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ORIGINAL ARTICLE

Impact of changes in fermentation time, volume of yeast, and mass of plantain pseudo-stem substrate on the simultaneous saccharification and fermentation potentials of African land snail digestive juice and yeast

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African land snail

Abstract This study was carried out to investigate the effect of variations in mass of plantain pseudo-stem waste, volume of yeast used, and fermentation time on the product yield resulting from simultaneous saccharification and fermentation using digestive juice of African land snail and yeast. The experiment was divided into three stages which included a total of fifty seven (57) experimental setups containing sixteen (19) different combinations of the varied substrates. The results show that by varying the mass of plantain pseudo-stem waste, the production of ethanol was optimized at a mass of 250 g, which yielded 125.6 ml ± 3.5 of distillate and a percentage ethanol composition of 25.0 ± 3.6. While varying the volume of yeast used between 50 and 250 ml, with 250 g of plantain pseudo-stem waste, 250 ml of snail digestive juice and 4 g garlic for 24 h, acetic acid was detected in the setup containing 200 ml of yeast, but was not detected in similar experimental setups containing 6 g garlic. The optimum ethanol production while varying the volume of yeast slurry was recorded to be 182.3 ml ± 4.9 of distillate with 28.0% ± 1.0 ethanol composition. Variations in fermentation periods had the greatest impact on the percentage composition of ethanol and the volume of ethanol produced showing the best fermentation period for obtaining optimal ethanol production to be at 96 h. These findings show that the best specifications for the optimum production of ethanol from a 250 g of plantain pseudo-stem waste using 250 ml snail digestive, are 200 ml of yeast slurry, 6 g of garlic to ferment for a period of 96 h.

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1. Introduction

Other reasons that have boosted the use of alternative sources of energy includes the fact that they pose less air pollution problems due to reduced emissions into the biosphere [23,24], and they are more environmentally friendly. Most of these wastes are composed majorly of cellulose. Utilization of lignocellulosic waste has been found efficient for the generation of substances used for electrical and thermal energy, as well as the generation of biofuels [15,20,44]. Plantain is one of the earliest cultivated crops of the musaceae family that is propagated by suckers. The plant grows to maturity in about four months, after which the fruit is harvested, and the aerial part is felled and left to rot away. Plantain pseudo-stems are fleshy and soft, they are actually the lower parts of the leaves folded together and structures with some intercellular openings are found within each fold; there is a large fleshy central core at the centre of the folds. The folds can easily be separated by hand. The core transports the nutrients from the roots to the upper part of the plant and finally terminates in fruit formation. The pseudo-stem contains much water that fills up the intercellular openings. The pseudo-stem except the core is made up of cellulosic filaments which are bonded together into cellulosic films by lignin and hemicelluloses [4]. The plantain pseudo-stem waste serves very little industrial purposes where its juice plays only significant roles in the manufacture of dyes and in herbal medicine. However, with 50% fibre [12] and quite unlike other agricultural wastes, the plantain pseudo-stem is left to rot away in their plantations due to its relatively very low appeal to grazing livestock as a result of high oxalate content. Consequently, the practice to utilize the pseudo-stem wastes of plantain plants for the production of ethanol as a lignocellulosic biomass ensures it overcomes the limitations of the first generation biofuel sources by not threatening food supplies and biodiversity. This implies that their usage can be extended above the thresholds of other food crop sources of bioethanol like sugar cane, corn, wheat, etc and still afford to sustainably supply a larger proportion of bioethanol even with greater environmental benefits. However, this type of waste poses some difficulties especially with extracting useful feedstocks from the fibrous biomass where the useful sugars are locked in by lignin, hemicelluloses and cellulose. It then requires an effective and efficient means to circumvent the recalcitrant nature of this waste at low cost, on time and with high yield while reducing to the barest minimum the possible occurrence of fermentation inhibitors.

The conversion of lignocellulosic biomass to ethanol entails the following steps: pretreatment, enzymatic degradation, fermentation and isolation of the ethanol. According to Balat [8] pretreatment serves the purpose of decreasing crystallinity, surface area enlargement, deconstruction of lignin barriers, and removal of hemicelluloses. Biodegradability of the cellulose tends to be the prominent difficulty, due to the presence of lignin in most cellulosic biomass [36]. A method of pretreatment would be considered ideal, if when applied, would deconstruct only the lignin moieties without the degradation of the cellulosic component. This is thus expected to cause an improvement on the sugars available, prevent carbohydrate breakdown and prevent the synthesis of inhibitory products all at minimal costs [56]. Most products that arise from lignin catabolism, causes an inhibition to the microorganisms responsible for the fermentation of sugar and cellulose through the provision of degrading enzymes. Hence, emphasis is laid on a pretreatment method that will in no way compromise the efficiency and optimal performance of these processes. With this, the availability of cellulose to degrading enzymes is enhanced, thus facilitating the catalysis of carbohydrates to fermentable sugars, with improved yield.

