

Effect of Maize/Millet Malt Hydrolysis and Baker's Yeast/Burukutu Starter Fermentation on the Yield of Ethanol from *Dioscorea Spp* (Yam)

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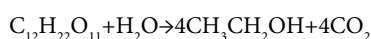
Abstract

The ethanol production ability of baker's yeast (*Saccharomyces cerevisiae*) and burukutu starter culture were examined on yam starch hydrolyzed with malts of maize and millet. Four substrate treatments which include yam starch (as control), yam starch hydrolyzed with millet malt (B), yam starch hydrolyzed with maize malt (C) and yam starch hydrolyzed with a mixture both millet and maize malt (D) were prepared in duplicate and made up to 600 ml. Baker's yeast (4.40 g) was inoculated on one set of four while burukutu starter was inoculated on the other set of four and fermented for a period of four days at a temperature of 26°C. The pH was observed to drop as fermentation period increased, the highest ethanol yield of up to 74.5 g/Kg was obtained from starch hydrolyzed with maize malt and inoculated with baker's yeast. Yam is a potential source of industrial scale ethanol production, there was a significant difference ($p < 0.05$) between maize and millet malts in ethanol yield but between baker's yeast and burukutu starter culture fermentation there was no significant difference ($p < 0.05$), the interaction between malts and the inoculum was significant ($p < 0.05$). Ethanol yield strongly depends on the hydrolysis of yam starch into fermentable sugar.

Keywords: Ethanol; Baker's yeast; Burukutu starter culture; Yam starch; Maize and millet malts

Introduction

Continuous search for renewable energy sources are ongoing. Key to this search is the discovery of biofuels which include biodiesels and bio-ethanol among others. Existing facts suggests that crude oil; the major source of organic fuel in Nigeria is gradually depleting and time will come when it will not sustain the nation or it will become scarce. Also, the global collaborative fight against greenhouse gas emission necessitates eco-compatible and sustainable alternative fuel sources. Ethanol is a byproduct of the metabolic process of yeast [1]. Biologically, ethanol is produced by microbial or enzymatic fermentation; a process that converts sugars to ethanol and carbon (IV) oxide, shown below:



Yam is a common root crop in Nigeria. The starch is large in particle and easily settles, and fat and protein content is small in quantity, thus good starch can be extracted comparatively easily. The starch then can be hydrolyzed, fermented and then distilled to produce ethanol. Due to lack of proper storage facilities, after harvest yam may get spoilt and end as waste; this spoilt yam can be converted to bioethanol production which can be used domestically in place of kerosine. The aim of this work is to examine the potency of yam in ethanol production, to compare the ethanol producing capacity of baker's yeast and burukutu starter and to establish the preferred source of malt enzyme between maize and millet for yam starch hydrolysis during ethanol production.

Materials and Methods

Sample preparation

The yam was peeled, blanched, dried at room temperature for 48 hours and ground to powder, 95.8 g was weighed and kept for each treatment.

Malting

Maize and millet were soaked in water separately for about two days, water was drained from them and they were covered with leaf for

four days to germinate into Malt, after which it was dried and crushed. 19.2 g was measured into various containers to be used as malting agent. The germination process creates several enzymes, notably alpha-amylase and beta-amylase, which was used to convert the starch in the yam into fermentable sugar.

Mashing

The dry-milled yam was mixed with water to which boiling water was added to gelatinize it. Making a paste which could cool and then dispensed into eight bottles such that each contained yam starch 95.8 g/500 ml; millet malt, maize malt and a mixture of both malts amounting to 19.2 g/100 ml were added to the different bottles respectively in duplicates. The process was allowed for about 2 hours.

Inoculation and incubation of the substrate

Yeast activation: The yeast cells were introduced to warm water containing sugar, it was observed after about 10 minutes to foam. The cooled mash contained in bottles were inoculated each with 4.40 g of both bakers and crude yeast (*Saccharomyces cerevisiae* and starter culture from burukutu producers), the control (unmalted yam starch) was also inoculated with yeast.

Fermentation

After inoculation, the samples were incubated for each inoculum to colonize the substrate at temperature of 26°C for 4 days.

