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Ethanol Yield of Three Varieties of Cassava (Odongbo, Ofege, and TMS 30572) Using α-Amylase from Germinated Paddy Rice and Yeast from Palm Wine

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***Abstract*—**A process of conversion of flour from three varieties of cassava, namely Odongbo, ofege and TMS30752 to ethanol using α-amylase locally sourced from germinated unhusked paddy rice and yeast isolated from palm wine was developed. It involves the germination of paddy rice for a period of 15days to produce α-amylase for starch hydrolysis and isolation of yeast from palm wine for fermentation. The results showed that optimum amylase yield of "ofada" rice paddy was at 6th day germination which was 576.9ml/g. Ethanol yield for TMS30572 (440.3%) was significantly higher than "Odongbo" (160.2%) and "Ofege'' (115.1%), Sugar conversion efficiency were 311.0%v/v, 268.2%v/v and 186.84%v/v for TMS30572, "Odongbo" and "Ofege" respectively. The ethanol boiling points were 78°C, 76°C and 80°C for TMS30572, "Odongbo" and "Ofege" respectively. This study showed that cassava varieties affects quality of ethanol produced and germination of "ofada" rice for 6 days ensures optimum production of crude amylase enzyme.

***Keywords*—**Cassava, ethanol, fermentation, hydrolysis, α-amylase.

I. Introduction

THE energy security, declining oil reserves and climate change has in recent time led to seeking alternative fuel sources and principally from ethanol [1]. The raw materials used depend on the country and include cereals, tubers and sugar crops. Although the potential effects of producing fuel ethanol from food crops on food security have been highlighted and emphasis is now on the use of non-food crops, especially cellulosic materials [2]-[4]. Efficient production of biofuel from cellulotic materials requires complex methods and expensive enzymatic hydrolysis [5]. Reference [6] has opined that nonfood crop production may also compete with food crop for the available farm land thereby affecting food security. Many Nations of the world are considering the cassava asa cheap source of feedstock for biofuel production [7],[8].

Reference [9] reported on the production of ethanol from cassava whey while [10] and [11] noted that cassava peel and cassava waste residues can be used for the production of ethanol. Several factors can help improve the profitability of ethanol process. These include development of high ethanol yielding cassava hybrid, better and lower cost enzymes etc. It is speculated that there is considerable variability within varieties for ethanol yield [5]. The current production technology involves the enzymatic hydrolysis of cassava flour to ethanol. In the processing, cassava flour was first cooked to gelatinization at a temperature of 80^0C for 15 minutes in a water bath. The liquefied cassava flour was hydrolysed by α – amylase enzyme at 80°C and pH 6.2. The hydrolysed cassava flour was saccharified by amyloglucosidase enzyme at pH 4.2. The saccharified product was then fermented using a strain of *Saccharomyces cerevisiae* (yeast) at 30°C for 72 hours incubation. The fermented mash was distilled at 78°C to recover the ethanol produced [13]. Many of these technologies are not suitable in the rural areas of developing countries. Amylase can be produced by a host organism such as yeast, fungi and bacteria [14], [15]. However, purified enzymes are very expensive and not easily assessable in the developing countries. And this has continually kept the price of bio-fuel higher than fossil fuel. The cost of cassava is very cheap in the rural areas and if the cost of substrate can be brought to affordable level, ethanol production can be done in the rural areas and this can be used for powering bio-fuel electricity generator and excess sold off to generate additional income for the farmers. Reference [6] has exploited the use of rice grain as koji for the extraction of amylase enzyme from *Aspergillus amadori*. Germinated rice paddy is known to contain some endogeneous enzymes used for the production of Japanese rice wine [16]. The possible use of some local enzyme substrates, such as rice grains for the production of ethanol from cassava still need to be considered. This work was designed to produce ethanol from cassava flour from three different varieties using crude enzyme from germinated rice paddy liquefaction and saccharification, and yeast from palm wine for fermentation.

II. Materials and Methods

A. Raw Materials

Cassava roots of three varieties ("Odongbo", ''Ofege" and TMS 30572) were obtained from the farm of the Univeristy of Agriculture, Abeokuta were used in this project. Rice paddy of the local "Ofada" rice in Abeokuta, Ogun State, Nigeria was used.