Saccharification refers to the synthesis of monosaccharides from the enzymatic degradation of complex polysaccharides. Historically, amorphous cellulose has been reported to be rapidly degraded to cellobiose by cellulases, while the saccharification of crystalline cellulose is much slower. *Archachatina marginata* (African land snail), a herbivore that naturally mostly consumes higher plants (Macrophytophage), is found mostly in East Africa but can be related to have originated from Tanzania and Kenya. *A. marginata* is a species termed to be highly invasive, and can form colonies from a single gravid individual. It feeds on vast majority of vegetables mostly and sometimes fruits. Ademolu et al. [2] while monitoring the availability of certain enzymes during some season induced conditions revealed that glycosidases in the foot muscles of *A. marginata* reduced significantly during aestivation. Adedire et al. [1] has revealed the rich composition of the gut of *A. marginata* with hydrolytic enzymes such as trypsin, lipases, alpha-glucosidases, proteases, and cellulases, while Fay [19] reported that since a carbohydrase is usually specific with reference to both the linkage and to the monosaccharide constituents of the polymer it splits, it is probable that most of the carbohydrates chosen for his study, were degraded by discrete enzyme systems of *A. marginata*. Strasdine and Whitaker [47], detected the mild presence of chitinase and cellulase activities in hepatopancreas of *Helix pomatia*; a European species of land snail. In addition, both cellulase and chitinase activities in the digestive juices (luminal fluid of the gut) increased in proportion to body weight and the total protein content of the hepatopancreas, but not in relation to increases in digestive juice bacterial counts when, for example, the snails emerge from hibernation and start eating. Strasdine and Whitaker, [47] thus concluded that the enzymes were endogenously produced.

Fermentation refers to the aggregate effect of yeast growth on monosaccharides, resulting to a spent growth, alcohol. *Saccharomyces cerevisiae* have been used frequently to ferment the monosaccharides present in corn, barley, wheat, and rice for the production of alcohol and rising of dough. The synthesis of fermentation inhibitors has been one of the major drawbacks of bioconversion of lignocellulosic biomass. The intolerance of alcohol concentration of greater than 10%V/V, poses a difficulty to the viability of fermenting microorganisms because of the tendency to produce ethanol concentrations of up to 17% V/V or higher [50]. More often, product inhibition is obtained during simultaneous saccharification and fermentation, where products of saccharification inhibit hydrolytic enzymes e.g. sugars like glucose and cellobiose on cellulase [6] while ethanol, a fermentation product, inhibits zymase. Larsson et al. [32] reported that formic acid bears the greatest inhibitory effect on *Saccharomyces*

cerevisiae followed by levulinic acid and then acetic acid, at concentrations greater than 100 mM. Therefore, most researchers now seek plant sources of phytochemicals that could specifically target and inhibit the enzymes that synthesize these fermentation inhibitors, in particular acetate. Kishimoto et al. [29] has reported a reduction in the levels of acetate and acetaldehyde, after the administration of garlic to experimental animals, with a consequent increase in ethanol concentration. Another study, also observed the change in rumen fermentation from acetogenic to glucogenic [39] while Rabinkov et al. [40] claimed that garlic extract proved useful in preventing the synthesis of acetaldehyde from ethanol by inhibiting selectively alcohol dehydrogenase. Also Reuter et al. [42] posited that by interacting with the sulphydryl group of proteins and some thiol containing enzymes like papain and alcohol dehydrogenases, the organo-sulphur constituents of garlic (allicin) effectively induce their antimicrobial effects. Notwithstanding the good knowledge of the biochemical processes provided by scientific processes [21], managing fermentation has been a process of balancing yeast metabolism and growth such that the products desired are generated within a specified time. Changes related to the volume of yeast, fermentation time, and mass of substrate are important factors among other factors that affect fermentation performance, and are considered to bear a significant effect on the product yield, but have received minimal interest. It is on this note that this study was carried out to evaluate the impact of changes in mass of plantain pseudo-stem substrate, volume of yeast, and fermentation time on the simultaneous saccharification and fermentation potentials of African land snail slime and yeast.

2. Materials and methods

2.1. Sample collection

The plantain pseudo-stem (PPS) waste samples used for this study were collected from a farmland at Irete in Owerri west L.G.A Imo state, and from plants that their fruits have been harvested and left for about 5 to 7 days. The samples collected were only made up of the fibrous matter excluding their core in the pseudo-stem of the plant. These samples were identified at the Plant Science and Biotechnology department Imo state University.

The garlic (*Allium sativum*) samples used for this study were purchased at Ekeonunwa Market Owerri and were identified at the Plant Science and Biotechnology department Imo state University.

The garlic was used for two purposes in this study

- As a snail irritant
- To inhibit the synthesis of fermentation inhibitors.

The African land snails (*A. marginata*) used for this study were purchased at Ekeonunwa market Owerri and identified at the department of Animal and Environmental Biology Imo State University Owerri.

The African land snails were used as sources of cellulase for the saccharification of plantain pseudo-stem wastes.

The yeast (*S. cerevisiae*) slurry (Brewer's yeast in liquid suspension to enable its fluidity) used for this study was obtained from the fermentation tank of Pabod breweries Trans-Amadi Port Harcourt Rivers State.

Yeast properties:
Viability: 99%
Consistency: 80%
Cell count using a haemocytometer: 2×10^{10} cell/ml

2.2. Pseudo-stem and garlic sample preparation

The fresh plantain pseudo-stem wastes were chopped into bits with a knife, oven dried at 50 °C, then ground properly using an electronic grinding machine (Dade:DFT-50), and sieved using a stainless steel sieve of 0.5 mm mesh, and afterwards transferred into a 1 dm^3 broad base plastic container. Next, the garlic samples were sundried and finely ground, then added to the container containing the finely ground plantain pseudo-stem waste.