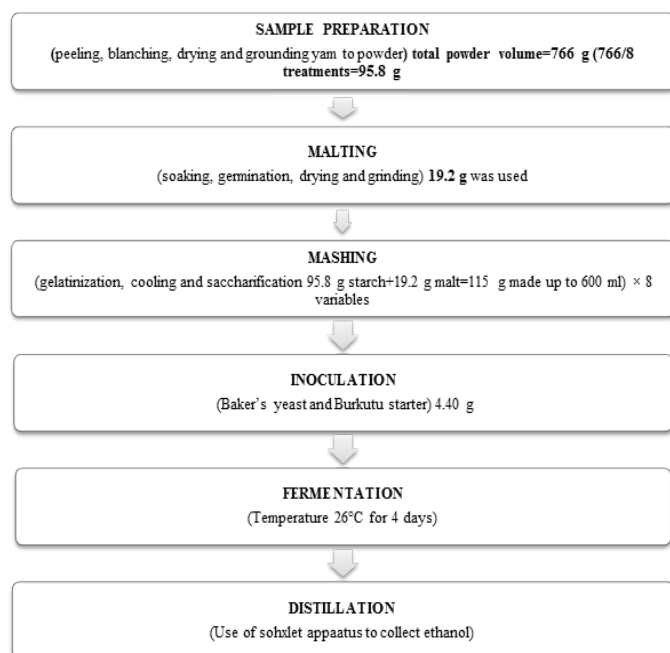
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Distillation



A sohxlet apparatus was used to collect the ethanol obtained by the processes above.

Treatments

1. Yam (95.8 g) with *Saccharomyces cerevisiae*,
2. Yam (95.8 g) with starter culture from burkutu producers,
3. Yam (95.8 g) saccharified with millet (19.2 g)+*Saccharomyces cerevisiae*,
4. Yam (95.8 g) saccharified with millet (19.2 g)+starter culture from burkutu producers,
5. Yam (95.8 g) saccharified with maize (19.2 g)+*Saccharomyces cerevisiae*,
6. Yam (95.8 g) saccharified with maize (19.2 g)+starter culture from burkutu producers,
7. Yam (95.8 g) saccharified with both millet (9.6 g) and maize (9.6 g)+*Saccharomyces cerevisiae*
8. Yam (95.8 g) saccharified with both millet (9.6 g) and maize (9.6 g)+starter culture from burkutu producers

Analysis

Sugar utilization rate: sugar disappearance was measured by the method below:

1. Density method after every 24 hours, CO₂ is released and then the mixture is mixed properly and the mass is taken in a constant volume of container 68 ml. the density of the liquid is obtained from Mass/constant Volume.

2. This value was converted to specific gravity by dividing the respective mixture densities by the density of equal volume of water (at temperature of 26°C=0.97 g/ml).

Alcohol content calculation (<http://www.beverageanswers.com>)

Original gravity-Terminal gravity=Density of CO₂ evolved (Kg/L)

The amount of CO₂ produced for every gram of ethanol produced during fermentation is 1.05

Amount of ethanol left (Kg/L)=1.05 × Density of CO₂ evolved

Amount of alcohol by mass (%)=Amount of ethanol left/ terminal density

Amount of alcohol by volume (%)=Amount of alcohol by mass/ density of alcohol (0.79)

1. (Ethanol percentage by mass/100) × Mass of substrate=Mass of substrate converted to ethanol.

2. pH of the mixture was taken during the 4 days' period.

3. The prevailing laboratory temperature during the study period was 26°C.

Results and Discussion

The specific gravity (SG) of the substrates (wort+inoculum) decreased as fermentation began until it didn't change any further after some days (Table 1) the greatest decrease in density was observe in wort treated with maize malt and bakers yeast with about 0.07 Kg/L deduction while the least was seen in the controls X1, X2 and B2 (0.02 Kg/L) this can be accounted for by higher starch content as compared to those treated with other malts. Decreased SG correlated with increased ethanol yield, it was observed to be highest after 24 hours and 48 hours, this agrees with Ref. [2] who observed steepest increase in ethanol production within the 24 hours, the authors also attributed decreased ethanol production to inhibitory activities of both ethanol and by-products in the fermentation medium.