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B. Methods

1. Alpha Amylase Production

The germination process followed the method of [22]; it involves the soaking of unparboiled paddy rice in clean water (600ml of water to 300g of paddy rice) for 24hours. The soaking water was drained off, the feeds were heaped to cone shape to generate heat (27^{o}C) before been spread thinly on a shallow tray for different period (0, 3, 6, 9, 12 and 15 days). The germinated paddy rice was dried in the oven at 50^{o}C for 10 to 14hours and then milled in mortar, to rice malt flour, which served as the crude enzyme source.

2. Determination of Amylase Yield in Rice Paddy (Ofada).

Rice malt 1g was dissolved in 10ml of extraction solution (1% Sodium Chloride, 0.02% Calcium Chloride and 0.02 % Sodium which was then made up to 100ml with distilled water) and left for 1h, the mixture was centrifuge at 1000G and enzyme supernatant was decanted. Absorbance of the resulting color solution was taken at 540nm wavelength against the blanks. The amount of Amylase yield was calculated using DNSA Reagent [16] from the formula below:

$$\text{Amylase yield (ml/g)} = \frac{\Delta A540}{\text{Incubation time}} x \frac{\text{Total Vol.in cell}}{\text{Aliquot assayed}} x \frac{\text{Extraction}}{\text{sample wt}} x \text{ dilution factor}$$

Rice malt = 1g in 10ml extraction solution
Aliquot assay = 0.1ml
Incubation time = 10min
Extract Vol. = 10ml
Sample wt = 1gm
Total vol. in cell = 23ml.

3. Hydrolysis of Cassava Flours and Ethanol Production

The sixth day germinated rice malt was used in this work as crude α-amylase, as it was found to have the highest α-amylase yield see Fig 1. Crude α-amylase (16gram) and 100gram cassava flour was processed to ethanol in 400ml of distilled water. The cassava flour was mixed with hot water at temperature 73^{o}C to give a final mash temperature of about 68^{o}C during which the viscosity of the cassava flour increases [15], cassava gel was finally formed at 65^{o}C, the starch level, sugar Brix and the sugar level was measured, during the hydrolysis. 20ml of yeast inoculate were inoculated into 400ml (pH 5.0) sterile glucose syrups ("Odongbo", "Ofege" and TMS30572) in 500ml conical flasks and were incubated on a Gyratory Shaker (100pm) for one day, followed by 3 days on stationary incubation at 30^{o}C. The pH, specific gravity, sugar content, ethanol content and TTA were monitored daily.

C. Analytical Methods

Starch concentrations were determined by iodometric method of AOAC 1990. Glucose assay was done using the DNSA method of [4]. The pH was measured using Jenway pH meter (Model 3015, serial No. 1647, UK).

Refratometer was used to measure the Brix, while Total acidity was determined by titration method. Total reducing sugar was measure by the method of [17]. Ethanol Yield (% w/v) was estimated according to [11]

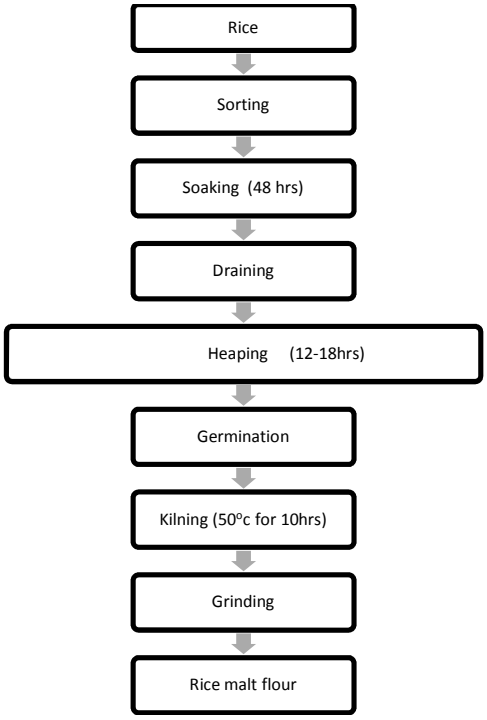

Fig. 1 Production of Rice Malt Flour

Thus:

$$\text{Ethanol yield} = \frac{\text{Ethanol produced}}{\text{Sugar consumed}} \times 100$$

Sugar conversion efficiency was calculated by % media sugar content at the start and the total amount of alcohol produced. [18].

$$\text{SCE} = \frac{\text{Percentage alcohol (w/v)}}{\text{Sugar content at the start}} \times 100$$

$$\text{SCE} = \frac{\text{w}}{\text{v}} x \frac{100}{0.504}$$

where:
0.504= Conversion efficiency factor.