2.3. Excision of digestive juice from snail (*A. marginata*)

The purchased and identified snails were separated into groups of eight snails in each group and put into a locally constructed horizontally partitioned plastic container with a perforated cover lid to allow the entry of oxygen. The partition is made up of a net to allow fluids pass through easily and settle at the bottom of the container. The snails were starved of food and water for 72 h, a compulsory procedure for generation of maximum quantity of digestive juice according to Walker et al. [53]. Then, freshly ground garlic of about 3grams was introduced into each group to serve as an irritant and afterwards left for an additional 72 h. After the 72 h, the digestive juice and slimy fluid settled at the bottom of the container were transferred into a beaker. Afterwards, the shells of the snails were broken and the digestive gland of the snails carefully and persistently rocked to secrete more. The snails were then cut open and rocked again in order to enable the foot muscles secrete enough slime for the experiment.

2.4. Preparation of yeast slurry

The acquired yeast slurry was refrigerated at 4 °C while occasionally opening the lid to release CO_2.

2.5. Pretreatment method

The hot water pretreatment method was used for this study. The ground and pre-weighed plantain pseudo-stem biomass to be pretreated were submerged in 500 ml of water in a fabricated stainless steel heating tank of 0.05 inch thickness with total volume of 1 dm^3 and a stirrer fitted on its lid. The heating tank was set on an electrothermal heating mantle and allowed to boil. At onset of boiling, a stop watch was adjusted for the required boiling time and after this time the heater was turned off and the heating tank allowed to cool.

2.6. Experimental design

This experiment consists of three stages

Stages	PPS (g)	SDJ	Yeast	Garlic	Time
1	50, 150, 250, 300, 350	250	100	4	24
2a	250	250	50, 100, 150, 200	4	24
2b	250	250	200, 250	6	24
3	250	250	200	6	24, 48, 72, 96, 120

*PPS means plantain pseudo-stem, SDJ means snail digestive juice.

2.7. Procedure

To the labelled Erlenmeyer flasks, the plantain pseudo-stem waste and garlic samples were added. Then to these substrates, excised snail digestive juice and yeast slurry were added. The resulting mixture in the flask was transferred into an electric blender and left to mix for 15minutes to ensure homogeneity of the mixture. After 15 mins of blending, the mixture was transferred back to the Erlenmeyer flasks, covered and left to stand for 24 h of time, occasionally releasing the accumulated CO_2.

On reaching the 24 h fermentation period, the mixture was sieved using a sieve cloth and the residue removed while the filtrate was prepared for further distillation. The filtrate collected in the beaker was further centrifuged (Coslab: Cle-110) at 2.5×10^3 RPM for 15 mins. While centrifuging, the hot plate was set at 78 °C and left for 15 mins. After centrifuging, the supernatant was collected and measured before distillation. The supernatant was distilled using a reflux condenser and the distillate collected and measured. The pH recorded from the fermentation mixtures ranged from 4.5 to 4.8 and temperature range of 34–37 °C

2.8. Analytical methods

The concentration of ethanol was estimated by spectrophotometric method of Caputi et al. [10].

Exactly 1 ml of the distillate was introduced into a distillation flask containing 30 ml of distilled water, and to it was added 25 ml of potassium dichromate solution (obtained from a mixture of 34 g of potassium dichromate in 500 ml of distilled water and 325 ml of conc. H_2SO_4, all made up to 1000 ml). Twenty millilitres of the resulting mixture was transferred to a test tube and incubated in a water bath at 60 °C for 20 min and afterwards cooled at room temperature and the volume made up to 50 ml. From this, 5 ml was measured out and diluted with 5 ml of distilled water and the absorbance determined at 600 nm using a spectrophotometer. The concentration of ethanol was determined from standard curve drawn using absolute ethanol, while the ethanol yield was calculated using the method of Yoswathana and Phuriphipat [55] shown below.

$$\text{Ethanol yield} = \frac{\text{Measured ethanol in sample}}{\text{Amount of initial sugar content} \times 0.5}$$

For determination of reducing sugars, the PPS samples were blended with sterile distilled water in ratio of 1:10 respectively using electric blender. To 1 cm^3 of the blended samples, 1 cm^3 of 3,5-dinitrosalicylic acid was added and boiled for about 5 mins before diluting with 10 cm^3 of distilled water. The absorbance was read at 540 nm using a spectrophotometer and concentration extrapolated using glucose as standard [5].

The cellulose content was determined using the standard procedure of Kürschner-Hanak, [31], while the qualitative acetic acid determination was carried out using lanthanum by a colorimetric microanalytical method [25], and% Saccharification evaluated using the description of Uma et al. [51] stated below.

$$\text{Saccharification } (\%) = \frac{\text{Reducing sugars} \times 0.9 \times 100}{\text{Cellulose content in pretreated substrate}}$$

Percentage yield was calculated as $\frac{V1}{V2} \times 100$ where V1 represents volume of the distillate, and V2 represents volume of the entire fermentation before distillation.

