UTILIZATION OF CASSAVA PEELS AS AN ALCOHOLIC FERMENTATION SUBSTRATE

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Abstract

Studies on the utilization of cassava peels (food waste) ns the substrate for fermentation using industrial yeast were carried out. The studies involved a submerged culture of *Saccharomyces uvarum* on pre-treated substrate at 32°C for 72 hours. The treated substrate at Oh contained maltose, raffinose, fructose and glucose, but contained no glucose by the end of the fermentation (72h). The highest yeast growth occurred in the first 48h with a value of 5 x 10 cfu/ml and a peak at 48h. Investigation of some growth parameters showed that increased substrate concentration from 6.7% to 13,3% and 20% (w/v) increased the yeast growth to 2.6 x iO^{10} cfu/ml and 5.0x10" cfu/ml, with 42.9% and 25.4% efficiency of substrate utilization respectively. In the absence of the nutrient supplement, yeast growth was 1.3 x IO^{7} cfu/ml with 52.3% efficiency of substrate utilization, while the efficiency ranged from 64.7% to 68.7% with increased inoculum size. The changes in substrate concentration, nutrient supplementation and inoculum size also affected ethanol yield and productivity.

Introduction

Biomass such as forest, cultivated energy crops, agricultural/animal residues, and industrial and domestic organic wastes can now be converted by physio-chemical and/or fermentation processes to clean fuels and petrochemical substitutes (Smith, 1981; Kaur, 1989). The three main renewable resources or biomass which form substrate for ethanol productions include:

(i) Cultivated energy crops;
(ii) Harvested natural vegetation, and
(iii) Agricultural and other organic wastes and animal residues (Clausen, Sitton and Gaddy, 1977 and Smiths, 1981),

Out of these three sources, the cheapest and most economical is the re-utilization of wastes which presently happens to receive a growing concern in the developing countries.

The feasibility of residue or waste utilization for production depends on a myriad of residue characteristics such as availability, supply, current usage, etc. (Detroy and Hesseltine, 1978; Sitton, Foutch, Book and Gaddy, 1979). Cassava peel, for instance is readily available, cheap and uncompetitive in countries where cassava is utilized as staple food, Nigeria being one of them.

It is about 10-13% of the tuber by weight (Tewe, Job, Loosli and Oyenuga, 1976 and Fetuga and Tewe, 1985), and the available metabolisation energy is between 1.52 and 2.16 Kcal/g (Longe, Famojuro and Oyenuga, 1977 and Tewe, 1984). The requirement to render it suitable for a fermentation substrate is saccharification to make the simple sugars available for use by the fermenting micro-organisms. Information on the studies of the utilization of cassava peels, as the fermentation substrate in a submerged culture is scanty. The aim of this study therefore was to investigate the possible usage of this food waste in alcoholic fermentation.

Materials and Methods Source of Cassava Peels

The peels of cassava *(Manihol escu/enla Granlz)* were collected from the processors as soon as they were peeled off. The peels which were obtained from various locations in Rivers State were pooled together, sorted and washed thoroughly to remove sand and debris.

After removing the brown out layer, the white fleshy material was dried in a hot-air oven at 60°C for 12h to a moisture content of 5-8% and then dry-milled with a moulinex coffee mill into a coarse powder. A fine flour of the peel was produced by sieving through a metal sieve of 0.5mm mesh pore size. The peel flour was then reconstituted to make a 6.7, 13.3 and 20% w/v substrates in different test tubes using

distilled water according to the method of Kaur (1989). The substrate (EH) containing 6.7% substrate was used as reference.

Pre-Treatment of Flour

The reconstituted substrate was sterilized by autoclaving at 115°C for 5 minutes. Then using a modified method of Dellweg and Luca (1988), 2ml of sterile termamyl enzyme (alpha-amylase) from Sigma Chemical Company, U.S.A. was added to 1 litre of the substrate in a conical flask. After adjusting the pH to 6.0 using 0.1M NaOH, it was heated in a water bath at 90°C for 30 minutes, cooled and supplemented (10%) by adding tryptone soy broth (OX1OD, England).

Substrate Fermentation

The test organism used for the fermentation was *Saccharomyces nvarum* which was maintained on Potato Dextross Agar slants (PDA - OXO ID, England) at 4°C.

