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Yield of Ethanol Produced from Waste Pod of Fluted Pumpkin (*Telfairia Occidentalis*) Using African Giant Snail (*Archachatina Marginata*) Slime and Yeast

Akwukwaegbu, P. I. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Peters, D. E.* Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Wegwu, M. O. Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Abstract: Yield of ethanol produced using biowaste from waste pod of fluted pumpkin (*Telfairia occidentalis*) was investigated. Breakdown of cellulose to glucose in the waste was achieved using cellulase from snail slime. Into holding tanks containing varying concentrations of wastes were added snail slime and yeast respectively. The control holding tank (group A) had neither yeast nor snail slime. All groups were fermented under anaerobic condition at (37oC) for 24hrs, 48hrs and 72hrs. Results of the cellulose content showed (20.56±0.58%). There was no ethanol (Et) produced in the control group (group A). Results of the yield of Et of the waste in the holding tanks containing snail slime alone and yeast alone were (1.45±0.10%) and (5.44±0.44%) respectively. The yield of Et produced decreased significantly ($p<0.05$) with increase in fermentation time. Also, increase in concentration of snail slime and yeast increased the yield of Et in the holding tanks. The holding tanks containing waste, yeast and snail slime together gave the highest yield of ethanol. The findings in this study suggest that a consortium of snail slime and yeast would yield higher percentage of ethanol than the duo acting independently.

Keywords: Biowaste; Holding tank; Ethanol; Fermentation.

1. Introduction

While the world is running short on fossil fuels, the production of solid waste and biowaste is growing steadily at the same time due to a growing world population and a rising standard of living in developing countries. The challenge of reducing greenhouse gas emissions has given rise to alternatives to fossil fuels. Global energy policy making responded to the urgent situation by setting up targets, like the European Union (EU) which is demanding a share of renewable fuels of at least 10 per cent of the fuel consumption in the EU by 2020 [1]. To answer the demand for new sources of energy and manage the growing amounts of waste, there has been research carried out on the utilization of waste for energy production in the past and will become more and more important in the future.

Biofuels produced from biomass such as plants or organic waste could help to reduce both the world's dependence on oil and CO_2 production. These biofuels have the potential to cut CO_2 emission because the plants they are made from use CO_2 as they grow [2].

Furthermore, lignocellulosic feedstock and waste can offer the potential to provide novel biofuels, the biofuels of the 'second generation' [3]. Second-generation biofuels produced from 'plant biomass' refers largely to lignocellulosic materials, as this makes up the majority of the cheap and abundant non-food materials available from plants. But, at present, the production of such fuels is not cost effective because there are a number of technical barriers that need to be overcome before their potential can be realized [4]. It is anticipated that, these 2nd generation biofuels could significantly reduce CO_2 production, do not compete with food crops and some types can offer better engine performance.

Once the pumpkin leaves and seeds are removed for various uses, the remaining recalcitrant stalks, pods and leaves are thrown into refuse bin, open dumps or ends up in a flowing stream. Preliminary investigations showed that several tons of these waste are produced daily in market places in Nigeria but scarcely useful and therefore create environmental nuisance. This practice can have serious implications on environment and health.

Utilization of biowastes as energy with high efficiency and rationality not only meets the demands for energy, but also provides a basis for environmental protection and sustainable development of the society [5, 6].

It has been reported that this waste serves as livestock meal but its digestibility is considered poor due to its anti-nutritional factors [7]. The proximate composition of *Telfairia occidentalis* coat has been given and as a result of this

*Corresponding Author

composition, it has been classified as a lignocellulosic material, hence the interest in finding out its feasibility of yielding ethanol biofuel.

According to Stichnothe and Azapagic [8] municipal waste and especially organic waste becomes due to its qualities increasingly interesting for the energy production industry, since the environmental and economical benefits of bioethanol derived from cultivated crops are questionable. Waste materials used as feedstock for the bioethanol production decrease the stress on landfills, increase the re-use of materials and reduce the greenhouse gas emissions from landfill sites. This paper was aimed at quantifying the yield of ethanol produced from waste pod of fluted pumpkin (*Telfairia occidentalis*) using African giant snail (*Archachatina marginata*) slime and yeast.

2. Materials and Method

2.1. Methods

2.2.1. Sample Collection

Pumpkin pod waste was collected from Obodo Ahiara community in Ahiazu-Mbaise L.G.A of Imo State, Nigeria. African giant snail was bought at Umuapu in Ohaji Egbema L.G.A of Imo State. The snail shell was broken and the slime extracted mechanically. Approximately 500g of solid waste was weighed using electronic weighing balance and used for proximate, phytochemical and cellulose analysis. The remaining solid waste was subjected to fermentation and distillation for ethanol extraction and determination in varying proportions.

