (ml)	DNS Reagent (ml)	Water (ml)
1	0.0	1.0	2.0	7.0
2	0.2	0.8	2.0	7.0
3	0.4	0.6	2.0	7.0
4	0.6	0.4	2.0	7.0
5	0.8	0.2	2.0	7.0
6	1.0	0.0	2.0	7.0

ii) A corresponding volume of distilled water, to make to 1 ml each was added to the tubes in (i) above: 1, 0.8, 0.6, 0.4, 0.2, and 0 ml.
iii) DNS reagent was added in 2 ml amounts to the test tubes. The solution was mixed well and the tubes were placed in boiling water bath for 5 minutes.
iv) The tubes were allowed to cool and 7 ml of distilled water added to each of them, and the absorbance read in a spectrophotometer at 540 nm using tube 1 as a blank (control).
v) The absorbance readings were plotted against glucose concentrations to get a standard curve.
vi) The curve was used to extrapolate the concentration of the unknown sample in mg/ml.

3) Testing for the Sample

One (1 ml) milliliter of the sample was dispensed in clean test tubes. Two milliliter (2ml) of dinitrosalicylic acid (DNS) reagent was added. The mixture was heated in a boiling water bath for 5 minutes. It was allowed to cool, after which 7ml of distilled water was added. The absorbance was measured with a UV-Spectrophotometer at 540nm, using blank as control.

RESULTS AND DISCUSSION

The absorbance values for the standard glucose solutions are represented in Table 1.The result for the reducing sugars in the calabash and fluted pumpkin pulp juices, with glucose as a standard, are low, with values as 3.22

mg/ml and 3.70 mg/ml for calabash and fluted pumpkin, respectively (Table 2.0). These values were extrapolated from standard glucose graph (Fig.1).

Table 1 Absorbance Values for Glucose Standard Curve

Concentration	Absorbance
0.2	0.046
0.4	0.121
0.6	0.193
0.8	0.298
1	0.353

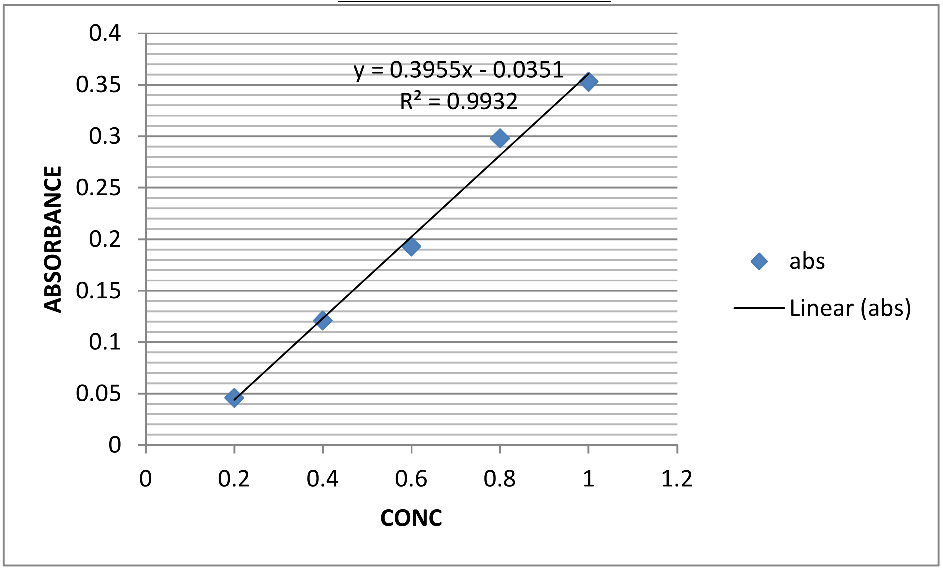

Fig. 1 Glucose Standard Curve

Table 2.0: Estimation of Reducing Sugar in Samples

Sample	Glucose (mg/ml)
Calabash juice	3.22 ± 0.20
Fluted pumpkin juice	3.70 ± 0.10

The quantity of sugar from this study is low, when compared with values from other fruits: *Citrus sinensis* 17mg/ml, *Citrus limetta* 21mg/ml and *Ananas comosus* 20mg/ml [6]. However, our result is higher than the reducing sugar obtained from sugar beet pulp juice 1.24mg/ml (Gumienna, *et al*., 2013). Also, the reducing sugar reported by Itelima, *et al*., (2013) as 0.63 mg/ml is lower than our results from this study. Coffee pulp hydrolysates have been reported to have fructose (0.9 – 3 g/l), glucose (1.3 – 6.31 g/l), and sucrose (0.08 – 3.96 g/l) [8].

The low sugar obtained from this study could be as a result of interferences with phytochemicals present in the samples. This is evident in the changes observed in colours of the juices during processing. Ejelonu, *et al*., (2011) reported the presence of saponins, flavonoid, cardenolides, tannins, phenol, and hydrogen cyanide from calabash fruit pulp. And also that sugars present in the Calabash fruit are sucrose 59.86% and Galactose 18.24% ,etc [9]. Fluted Pumpkin pulp, on the other hand, has pectin content of 1.0% [10]. Other phytochemicals such as alkaloids, flavonoids, phenols, saponins and tannins have been reported in fluted pumpkin seed shell [11].

CONCLUSION

This study is the first stage of research into the production of bioethanol from Calabash and Fluted Pumpkin pulp juices. It has confirmed that they contain reducing sugars in small amounts, and may require optimization processes to make the process profitable.

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