1. Sugar Consumed

Is calculated by subtracting the initial sugar during hydrolyzes from sugar after fermentation.

Sugar consumed = Initial sugar – sugar after fermentation.

2. Fermentation efficiency:

This is calculated by % media sugar content at the start and the sugar consumed.

III. Result and Discussion

A. Paddy Rice Germination (Enzymes Source)

The rice grain contains many endogenous enzymes, and it is

smaller to other cereal grains, relatives to the biosynthesis of the major seed components (Starch, protein and liquid). The germination process is as illustrated in Fig. 1. The paddy rice used in this work was yet to be subjected to parboiling or other processing. The paddy rice was cleaned and soaked in clean water (600ml of water to 300g of paddy rice) for 24hours. The soaking water was drained off, the feeds are first heaped to cone shape to generate heat (27^{o}C) before been spread thinly on a shallow tray for different period (0, 3, 6, 9, 12 and 15 days). Thus some enzymatic changes occurred that affect the quality [18]. After the germination, the germinated paddy rice was dried in the oven at 50^{o}C for 10 to 14hours and then milled in mortar, to rice malt flour, which served as the crude enzyme source.

B. Yield of α-Amylase from Ofada Rice Paddy

The amylase yield of the ofada rice paddy germination is presented in Fig. 2. The enzyme yield gave varying amylase (ml/g) at intervals of 3 days to 15 days. The results showed that the α-amylase yield increased from day 1 (3.12ml/g) and got to the peak (the optimum yield) at day 6 (576.9ml/g), which indicated that the germination process is very necessary to yield amylase. Amylase is without doubt the most important enzyme in malt. They are responsible for degradation of starch during mashing. The trend in the increase of α-amylase in this study agrees with the report of [19] who suggested that 7 days germination would be the optimum for α-amylase yield in plant product.

C. Changes in Slurry Parameters during Hydrolysis

The changes occurring during hydrolysis of the three varieties of cassava flour are presented in Table I. The pH and starch decreased with time while Brix and sugar level increased at an hour interval for seven hours. At the end of 7 hours of the starch hydrolysis, the pH of TMS30572 stood at 5.00±0.12, significantly higher than that of Ofege and Odongbo, indicating that TMS30572 is more resistant to pH change than the other varieties.

The level of starch content in the slurry also reduced significantly during the seven hours of hydrolysis, indicative of effective convention of starch to glucose syrup. Ofege variety had the lowest level of starch at the end hydrolysis period, suggesting that the starch granules of the variety are more susceptible to α-amylase degradation than the two other varieties. As it may be expected the sugar content and the Brix increased as the period of hydrolysis increased. The starches in the flours were successfully hydrolysed as revealed by the level of starch content that remained after the period of hydrolysis. Meanwhile, [8] reported that no starch remained after the hydrolysis of yam and cocoyam flours with a combine effect of α-amylase and glucoamylase enzymes. This observed deference might be because; only amylase from rice paddy was used for both hydrolysis and saccharification. And the result nonetheless showed that the flours were not resistant to the α-amylase derivable from locally available Ofada rice paddy.

The high convertible starch content of this cassava flour will produce more fermentable sugar and therefore more alcohol.