2.2.2. Experimental Design

Pumpkin pods were first collected from storage bans and ground into a fine mixture using mortar and pestle. Varying weights of 250g, 500g and 750g of solid waste were chosen for the 1st, 2nd and 3rd determinations respectively. A total of seven groups labeled A-G were set up as explained below. Group A (pumpkin pod (Pp) alone); group B (snail slime (SL) plus Pp); group C (yeast (Y) plus Pp); group D- D1, D2, D3 (25ml of (SL) plus 7.5g of (Y)); Group E- E1, E2, E3 (50ml of SL plus 15g of Y); group F- F1, F2, F3 (75ml of SL plus 22.5g of Y) and group G- G1, G2, G3 (100ml of SL plus 30g of Y). All groups were fermented under anaerobic condition at 37^{o}C for 24hrs, 48hrs and 72hrs. The pH of the solution before and after centrifuging was 5.4 and 5.2 respectively [9].

2.2.3. Sample Extraction for Ethanol Determination

Each sample container was squashed, filtered with cheese filter cloth to obtain the fermented liquid samples and centrifuged at 2500rpm for 15 minutes to obtain 200ml of supernatant. Afterwards, the sample (supernatant) was decanted and made ready for distillation.

2.2.4. Estimation of Ethanol by Soxhlet Distillation [10]

The samples were transferred into the soxhlet flask and placed on a heating mantle. The soxhlet condenser together with the entire components of the soxhlet apparatus was then set up. The heating mantle was set at a temperature of 78^{o}C (boiling point of ethanol) for 1hr and the content of the flask was left to distill as ethanol was collected overhead.

The percentage (%) yield of ethanol, related to the volume of sample (supernatant) used was calculated as follow;

$$(\%)\ \textbf{yield of ethanol} = \frac{\textbf{volume of ethanol produced}}{\textbf{volume of sample used}} \times \frac{\mathbf{100}}{\mathbf{1}} = \frac{\mathbf{W_1 - W_0}}{\mathbf{W_2 - W_0}}$$

2.2.5. Method of Data Analysis

All data collected were subjected to descriptive and one way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS), Inc.20.0 software. All data were represented in mean ± standard deviation (M±S.D). Confident level of determination ($p<0.05$).