2.9. Statistical analysis

The data obtained from the analysis was subjected to statistical analysis of variance (ANOVA) using the SPSS® statistical package (version 20) from IBM® Inc, USA and the means were compared with least standard deviation (LSD) test at 95% confidence interval. The values obtained from the analysis were thus expressed as mean ± standard deviation.

3. Results and discussion

The results of the cellulose content, amount of reducing sugars and percentage saccharification derivable from plantain pseudo-stem waste using 250 ml of snail digestive juice, and 100 ml of *S. cerevisiae* are shown in Table 1. The results show the least cellulose content obtained after pretreatment of 50 g PPS and adding snail digestive juice and yeast, was 9.1% ± 0.8, while no significant change was recorded for both the cellulose content and amount of reducing sugars, on increment of the plantain biomass from 250 g to 350 g. These results are in agreement with the reports that at very low substrate concentration, the yeast starves and productivity decreases [33]. In general, addition of snail digestive juice had a positive correlation with the release of sugars. The significant difference recorded for the amount of reducing sugars of the pretreated 50 g of PPS and the succeeding experimental setup containing SDJ in Table 1, implies the effective potentials of SDJ in saccharifying the PPS substrate used, while the significant decrease in the amount of reducing sugars after the addition of yeast suggest that the synthesized sugars were utilizable by the yeast cells. On a similar note, Itelima et al. [27] posited that the after substrate hydrolysis by *A. niger*, the susceptibility of sugars to the fermentation activity of *S. cerevisiae* depends on the sugar composition. The cellulose content of PPS recorded in this study 25.5% ± 2.4 was comparable to the values reported for banana pseudo-stem waste 22.8% ± 2.3 [38], but lower than the cellulose content of sugarcane bagasse, pretreated by steam explosion containing 49.89% [22]. The reduc-

Table 1 Effect of varying masses of substrates on cellulose content, reducing sugars, saccharification and ethanol yield of plantain pseudo-stem waste using 250 ml of SDJ and 100 ml yeast.

Parameters	Cellulose (%)	Amt. of reducing sugars	% Saccharification
50 g of PPS	25.5 ± 2.4[a]	5.1 ± 0.4[a]	18
Pretreated 50 g PPS	52.5 ± 3.3[b]	4.9 ± 0.2[a]	8.4
Pretreated 50 g PPS + SDJ	9.1 ± 0.8[c]	50.7 ± 0.4[b]	501
Pretreated 50 g PPS + SDJ + yeast	8.8 ± 1.0[c]	12.5 ± 1.2[c]	127.8
Pretreated 150 g PPS + SDJ + yeast	39.9 ± 2.9[d]	27.2 ± 2.7[d]	61.3
Pretreated 250 g PPS + SDJ + yeast	42.0 ± 0.3[de]	36.6 ± 2.0[e]	78.4
Pretreated 300 g PPS + SDJ + yeast	44.0 ± 0.2[de]	33.9 ± 2.9[e]	69.3
Pretreated 350 g PPS + SDJ + yeast	45.7 ± 0.3[e]	35.0 ± 0.8[e]	68.9

Values represent means and standard deviations of triplicate determinations.
Values bearing similar superscript(s) letters (a–e) down the column denotes no significant ($P < 0.05$) change using the least standard deviations (LSD).
SDJ-Snail digestive juice, PPS-Plantain pseudo-stem.

ing sugar content of PPS waste 5.1 mg/ml ± 0.4 recorded in this study was found to be lower than the reducing sugar content in sunflower head waste 26.73 mg/ml [45]. Pretreated oil palm branches had more cellulose 77.50% [17] than the values obtained for PPS (52.5% ± 3.3) recorded in this study while the experimental setup lacking yeast showed the highest percentage of saccharification obtained in this study.

The results in Table 2 show the effect of alterations in mass of plantain pseudo-stem waste on the volume of distillate, percentage yield and percentage ethanol by volume, using 250 ml of snail digestive juice, 100 ml of brewer's yeast, and 4 g garlic for 24 h. The best levels for volume of distillate and percentage yield were obtained in this study when the mass of plantain pseudo-stem waste was 250 g. There were no significant changes in results recorded between masses 300 g and 350 g. Ishmayana et al. [26] justified the fact that it is more preferable to obtain a high sugar concentration during industrial bioethanol production due to the potentials to enhance the ethanol concentration generated after the completion of the fermentation process. He however observed that sugar concentration above the optimum amounts compromises the viability of the yeast cells by exposing them to high osmotic stress, thus affecting the fermentation performance. Also, it was observed that the percentage ethanol by volume remained unchanged as the source of cellulose increased from 150 to 350 g, indicating a sufficient amount of fermentable sugars for the amount of yeast available per that fermentation time (24 h).

From the results shown in Fig. 1 for the ethanol yield of varying masses of plantain pseudo-stem waste using 250 ml of SDJ and 100 ml yeast, increment of the biomass from 50 g to 150 g consequently increased the yield of ethanol which remained fairly uniform as the substrate mass was increased to 350 g. The deduction that uniformity was achieved as the mass of substrate attained 350 g from 250 g, is shown in Fig. 1.