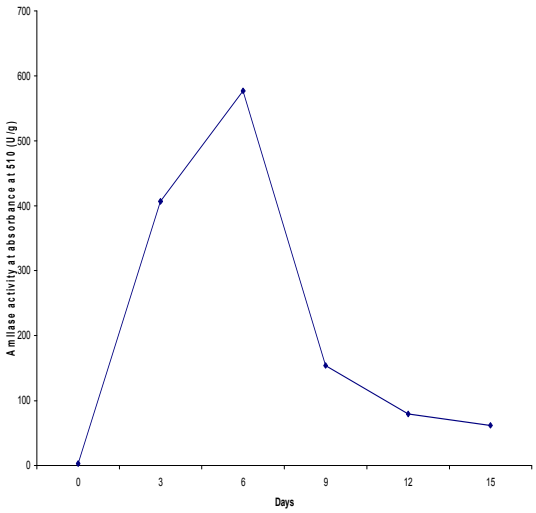

Fig. 2 Amylase yield of Rice

D. Changes in Hydrolysate Parameter duringFermentation

The changes in hydrolysed cassava in flour fermentation at 30^{o}C for 4 days shows that; there was consistent decrease in the pH level of all samples from day 1 to day 4 (Table II). The reduction of pH indicates an increase in acidity of the samples fermented (4.40 to 3.80 for odongbo; 4.60 to 3.85 for Ofege; and 5.00 to 3.99 for TMS30572).

TABLE I
CHANGES IN SLURRY PARAMETERS DURING HYDROLYSIS

Parameter	Time(hrs)	Odongbo	Ofege	TMS 30752
pH	1	5.63 ± 0.02^{ay}	6.38 ± 0.02^{ax}	5.52 ± 0.02^{az}
	2	5.46 ± 0.03^{bz}	6.41 ± 0.02^{ax}	5.51 ± 0.02^{ay}
	3	5.26 ± 0.01^{cz}	6.01 ± 0.02^{bx}	5.41 ± 0.01^{by}
	4	5.10 ± 0.02^{dz}	5.75 ± 0.03^{cx}	5.32 ± 0.02^{cy}
	5	5.01 ± 0.01^{ez}	5.42 ± 0.03^{dx}	5.25 ± 0.03^{dy}
	6	4.85 ± 0.03^{fz}	5.02 ± 0.02^{ex}	5.08 ± 0.02^{ey}
	7	4.55 ± 0.05^{gz}	4.83 ± 0.02^{fy}	5.21 ± 0.02^{dx}
		4.42 ± 0.03^{hz}	4.61 ± 0.02^{gy}	5.00 ± 0.03^{fx}
Starch	1	0.74 ± 0.03^{ax}	0.76 ± 0.01^{ax}	0.73 ± 0.01^{ax}
	2	0.57 ± 0.02^{by}	0.63 ± 0.02^{bx}	0.55 ± 0.02^{by}
	3	0.38 ± 0.03^{cy}	0.34 ± 0.02^{cz}	0.46 ± 0.02^{ex}
	4	0.28 ± 0.04^{dy}	0.22 ± 0.03^{dz}	0.39 ± 0.02^{dx}
	5	0.12 ± 0.03^{ey}	0.10 ± 0.02^{ey}	0.26 ± 0.02^{ex}
	6	0.08 ± 0.01^{fy}	0.07 ± 0.02^{fy}	0.12 ± 0.02^{fx}
	7	0.07 ± 0.02^{fy}	0.05 ± 0.01^{fy}	0.10 ± 0.01^{fx}
Sugar	1	0.16 ± 0.02^{fy}	0.18 ± 0.01^{gx}	0.09 ± 0.02^{fz}
	2	0.03 ± 0.02^{gy}	0.26 ± 0.03^{fx}	0.26 ± 0.02^{ex}
	3	0.45 ± 0.01^{ez}	0.39 ± 0.04^{ey}	0.42 ± 0.02^{dx}
	4	0.51 ± 0.01^{dx}	0.48 ± 0.02^{dy}	0.44 ± 0.01^{dz}
	5	0.67 ± 0.02^{cx}	0.63 ± 0.02^{cy}	0.57 ± 0.01^{cz}
	6	0.74 ± 0.02^{bx}	0.71 ± 0.02^{by}	0.71 ± 0.02^{by}
	7	0.79 ± 0.03^{ax}	0.76 ± 0.01^{ay}	0.78 ± 0.02^{ax}
Sugar brix	1	2.51 ± 0.06^{gx}	2.02 ± 0.03^{gy}	2.05 ± 0.09^{gy}
	2	8.05 ± 0.05^{fx}	4.05 ± 0.05^{fz}	5.05 ± 0.05^{fy}
	3	12.05 ± 0.05^{ex}	8.05 ± 0.05^{ez}	9.03 ± 0.03^{ey}
	4	17.03 ± 0.10^{dx}	13.05 ± 0.10^{dy}	12.05 ± 0.05^{dz}
	5	20.05 ± 0.05^{cx}	17.13 ± 0.13^{cy}	17.05 ± 0.05^{cy}
	6	22.03 ± 0.06^{bx}	20.05 ± 0.05^{by}	23.05 ± 0.18^{bx}
	7	24.07 ± 0.08^{ax}	22.10 ± 0.13^{ay}	25.02 ± 0.03^{ax}