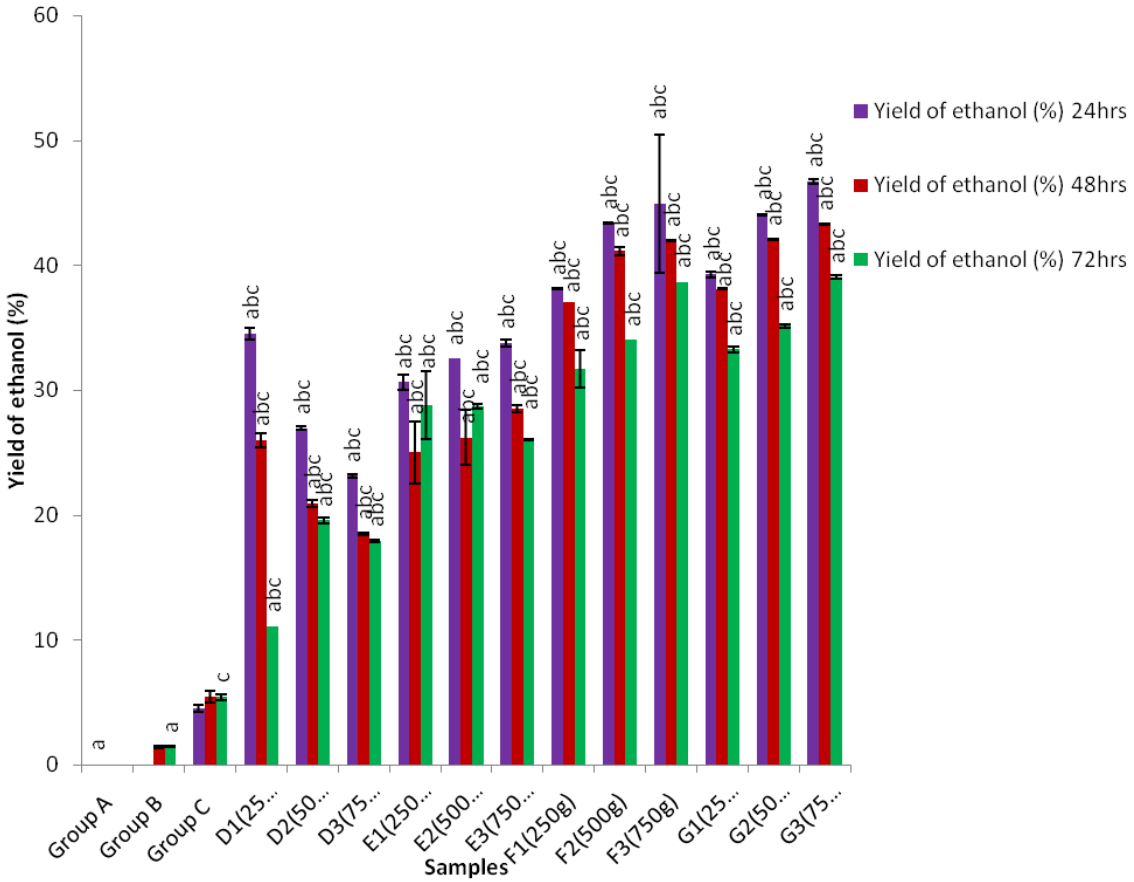
3. Results

Table-1. Cellulose content of fluted pumpkin (*Telfairia occidentalis*) pod waste

Cellulose content	Pumpkin pod waste (%)
Cellulose	20.56±0.58

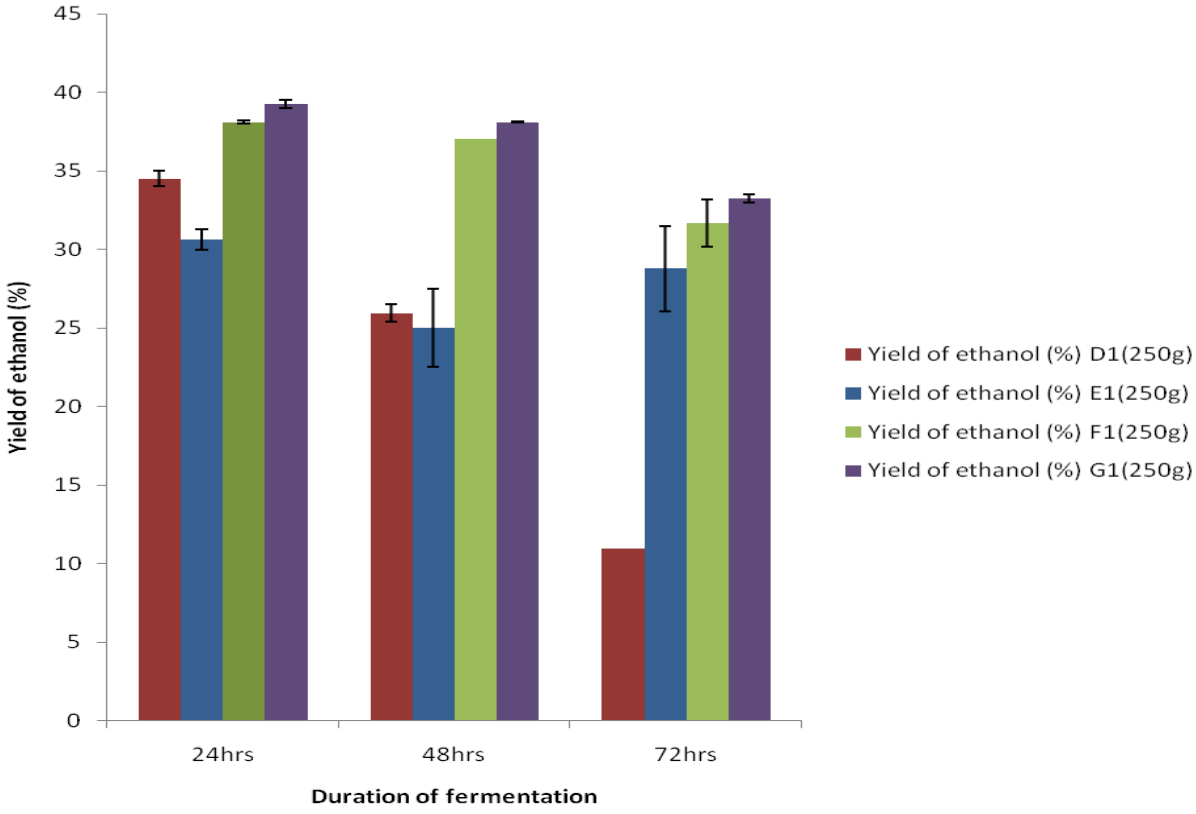
Value is mean ± standard deviation (M±S.D) of triplicate determination (n=3).