The results of the effect of variations in volumes of brewer's yeast on cellulose content, reducing sugars, and saccharification using 250 g of plantain pseudo-stem waste, 250 ml of snail digestive juice and 4 g/6 g garlic for 24 h are shown in Table 3. The residual cellulose contents were unaffected by the variations in amount of yeast used thus showing that yeast possibly do not utilize cellulose. However, the amount of reducing sugars significantly decreased with the addition of 50 ml of yeast slurry, attaining uniformity at 200 ml of yeast (29.9 ± 1.1 mg/ml). No significant change was recorded for the residual reducing sugar contents, between the experimental setups containing 150 ml and 200 ml of yeasts. However, it was observed that the increment of mass of garlic from 4 g to 6 g significantly decreased the amount of reducing sugars (24.7 ± 0.3) suggesting that addition of garlic enhanced sugar utilization by yeast. From the results in Table 3, it is expected that the amount of initial reducing sugar was above the recorded values of the residual sugar content. These results were in agreement with the findings of Hashem et al. [24] that reported that the productivity of the ethanol producers slightly decreased when the sugar concentration increased up to 30 or 35%. Reddy et al. [41] reported that the increase in the sugar concentration will decrease the sugar utilization, which results in reduction of the total ethanol production. This

Table 2 Effect of varying masses of plantain pseudo-stem waste on the volume of distillate, percentage yield and percentage ethanol by volume, using 250 ml of snail digestive juice, 100 ml of brewer's yeast, and 4 g garlic for 24 h.

Performance parameters	50 g Plantain stem waste	150 g Plantain stem waste	250 g Plantain stem waste	300 g Plantain stem waste	350 g Plantain stem waste
Vol. of distillate (ml)	56.0 ± 3.5[a]	101.3 ± 1.5[b]	125.6 ± 3.5[c]	133.0 ± 3.0[c]	133.3 ± 6.1[c]
Percentage yield (%)	35.4 ± 2.1[a]	42.8 ± 0.4[b]	47.4 ± 2.1[c]	48.4 ± 1.3[c]	48.9 ± 1.5[c]
Ethanol (% W/V)	10.6 ± 2.1[a]	23.0 ± 3.0[b]	25.0 ± 3.6[b]	24.0 ± 2.6[b]	25.0 ± 2.0[b]
CH_3COOH	–	–	–	–	–

Values are means and standard deviations of triplicate determination.
Figures across the column bearing similar superscript(s) letters (a–c) mean there is no significant ($P < 0.05$) difference between them. The sign – indicates absence.

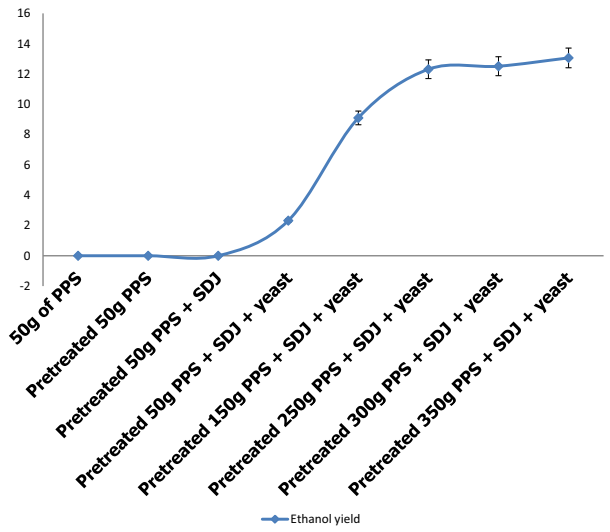

Figure 1 Ethanol yield of varying masses of plantain pseudo-stem waste using 250 ml of SDJ and 100 ml yeast.

reduction could be due to several reasons including the production of other compounds like glycerol or acetic acid. Also, the intracellular ethanol, which may be increased by increasing ethanol production at high sugar concentration, exerts high toxicity on yeast and the nutrient may be deficient at the final stage of fermentation [46]. All these factors lead to stopping the fermentation process and ethanol formation at the final stage of fermentation. Optimal saccharification was achieved using 50 ml of yeast slurry.

The effect of varying volumes of brewer's yeast on the volume of distillate produced, percentage yield and percentage ethanol by volume, using 250 g of plantain pseudo-stem waste, 250 ml of snail digestive juice and 4 g/6 g garlic for 24 h were shown in Table 4. The results on the effect of increase in yeast concentration on the rate of fermentation revealed that though there was a wide range of volume over which the yeast (enzyme) is seen active, there is a narrow range over which the activity is maximum, monitored through the resulting volume and percentage ethanol by volume for each distillate. Ethanol causes the inhibition of cell viability and growth, and rate of fermentation, depending on the parameter being assessed [37]. Also, a general method for the quantification of ethanol tolerance is yet to be established because studying the capacity of yeast to tolerate ethanol is quite difficult due to numerous inhibitory effects of ethanol on this organism [14,13]. The results of this study show that by increasing the volume of yeast from 150 ml to 200 ml caused no significant change in the resulting volume of distillate and percentage ethanol by volume, but induced the synthesis of acetic acid at 200 ml with 4 g garlic. The synthesis of acetic acid due to the increase in volume of ethanol is in accordance with the suggestions that notwithstanding that the capacity to tolerate ethanol is strain dependent, *Saccharomyces* sp. is adjudged to be unable to tolerate a modest concentration of ethanol [49] [9]. Casey and Ingledew [11] observed that at an ethanol concentration of 20% (v/v) the capacity of *S. cerevisiae* to undergo fermentation is inhibited but yeast growth inhibition occurs at a much lower concentration. Hypothetically, ethanol must have inhibited alcohol dehydrogenase 1 and activated alcohol dehydrogenase 2 responsible for the oxidation of ethanol to acetaldehyde and further oxidation to acetic acid by aldehyde dehydrogenase. Following the consequent increase in mass of garlic by 2 g (i.e. from 4 g to 6 g) from the results of this study shown in Table 4, it could be seen that the formation of acetic acid was inhibited with a concomitant significant ($p < 0.05$) increase in the volume of distillate but not for the percentage yield of ethanol. One would suggest from this that the increase in the allicin content from the 4 g to 6 g garlic enabled the previously insufficient allicin content at 4 g to inhibit the increased concentration of aldehyde dehydrogenase brought about by the suppression of alcohol dehydrogenase 1 by ethanol. It was further shown that when the mass of garlic was increased to 6 g, a significant increase in the volume of distillate was obtained, with no further increase in ethanol content resulting from increasing the volume of yeast to 250 ml at 6 g of garlic. This level implies the optimal biomass to yeast to garlic ratio for the production of ethanol.