a,b,c,d,e: Means down the same column with same superscripts are not significant (P>0.05)

x,y,z: Means along the same row with same superscripts are not significant (P>0.05)

Yeasts are also found in some fermented foods. Yeast are known to produce acids such as acetic, pyruvic, propionic and butyric during fermentation [22]. Hence, the reduction in the pH of the fermenting medium, the total sugar increased in all varieties for the first 36 hours from 0.48 to 2.18 in Odongbo; 0.49 to 2.29 in Ofege and 0.73 to 3.13 in TMS30752, and a general decline to 0.91, 1.04 and 0.89 respectively for Odongbo, Ofege and TMS30752 at the 96th hour. This trend seems to balance between the two contradictory views of researchers on the performance of sugar during fermentation of hydrolysed starch.

TABLE II
CHANGES IN SLURRY PARAMETERS DURING FERMENTATION

Parameter	Time (hrs)	Odongbo	Ofege	TMS 30752
pH	0	4.44 ± 0.04^{az}	4.62 ± 0.23^{ay}	5.02 ± 0.02^{bx}
	24	4.24 ± 0.04^{by}	4.23 ± 0.03^{by}	5.52 ± 0.03^{ax}
	48	4.12 ± 0.03^{cx}	4.03 ± 0.04^{cy}	4.13 ± 0.03^{cx}
	72	3.93 ± 0.02^{dy}	3.91 ± 0.02^{dy}	4.15 ± 0.04^{cx}
	96	3.83 ± 0.03^{ey}	3.85 ± 0.03^{ey}	3.96 ± 0.03^{dx}
Sugar	0	0.48 ± 0.03^{dy}	0.49±0.02dy	0.73 ± 0.03^{dx}
	24	0.34 ± 0.02^{ey}	0.32±0.03ey	0.45 ± 0.03^{ex}
	48	2.18 ± 0.04^{az}	2.27±0.02ay	3.14 ± 0.04^{ax}
	72	1.25 ± 0.03^{bz}	1.46±0.03by	1.73 ± 0.03^{bx}
	96	0.91 ± 0.02^{cz}	1.04±0.02cy	1.39 ± 0.04^{cx}
Sugar brix	0	24.03 ± 0.50^{ax}	22.00 ± 0.25^{ay}	25.13 ± 0.12^{ax}
	24	17.08 ± 0.53^{bxy}	16.07 ± 0.08^{by}	18.00 ± 0.10^{bx}
	48	12.32 ± 0.38^{cx}	9.10 ± 0.13^{cz}	11.06 ± 0.08^{cy}
	72	6.03 ± 0.45^{dx}	3.13 ± 0.12^{dy}	6.03 ± 0.50^{dx}
	96	3.00 ± 0.35^{ey}	1.08 ± 0.78^{ez}	4.05±0.05ex
Total acidity	0	0.09 ± 0.01^{by}	0.07±0.01exy	0.05 ± 0.02^{dx}
	24	0.21 ± 0.08^{ax}	0.12±0.01dy	0.12 ± 0.02^{cy}
	48	0.22±0.01ax	0.19±0.02cy	0.18 ± 0.01^{by}
	72	0.22±0.01ax	0.22±0.01bx	0.22 ± 0.01^{ax}
	96	0.24±0.22ax	0.25±0.02ax	0.22 ± 0.01^{ay}