Fig-1. Yield of ethanol at varying fermentation times



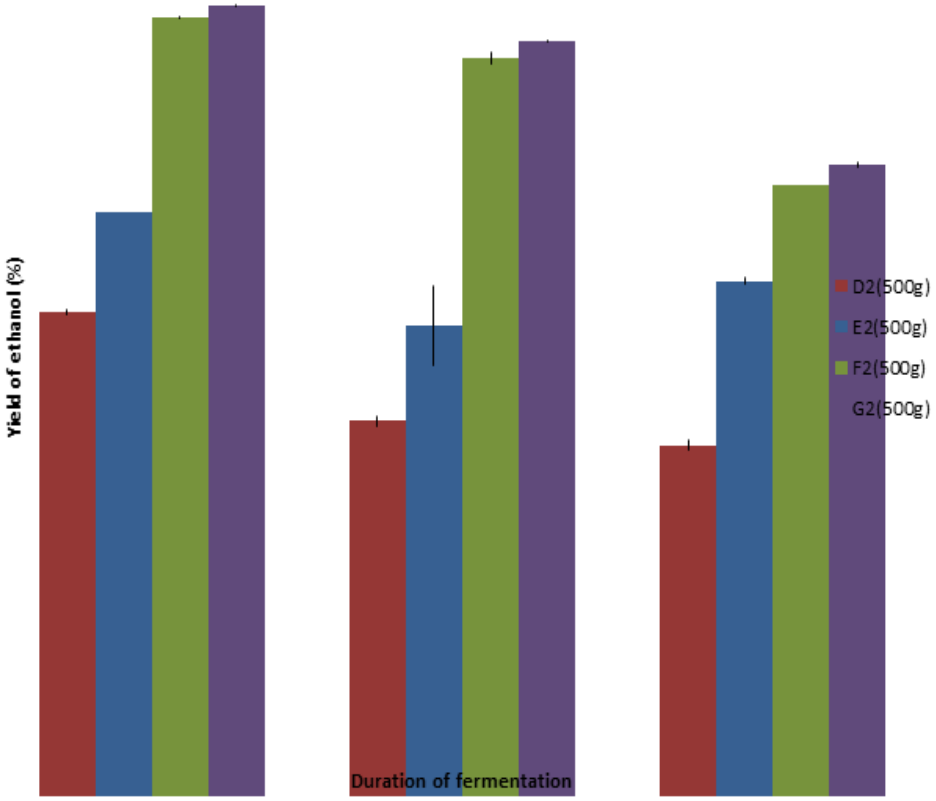
Data are mean ± standard deviation (M±S.D) of triplicate determinations. Superscripts a, b, c indicate significant difference ($p<0.05$) when compared to groups A, B and C respectively.