The results of the ethanol yield of fermented PPS waste with varied quantities of yeast slurry, shown in Fig. 2 indicate that optimal fermentation is achieved using 200 ml of yeast with 6 g garlic that remained fairly uniform with the results obtained using 250 ml of yeast and 6 g garlic. However, an obvious rise in ethanol yield was shown on increment of the garlic used from 4 to 6 g. Osunkoya and Okwudinka [35] after fermenting different cassava cultivars similarly reported that increasing the amount of yeast used affects the yield of ethanol produced. However, it has been reported that the increase in the quantity of yeast does not favour the fermentation process initially but with the continuous increase, the yeast activity

Table 3 Effect of varying volumes of brewer's yeast on cellulose content, reducing sugars, saccharification and ethanol yield using 250 g of plantain pseudo-stem waste, 250 ml of snail digestive juice and 4 g/6 g garlic for 24 h.

Parameters	Cellulose	Amt. of reducing sugars	% Saccharification
250 g PPS + SDJ + 50 ml yeast + 4 g garlic	42.0 ± 0.3[a]	41.6 ± 0.9[a]	89.1
250 g PPS + SDJ + 100 ml yeast + 4 g garlic	42.0 ± 1.4[a]	37.2 ± 1.7[b]	69
250 g PPS + SDJ + 150 ml yeast + 4 g garlic	41.6 ± 1.1[a]	29.8 ± 0.8[c]	64.5
250 g PPS + SDJ + 200 ml yeast + 4 g garlic	41.3 ± 1.2[a]	29.9 ± 1.1[c]	65.2
250 g PPS + SDJ + 200 ml yeast + 6 g garlic	43.0 ± 1.2[a]	24.7 ± 0.3[d]	51.9
250 g PPS + SDJ + 250 ml yeast + 6 g garlic	44.2 ± 1.4[a]	24.3 ± 0.8[d]	49.5

Values represent means and standard deviations of triplicate determinations.
Values bearing similar superscript(s) letters (a–d) down the column denotes no significant ($P < 0.05$) change using the least standard deviations (LSD).
SDJ-Snail digestive juice, PPS-Plantain pseudo-stem.

Table 4 Effect of varying volumes of brewer's yeast on the volume of distillate produced, percentage yield and percentage ethanol by volume, using 250 g of plantain pseudo-stem waste, 250 ml of snail digestive juice and 4 g/6 g garlic for 24 h.

Performance parameters	50 ml of Yeast with 4 g Garlic	100 ml of Yeast with 4 g Garlic	150 ml of Yeast with 4 g Garlic	200 ml of Yeast with 4 g Garlic	200 ml of Yeast with 6 g Garlic	250 ml of Yeast with 6 g Garlic
Vol. of distillate (ml)	75.0 ± 6.0^{a}	133.3 ± 6.1^{b}	164.3 ± 4.5^{c}	161.3 ± 3.5^{c}	182.3 ± 4.9^{d}	183.3 ± 3.1^{d}
Percentage yield (%)	36.0 ± 3.1^{a}	48.9 ± 1.5^{bc}	51.4 ± 0.8^{b}	45.0 ± 0.9^{c}	46.6 ± 0.8^{c}	46.8 ± 0.6^{c}
Ethanol (% W/V)	23.0 ± 2.6^{a}	25.0 ± 2.0^{ab}	26.3 ± 1.5^{ab}	26.3 ± 2.1^{ab}	28.0 ± 1.0^{b}	27.0 ± 1.0^{ab}
CH_3COOH	–	–	–	+	–	–

Values are mean and standard deviation of triplicate determination.
Figures across the column bearing similar superscript(s) (a-d) letters mean there is no significant ($P < 0.05$) difference between them. The sign – indicates absence + indicates absence.

