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Preliminary studies on ethanol production from *Garcinia kola* (bitter kola) pod: Effect of sacharification and different treatments on ethanol yield

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Abstract

A study on yeast fermentation of bitter kola pod(agricultural waste) was carried out using dried active bakers' yeast (*Saccharomyces cerevisiae*)and brewer's yeast (*Saccharomyces carlsbergensis*) Effects of saccharification using *Aspergillus niger* and different treatments on the waste as they relate to the optimization of the ethanol production were investigated. The reducing sugar in the waste was increased after 24-48 hours of sacharification using *Aspergillus niger*, fresh bitter kola waste recorded 4.4 - 8.0g/100g of the waste, while the partially fermented bitter kola waste gave the value in the range of 5.6 - 6.8g/100g. Baker's yeast gave a higher ethanol yield than Brewer's yeast. Different treatments of bitter kola pod revealed that addition of nutrient supplement enhanced the ethanol yield; however, 48 hours of saccharification significantly improved the ethanol yield at $p \leq 0.05$.

Keywords: bitter kola waste, Baker's and Brewer's yeast, saccharification, *Aspergillus niger*, Treatments

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INTRODUCTION

The conversion of wood or agricultural residues to ethanol and industrial chemicals is an attractive option for utilizing all major components of biomass to produce a liquid automotive fuel and for environmental remediation[1,2,3,4]. The increase need for ethanol as a universal energy source has stimulated worldwide investigations, not only with respect to high ethanol yielding strains, but also to cheaper raw materials[5]. Apart from fermentation of carbohydrates, alcohol can also be produced industrially by the hydration of ethylene, which is a product of petroleum industry. Because petroleum is a non-renewable resource, studies have recently been focused on getting alcohol through renewable resources such as agricultural sources[6,7,8].

Garcinia kola (bitter kola) is a tropical plant that grows well in Nigeria, producing fruit that is usually reddish-yellow when ripe. It is however cultivated in the Western and Eastern part of Nigeria. The fruit itself is edible[9]. In Eastern part of Nigeria, children are often seen licking the fruit pulp. Apart from this use, the fruit is allowed to soften for the seeds to be removed and the pod wasted. In this work, Bakers' and Brewers' yeast were used to ferment the sugar in the fruit waste to produce ethanol.

MATERIALS AND METHODS

The ripe fruit of *Garcinia kola* (bitter kola) were obtained from Nnewi, Anambra State of Nigeria. The seeds were removed. One kilogram of the pod was weighed and frozen in a freezer. This was pulverized with 1 litre of distilled water using blender. It was packaged in a plastic bucket and stored in the freezer for subsequent use.

Another one kilogram of the waste sample was weighed into a drying flat tray and kept on the bench in the laboratory for four days to allow primary fermentation to take place. This was then frozen in the freezer, pound and then pulverized with 1 litre distilled water using a blender. It was labeled partially fermented bitter kola and stored in the freezer for subsequent experiment.

All chemicals used were of analytical grade and products of British Drug House (BDH) Poole, England; Aldrich Ltd. and SIGMA Chemicals (U.S.A.). Dried active bakers' yeast is the product of GYMA CARPENTRAS TEWEX 431184 France and was purchased from Central Market, Zaria, while the brewers' yeast was obtained from Nigerian Breweries, Plc. Kaduna.

The total reducing sugar content of this syrup was determined using dinitrosalicylic (DNS) methods as described by Miller[10] with slight modifications. The pH of the syrup was determined using digital pH meter. The effects of saccharification using *Aspergillus niger* on the amount of reducing sugar was also investigated on the waste.

Fermentation

The substrate [100g] was weighed into a 250cm^3 conical flask and diluted in ratio 1:1 with distilled water. The pH of the broth was adjusted to 4.5 with 0.5M NaOH. The syrup was then pasteurized by boiling for 15 minutes on a boiling water bath and allowed to cool before inoculated with 5 and 10g bakers' and 1.09 and 3.56 x 10^6 yeast cell/10ml of brewers' yeast respectively. The syrup was then fermented for 3 days at 30^{o}C. Effect of concentration of the yeast on the percentage ethanol yield was determined and then different treatments were carried out on the fruit waste to determine their effects on the percentage ethanol yield .At the end of fermentation, alcohol was recovered by simple batch distillation using laboratory distillation unit. The alcohol content was determined by the determination of the specific gravity using a standard alcohol density table[11].

RESULTS AND DISCUSSION

The values of the reducing sugar obtained from the bitter kola waste in this study are shown in Table 1. The fresh bitter kola recorded the value of 5.8 g/100g before it was saccharified and the highest value of 8.0g/100g was recorded after 48 hours of saccharification which was later reduced by almost 50% after 72 hours of saccharification.