Fig-2A. Yield of ethanol at varying concentrations of snail slime and yeast



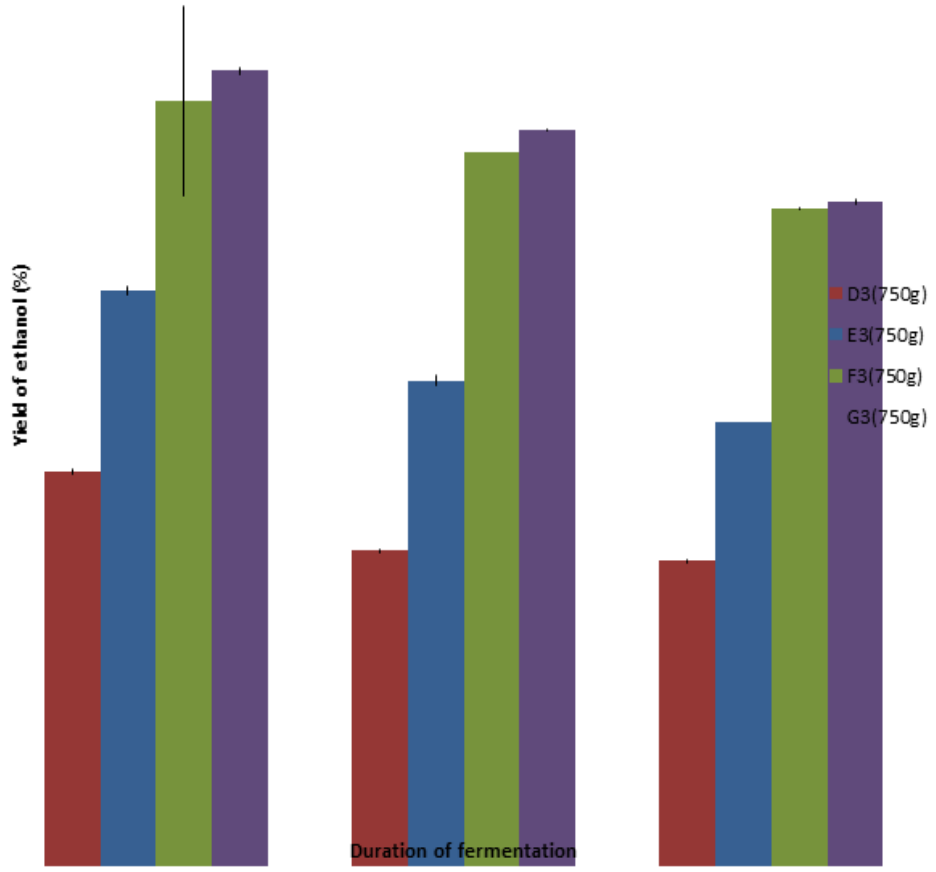
Data are mean ± standard deviation (M±S.D) of triplicate determinations.

Fig-2B. Yield of ethanol at varying concentrations of snail slime and yeast



Data are mean ± standard deviation (M±S.D) of triplicate determinations.

Fig-2C. Yield of ethanol at varying concentrations of snail slime and yeast treated with 500g pumpkin pod waste.



Data are mean ± standard deviation (M±S.D) of triplicate determinations.

4. Discussion and Conclusion

The cellulose content in fluted pumpkin waste was found to be 20.56% (Table 1). The cellulose contents together with the fibre content of fluted pumpkin provide substrate for cellulase action. Snail slime was able to degrade pumpkin pod waste because of the substantial amount of cellulose and this to a large extent influenced our choice of lignocelluloses waste.

The yield of ethanol produced at varying fermentation time was represented in Figure 1. It was observed that the yield of ethanol decreased significantly with fermentation time which could be as a result of decrease in yeast action on the fluted pumpkin pod waste as fermentation continued. There was no ethanol produced in group A while very little ethanol was produced in groups B and C. There were significant differences ($p<0.05$) in the yield of ethanol from groups D-G when compared to groups A, B and C respectively (Fig 1). Ethanol was produced in group B (pumpkin pod waste and snail slime only) probably due to the presence of amylase, α-glucosidase, protease and lipase in addition to cellulase found in the gut regions of digestive track of African giant snail [11]. The quantity of yeast present in the sample to sustain the fermentation process decreased.

Figures (2A, 2B and 2C) showed the yield of ethanol at varying concentrations of snail slime and yeast for the varying weights of fluted pumpkin pod waste taken. It was observed that the yield of ethanol increased with increase in concentration of snail slime and yeast which shows that snail slime and yeast act synergistically to bring about yield of ethanol. It was equally observed that there was no marked change in the yield of ethanol in group F and group G which represents the groups with the second to the highest and highest concentrations of snail slime and yeast respectively. This could be that at this point, the enzyme had attained saturation point and the active sites were fully occupied.

The percentage bioethanol yield from pumpkin pod waste system was very low generally when compared to the E 85 (85% ethanol) utilized in fuel flexible vehicles (FFV) in Brazil and China which has the capacity to reduce green house gas effect, particulate and sulfate emissions by 10, 20 and 80 %, respectively. The low yield of ethanol may be due to acidic nature of the waste and solution. The pH was measured over the time of analysis. The pH before and after centrifuging was 5.4 and 5.2 respectively. Though the acidic pH was necessary to prevent microbial activities in the containers which could have inhibited the fermentation process, however, it may have contributed to lower percentage yield of ethanol from the waste. Acids and bases are known to delignify plant cell structures. It could as well be as a result of high content of indigestible fibre waste (hemicelluloses and lignin) among others in fluted pumpkin waste pod, which are structural polysaccharides and very difficult to biodegrade.

From this study, it is concluded that snail slime and yeast act synergistically to enhance higher yield of ethanol. Also, increasing the concentration of the snail slime and yeast brought about increased yield in ethanol from the waste. Maximum yield of ethanol was obtained after fermentation for 2-3 days.

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