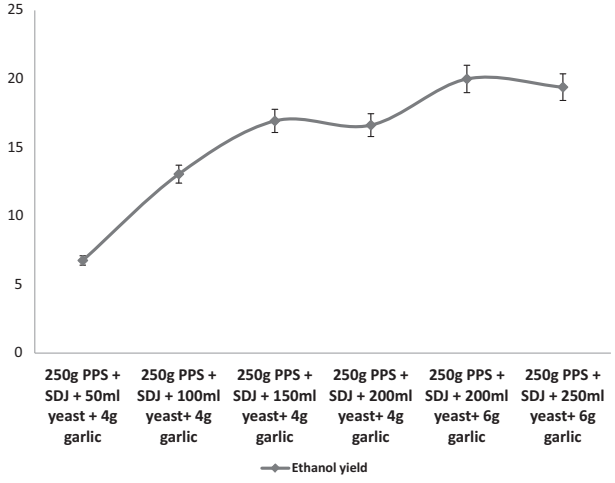

Figure 2 Ethanol yield of fermentation setups with varied quantities of yeast slurry.

increases thereby increasing the yield [35]. This observation was in contrast to the findings of this present study for PPS where an initial and continuous increase in ethanol yield was recorded until a uniform ethanol yield was obtained between 200 ml and 250 ml as shown in Fig. 2. The basis for the decline in fermentation rate resulting from the increase in yeast quantity from 200 ml to 250 ml was not fully understood. Evidence has been accumulating which indicates that decline in fermentative activity may not only be attributed to the presence of ethanol. Dombek and Ingram [16] posited that by removing a fermentative broth containing ethanol with fresh medium and replacing them with one lacking ethanol did not restore fermentative activity immediately. Further investigations are thus needed to ascertain the cause of the decline in the growth of these yeast cells.

Similar to the effect of varied volume of yeast used, presented in Table 4, the cellulose contents were unaffected by variations in fermentation periods, as shown in Table 5, while the amount of reducing sugars continuously increased with increment in fermentation period from 24 h to 96 h. No significant change in amount of reducing sugars was obtained by increment to 120 h fermentation, showing optimum fermentation period to be 4 days. Ado et al. [3] similarly reported a continuous significant reduction in the amount of reducing sugars as the fermentation period increased. The effect of reduced sugar concentration and ethanol inhibition becomes however important after optimum fermentation time. The fermentation continued at a decreasing rate until it leveled off between 4 and 5 days, as indicated by the statistically comparable amount of reducing sugars obtained after 96h and 120h of fermentation (Table 5). Fermentation time also varies depending on the yeast strains, and substrates used as sources of sugars. Arifa et al. [7] showed a similar analysis of residual sugar concentration from sugarcane molasses fermented by *S. cerevisiae,* reducing with further increase in time period resulting in a steady decrease ethanol production.

Fig. 2 shows the ethanol yield of fermentation setups containing 200 ml of brewer's yeast, 250 g of Plantain pseudo-stem waste, 250 ml of snail digestive juice and 6 g garlic. An

Table 5 Effect of varying fermentation periods on the cellulose content, reducing sugars, and saccharification using 200 ml of brewer's yeast, 250 g of Plantain pseudo-stem waste, 250 ml of snail digestive juice and 6 g garlic.

Parameters	Cellulose	Amt. of reducing sugars	% Saccharification
250 g PPS + SDJ + 200 ml yeast + 6 g garlic on 24 h fermentation	43.0 ± 1.2^{a}	24.7 ± 0.3^{a}	51.6
250 g PPS + SDJ + 200 ml yeast + 6 g garlic on 48 h fermentation	41.0 ± 0.6^{a}	20.1 ± 0.9^{b}	44.1
250 g PPS + SDJ + 200 ml yeast + 6 g garlic on 72 h fermentation	42.5 ± 1.8^{a}	17.2 ± 0.3^{c}	36.4
250 g PPS + SDJ + 200 ml yeast + 6 g garlic on 96 h fermentation	42.0 ± 1.0^{a}	11.1 ± 0.4^{d}	23.7
250 g PPS + SDJ + 200 ml yeast + 6 g garlic on 120 h fermentation	41.5 ± 1.0^{a}	12.0 ± 0.5^{d}	26.0

Values represent means and standard deviations of triplicate determinations.
Values bearing similar superscript(s) letters (a–d) down the column denotes no significant ($P < 0.05$) change using the least standard deviations (LSD).
SDJ-Snail digestive juice, PPS-Plantain pseudo-stem.

Table 6 Effect of varying fermentation periods on the volume of distillate, percentage yield and percentage ethanol by volume, using 200 ml of brewer's yeast, 250 g of Plantain pseudo-stem waste, 250 ml of snail digestive juice and 6 g garlic.

Performance parameters	24 h fermentation	48 h fermentation	72 h fermentation	96 h fermentation	120 h fermentation
Vol. of distillate (ml)	182.3 ± 4.9^{a}	137.3 ± 6.7^{b}	104.7 ± 3.1^{c}	98.3 ± 5.7^{c}	82.3 ± 1.2^{d}
Percentage yield (%)	46.6 ± 0.8^{a}	38.5 ± 2.6^{bc}	33.4 ± 0.6^{c}	41.0 ± 3.9^{ab}	35.2 ± 1.9^{bc}
Ethanol (% W/V)	28.0 ± 1.0^{a}	46.3 ± 2.1^{b}	70.3 ± 2.3^{c}	79.3 ± 1.5^{d}	79.7 ± 2.5^{d}
CH_3COOH	–	–	–	–	–