The yield of ethanol was calculated in equivalent of one 1 Kg starting material, and it was seen that yam starch hydrolyzed with maize malt gave the highest yield (74.5 g/Kg wort) (Table 2). The control (only starch) showed ethanol yield 3.06% and 3.02% respectively which could be attributed to glucoamylase and α-amylase activities of yeast strains that hydrolyzed the starch to fermentable sugars, this agrees with Ref. [3].

The pH was generally observed to decrease as fermentation continued (Figure 1) this can be attributed to the presence insoluble calcium phosphate present in the water which complexes on heating resulting in decrease in pH making enzymes like beta-amylase activity higher, this enables organisms that function maximally at low pH to metabolize their substrate. Decrease in pH with ethanol fermentation has been reported [2,4].

This experiment shows that maize and millet malts can be used as alternative sources of starch hydrolysing enzyme. Maize malt was seen to have hydrolysed more starch to fermentable sugar as its total dissolved solid reading was higher this could be attributed to the presence or levels of any of the following enzyme amyloglycosidase, glucanases, debranching enzymes, pentosanase and xylanase which are all specific and add to the release of fermentable sugars. During the study period, alcohol yield with malt was observed to reduce in the order Maize>Maize+Millet>Millet (Figures 2-10).

The peak production period for *Sacharomycis cerevisiae* was observed to be after 24 hours while that for burukutu starter culture was spread up to 72 hours. *Saccharomycis cereviciae* gave an overall higher yield of ethanol (74.5 g/kg) than the burukutu starter culture (52.1 g/kg). This supports the speculation that burukutu is a low alcohol drink. This observation may be due to interference and competition among

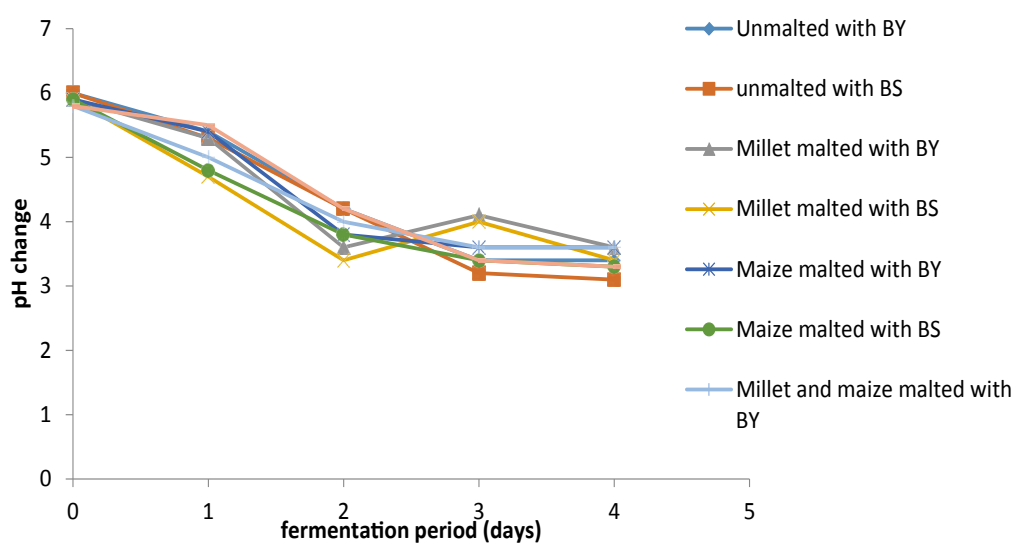


Figure 1: Change in pH during fermentation period.