Reference [23] postulated that there would be increase in the level of reducing sugars and total sugars, however, some studies showed the opposite, that is a decrease in reducing sugar [24], and total sugars [25]. The cell growth of the slurry as fermentation progresses is presented in Fig.2. Cell growth of the slurry as fermentation progresses is presented in Fig .2. At the end of the 4th day the cell growth of TMS30752 was significantly higher than that of Odongbo and Ofege. The reason for this is not presently clear, as there was nothing that warranted thisphenomenon in constituent of the said variety. The increase in cell growth confirms high activity of yeast and the suitability of the medium to support yeast activity. Reference [21] opinioned that sluggish yeast growth will cause delay in fermentation and give room for infection which may reduce alcohol yield.

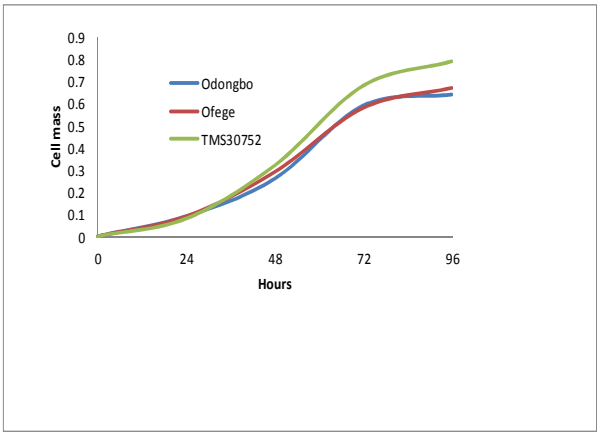

Fig. 3 Changes in cell growth of *Saccharomyces cerevisiae* from palmwine using various hydrolysates during fermentation

The changes in ethanol content and ethanol yield as against the quantity of sugar consumed during fermentation are presented in Table III. The quantity of ethanol produced by TMS30572 (39.19w/v) was significantly ($p<0.05$) higher than both Ofege and Odongbo. The quantity of sugar consumed in the course of fermentation also shows that TMS30572 has the highest yield of ethanol 440% as against 122.6%. The level of ethanol yield in all of the three varieties notwithstanding was higher than the yield from cocoyam and yam flour used for ethanol production [2]. The specific gravity of the ethanol was in the range of acceptable level of pure ethanol, while the color of the ethanol colorless and devoid of residue. Ethanol production from cassava is an old technology that has been effectively researched and has been already implemented for production of ethanol at a commercial level in many industrialized nations. Meanwhile, in many emerging economies, commercial production of ethanol as bio-fuel is not economical and competitive, due to the technologies and ingredients involved. The use of locally sourced enzymes and improved cassava hybrid like TMS30572 that are readily available to rural farmers as is being revealed by this study, will not only make the production of ethanol a practicable ventures by rural farmers, but will also make ethanol abundance through the pull from many small scale farmers to a central rectification facility.

TABLE III
PARAMETERS ON ETHANOL PRODUCED AT DAY 6 OF GERMINATION

Characteristics	"Odongbo"	"Ofege"	TMS30572
Sugar consumed (mg/ml)	20.26	18.00	8.90
Sugar conversion efficiency %	268.2	186.84	311.0
Fermentation efficiency at 4 day (%)	95.70	94.54	90.91
Ethanol produced	32.45wlv	23.30wlv	39.19w/v
Ethanol yield (%)	160.2	115.1	440.3
Specific Gravity (kg/l)	0.9572	0.9696	0.9465
% Alcohol (w/v)	32.45	23.30	39.19
Boiling pt (^{0}C)	76	80	78
Sugar at 20^{0}C % Sucrose	10.8	6	7.9
Refractive Index at 20^o	1.3495	1.3420	1.3445
Color	colorless	colorless	colorless
Residue	NiL	NiL	NiL

IV. Conclusion

In this work, we have been able to describe a system where locally sourced enzymes from germinated paddy rice and yeast isolated from palm wine could give a high yield of ethanol from three varieties of cassava. The study also shows that, apart from the enzyme from the germinated rice, no additional enzyme is required before the hydrolyzed starches are fermented to ethanol, hence reducing the overall cost of production.

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Statement of Competing Interest

The authors have no competing interest

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