Values are mean and standard deviation of triplicate determination.
Figures across the column bearing similar superscript(s) (a–d) letters mean there is no significant ($P < 0.05$) difference between them. The sign – indicates absence.

obvious steady rise in ethanol yield was recorded with each increase in fermentation period until 96 h. From the results presented in Fig. 2, optimal fermentation period for ethanol yield was recorded at 96 h. Similarly, Ado et al. [3] reported a gradual increase in the yield of ethanol generated from fermentation of cassava starch, but however recorded optimal fermentation at the fifth day. The results of this study were also in agreement with the reports of Osunkoya and Okwudinka, [35] that posited an increase in ethanol yield with an increasing fermentation period. Itelima [27] reported that the ethanol yield gradually increases as the fermentation period increases from the first day to the seventh day which cannot be concluded from this study, however, a slight decline was obvious as the fermentation period approached 120 h as shown in Fig. 3.

Table 6 shows the effects of varying fermentation periods on the volume of distillate, percentage yield and percentage ethanol by volume, using 200 ml of brewer's yeast, 250 g of Plantain pseudo-stem waste, 250 ml of snail digestive juice and 6 g garlic. Continuous increment in fermentation time led to a significant increase in quantity of ethanol produced from 24 to 96 h. No significant change was recorded in the concentration of ethanol on increment of the fermentation period from 96 to 120 h, however there was a significant decrease in volume of distillate obtained, as shown in Table 6. Fermentation time is a very important factor from an economic point of view in ethanol production [24]. Similar to the findings of this study, Hashem et al. [24] observed the effect of varying fermentation periods on the efficiency in ethanol production of very high ethanol producers and thermotolerant strains, and revealed that the ethanol yield gradually increased with time but however recorded optimal results between 60 and 72 h with a dramatically decreased ethanol production with further extension of fermentation periods. Also, optimum fermentation periods for ethanol production by different strains of *K. marxianus* according to Suryawati et al. [48] and Faga et al. [18] was 72 h which was below the optimum fermentation period for *S. cerevisiae* reported in this study. The findings of this study, agree with the fact that yeast consumes all of the dissolved oxygen from the medium usually within the first hours of fermentation, thus recording rapid ethanol production within the first few hours. This initial aerobic condition, known as the glycolytic pathway or Embden-Meyerhof-Parnas route catabolizes the six carbon sugars (glucose) to pyruvic acid. The synthesized pyruvate is thus completely oxidized by the yeast cell to CO_2 and water with the release of energy for other metabolic processes. Only in the absence of oxygen, the pyruvate is converted into ethanol and CO_2 primarily by way of acetaldehyde [34]. Though, according to Van Dijken et al. [52] *S. cerevisiae*, displays the Crabtree effect such that most of the pyruvate generated from glycolysis, even in the presence of oxygen, it is channelled to fermentation. The ethanol content of PPS wastes obtained in this study was higher than that for optimally fermented banana, plantain, and pineapple peels [27]**.** The reported ethanol concentration of sugarcane bagasse by Wanderley et al. [54] was lower than the optimal ethanol concentration obtained after 96 h in this present study, but however comparable to the results of 150 g of plantain fermented for 24 h (Table 2). Also, the ethanol content obtained from the carbohydrate component of newspaper by Kuhad et al., [30] and delignified sugarcane bagasse by Santos et al. [43] was also found lower than the optimal ethanol content of PPS after 96 h in this study but comparable to the ethanol content obtained from 250 g of PPS fermented with 50 ml of yeast for 24 h (Table 4). However, from the findings of Janani et al. [28] the ethanol yield obtained from fruits; grape, papaya, apple, and banana were comparable to the results of this present study obtained for the 150 g PPS at 24 h (Fig. 1).

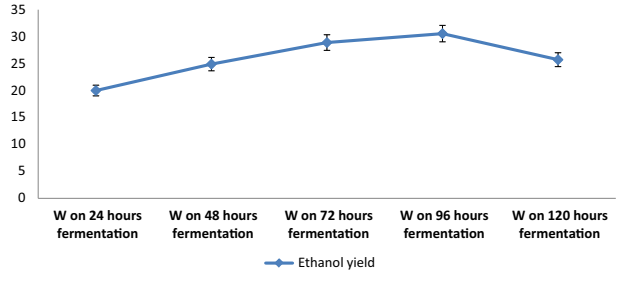

Figure 3 Ethanol yield of fermentation setups with variations in fermentation time * W represents 250 g PPS + SDJ + 200 ml yeast + 6 g garlic.

4. Conclusion

By varying the substrate mass fermented by yeast after addition of digestive juice, the best ethanol content 25% ± 3.6 in 125.6 ml ± 3.5 distillate, 42.0% ± 0.3 residual cellulose and 36.6 mg/ml ± 2.0 was obtained using 250 g of PPS. The optimal ethanol content produced from fermented PPS with varied yeast contents was 28.0% ± 1.0 w/v of 182.3 ml ± 49 at 200 ml with 6 g of garlic, while the most suitable fermentation period produced an ethanol content of 79.3% ± 1.5 of 98.3 ml ± 5.7 distillate after 96 h. This thus indicates that these factors that include variations in mass of substrate, volume of yeast and fermentation time, all contribute significantly

to fermentation performance, and hence should be considered important for the optimization of fermentation products.

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