Table 1: Effects of Saccharification on the Amount (g/100g) of Reducing Sugar in the Bitter Kola Waste. (n = 3, ± SD)

Period of Saccharification	Reducing Sugar (g/100g)	
	Fresh bitter kola	Partially fermented bitter kola
0 hour	5.8 ± 0.28	5.6 ± 0.13
24 hours	6.0 ± 0.19	6.4 ± 0.22
48 hours	8.0 ± 0.21	6.8 ± 0.18
72 hours	4.4 ± 0.17	6.2 ± 0.08

Table 2: Effects of Saccharification and simultaneous fermentation on the percentage ethanol yield ($^{v}/_{v}$) of the bitter kola waste. (n = 3, ± SD)

Substrate	Percentage ethanol yield ($^{v}/_{v}$)			
	Bakers' yeast		Brewers' yeast	
	0 hour	24 hours	0 hour	24 hours
Fresh bitter kola	2.10 ± 0.03	2.16 ± 0.01	1.89 ± 0.02	2.05 ± 0.04
Partially fermented bitter kola	1.63 ± 0.01	1.78 ± 0.06	1.52 ± 0.02	1.70 ± 0.01

Nutrient Supplements include 0.1g KH_2PO_4, 0.5g $CaCl_2$, 0.05g $MgSO_4$, 0.1g $NaSO_4$, 0.1g $(NH_4)_2SO_4$, 5g/100g (5%) bakers' yeast, 1.09 x 10^6 yeast cell/10ml brewers' yeast, 1.95 x 10^6 yeast cell/5ml *Aspergillus niger*; 0 hour means *A. niger* and the inoculum were added simultaneously; 24 hours means the inoculum was added after 24 hours after the addition of *A. niger*.

The partially fermented bitter kola recorded no much difference among the values recorded for the unsaccharified and the saccharified sample. The value recorded before saccharification was 5.6g/100g and 6.8g/100g after 48 hours of saccharification which was the highest. It would appear that the nature of carbohydrate in the pod was not amenable to digestion by the enzyme produced by the A. niger, perhabs the pod needed some chemical treatments prior to fungal treatment.

From the work carried out on these two substrates it was observed that when the bakers' yeast was used as inoculum the yield recorded (2.08, and 2.18% ($^{v}/_{v}$) for fresh and partially fermented bitter kola respectively and was significantly ($P< 0.05$) higher than the one obtained (0.90 and 0.92% ($^{v}/_{v}$) when Brewers' yeast was used on fresh and partially fermented bitter kola respectively. It was also noted that the higher the concentrations of these yeasts used, the higher the percentage ethanol obtained. This is in agreement with the trend reported by Adams and Flynn[12].

Table 2 shows the percentage ethanol yield from the fermentation of the bitter kola waste using simultaneous saccharification and fermentation for 24 hours. It was observed that, the percentage ethanol yield is significantly higher than the value obtained for the unsaccharified sample. The yield recorded from 24 hours saccharification before the inoculation was also higher than the yield obtained from simultaneous saccharification and fermentation (0 hour).

However, when different treatments were carried out on the bitter kola wastes before fermentation, it was observed (Table 3) that, the percentage ethanol yield was enhanced by the addition of nutrient supplements (salts, footnote Table 2). It was also noted that, the yield was significantly ($P< 0.05$) improved when the wastes were saccharified. The yield obtained after 48 hours of saccharification is higher than the one recorded after 24 hours (Table 2). This may be due to more fermentable sugar available in the waste after a longer time of saccharification.

Table 3: Effect of Different Treatments of the Substrates on the Ethanol Yield % ($^v/_v$). (n=3, ± SD)

Treatment	% Ethanol Yield ($^v/_v$)	
	Fresh Bitter Kola	Partially Fermented Bitter Kola
A	1.09 ± 0.03	1.12 ± 0.04
B	1.28 ± 0.03	1.25 ± 0.01
C	2.14 ± 0.02	2.20 ± 0.01
D	2.21 ± 0.03	2.28 ± 0.02
E	1.49 ± 0.01	1.18 ± 0.03
F	2.22 ± 0.04	1.97 ± 0.03
G	1.80 ± 0.03	1.50 ± 0.06
H	2.33 ± 0.01	2.31 ± 0.02

A = Substrate + Brewer's yeast (3.56 x 10^6 yeast cell/10ml) added without nutrient supplement.

B = Substrate + Brewers' yeast (3.56 x 106 yeast cell/10ml) added with nutrient supplement listed in Table 2.

C = Substrate + 10g Bakers' yeast without nutrient supplements.

D = Substrate + 10g Bakers' yeast + nutrient supplement.

E = Substrate + *Aspergillus niger* (mixed culture) at 0 hour + Brewers' yeast + nutrient supplement.

F = Substrate + 10g Bakers' yeast + *Aspergillus* (1.09 x 10^6 yeast cell/5ml) at 0 hour.

G = Substrate + *Aspergillus niger* (1.09 x 10^6 yeast cell/5ml) + Brewers' yeast (3.56 x 10^6 yeast cell/10ml) after 48 hours of saccharification.

H = Substrate + *Aspergillus niger* (1.09 x 10^6 yeast cell/10ml) + 10g Bakers' yeast after 48 hours of saccahrification.

CONCLUSION

The results of this work show that, alcohol can be produced from these agricultural wastes. The alcohol yield was significantly ($p< 0.05$) increased by the addition of nutrient supplements to the wastes and saccharification with *Aspergillus niger*. It is also noted that for optimum yield of ethanol from these wastes, bakers' yeast is most preferred than brewers' yeast. Presently research is directed towards the use of other saccharification methods and genetically engineered microrganisms to optimize the ethanol yield.

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