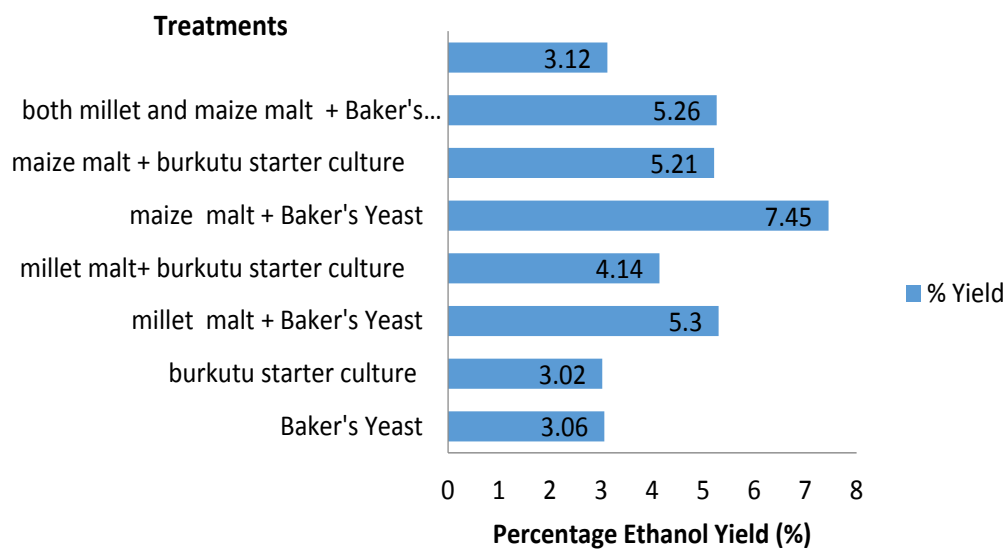


Figure 2: Percentage Ethanol Yield (%) for different treatments.



Figure 3: Yam flour.



Figure 4: Malted maize.



Figure 5: Malted millet.



Figure 6: Prepared malts.



Figure 7: Gelatinized yam starch.



Figure 8: pH meter.



Figure 9: Bioreactor, substrate and fermenters.



Figure 10: Fermentation started.

Treatments Time	Unmalted		Millet malt		Maize malt		Millet and Maize malt	
	BY	BS	BY	BS	BY	BS	BY	BS
Before incubation	1.06	1.06	1.04	1.04	1.04	1.04	1.03	1.03
24 hr	1.05	1.05	1.01	1.03	0.99	1.03	1.01	1.03
48 hr	1.04	1.04	0.99	1.02	0.99	1.02	1.00	1.02
72 hr	1.03	1.04	0.99	1.01	0.98	1.00	0.99	1.01
96 hr	1.03	1.03	0.99	1.00	0.97	0.99	0.98	1.00

Key: BY: Inoculated with Baker's Yeast; BS: Inoculated with Burukutu starter

Table 1: Specific gravity of wort during the fermentation period.

Treatments Time	Unmalted		Millet malt		Maize malt		Millet and Maize malt	
	BY	BS	BY	BS	BY	BS	BY	BS
Before incubation	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24 hr	10.0	10.0	31.2	10.2	53.0	10.2	20.8	0.00
48 hr	20.1	20.1	52.4	20.5	53.0	20.5	31.3	10.3
72 hr	30.3	20.1	52.4	30.9	63.7	41.5	41.9	20.7
96 hr	30.6 ^a	30.2 ^a	53.0 ^b	41.4 ^c	74.5 ^b	52.1 ^c	52.6 ^b	31.2 ^c

Key: BY: Inoculated with Baker's Yeast; BS: Inoculated with Burukutu starter; Values followed by the same letter on a row based under same malt treatment indicate no significant ($p < 0.05$) difference in ethanol yield between inoculums after day four of fermentation.

Table 2: Ethanol yield (g/Kg yam).

the microorganisms present in the burukutu starters, the scope of this research did not include isolation and characterisation of microbes of burukutu starter. In a similar research, the yield of ethanol from the 500 g of flour was 130 g for yam and 117 g for cocoyam respectively, hence our experiment produced higher yield in comparison too [4].

Going by the volume recovered we get % ethanol ranging from 13.89% for X2 to 40% for C2 respectively, this figure is not possible as stated by Ref. [5] that most ethanol-tolerant strains of yeast can survive up to approximately 15% ethanol by volume. This is not pure ethanol, it contains other solvents that can vapourise at any temperature before 78°C.

Conclusion

Yam is a viable source of ethanol, there was a significant difference ($p < 0.05$) between maize and millet malts in ethanol yield but between baker's yeast and burukutu starter cultures fermentation there was no significant difference ($p < 0.05$), the interaction between malts and the inoculums showed a significant difference ($p < 0.05$). Maize malt had

the best hydrolyzing ability, and gave highest yield when fermented with baker's yeast.

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