The inoculum was prepared using the modified method of Kaur (1989) in which two loopfuls of the yeast culture from the maintenance slants were inoculated into sterile 10ml of tryptone soy broth (TSB - OXO1D, England), in test tubes and incubated at 32°C for 48h with regular shaking at 6 hourly interval. The pre-treated and supplemented substrate (100ml) was then inoculated with 2 ml, 4 ml and 8ml of the yeast inoculum in separate flasks containing averages of 3.3×10^4, 9.2×10^7 and 2.3×10^{14} cfu/ml respectively and incubated at 32°C or 3 days during which analyses were carried out every 24h. A separate set up with no supplement was inoculated with 2ml inoculum and also incubated as above for analyses. .

Determination of Yeast Populations

This was done using the standard spread plate technique as described by Harrigans and Me Cance (1976) and Cruickshank, Duguid, Marmion and Swain (1980)

Chemical Analyses

The fermented substrate were first centrifuged at 300 x g for 25 minutes using the MSE MINOR 35 table centrifuge, and then the supernatants were used for the analyses at 24h intervals. The changes in reducing sugar concentrations were determined using the dinitrosalicylic acid (DSN reagent) method of Miller (1959).

The efficiency of substrate utilization was obtained by the method of Ofuya and Nwajiuba (1990), calculated as follows:

Amount of reducing sugar utilized - Original concentration - amount utilized

$$\text{Efficiency (\%)} = \frac{\text{concenttation for reducing sugar utilized} \times}{\text{original concentration of reducing sugar}} \quad \frac{100}{1}$$

Ethanol production was determined by the tilrimetirc method of Tietz (1976). This method is based on the fact that ethanol released from the sample is absorbed in an acid dichromate solution and, then oxidized by the solution to ethanoic acid. Then ethanol yield was obtained by the method of Kaur (1989) in which Yield was calculated as:

$$\text{Yield} = \frac{\text{Concentration of ethanol produced (g/1)}}{\text{Concentration of substrate (reducing sugar) utilized (g/1)}}$$

The productivity on the other hand was calculated as:

$$\frac{\text{Concentration\_pf\_ethan\_o1 produced}}{\text{Time of fermentation}} \quad (g/i/h)$$

(Kaur, 1989)

Analysis of sugars by paper chromatography was done using the method of Aliens, Grimshaw, Parkinson and

Quaramby (1974) and Pavia, Lampman and Kriz (1976).

The sugars were identified by comparing their R_f value with those of the standard sugar solution.

Results and Discussion

The growth of the test organism (yeast) occurred throughout the period of fermentation, with the highest growth occurring in the first 48h. At Oh, the substrate (EH) with 2ml inoculum and 6.7% w/v substrate (which served as reference) contained an average of $Logi_a 4.5 \pm 0.1$ cfu/ml.

By the end of the fermentation (7211), the population has increased to Logio7.7+2.1 cfu/nl ($5xl0^7$cfu/ml) which was also the peak (Figure 1). Other substrates with equivalent inoculum had peaks at 48h.

The effect of substrate concentration and supplementation on yeast growth is shown on Table 1. In comparison with substrate EH, growth occurred most in substrate W (with 20% substrate). The changes in the reducing sugar levels in the various substrates using 2ml inoculum are showm in Table 2. The substrate with 13.3% w/v concentration released 63g/l reducing sugar after treatment, and this also was the value at Oh. By the end of fermentation (72h), 27 g/1 was utilized. On the other hand, 118 g/1 reducing sugar was present at Oh in substrate W (20% substrate), and of which level decreased to 88g/l by 72h. The substrate without supplement (X) had 33 g/1 at Oh as in substrate EH, however, 17g/l was utilized as against 20.5g which was utilized in EH. With the increase in inoculum size to 4ml (Y) and 8ml (Z), 22g/l and 23 g/1 respectively were utilized. The efficiency of substrate utilization for substrates EH (reference), V.W and X were 61.2%, 42.9%. 25.4% respectively. This shows that the substrate without supplement (X) had a relatively higher efficiency of utilization (Figure 2). The substrates with increased inoculum size (Y and Z) hat[1] efficiencies of substrate utilization of 64.7% and 68.7% respectively.

Table 1: Changesjn the Yeast Populations in the Various Substrates

Fermentation Time(h)	Yeast counts in the different substrates (cfu/ml)			
	EH	V	W	X
0	$Log_{10}4.5$ (±1.6)	$Log_{10}4.4$ (+ 1.5)	$Log_{10}4.5$ (±1.6)	$Logi_n 4.6$ (+1.7)
72	$Log_{10}7.7$ (+2.1)	$Log_{10}10.4$ (±3.3)	$Logi_0 11.7$ (±3.5)	$Log_{10}7.1$ (±2.0)
Growth Increase	$Log_{10}3.2$	$Log_{10}6.0$	$Logi_0 7.2$	$Log_{10}2.5$

Values in parenthesis represent standard deviation

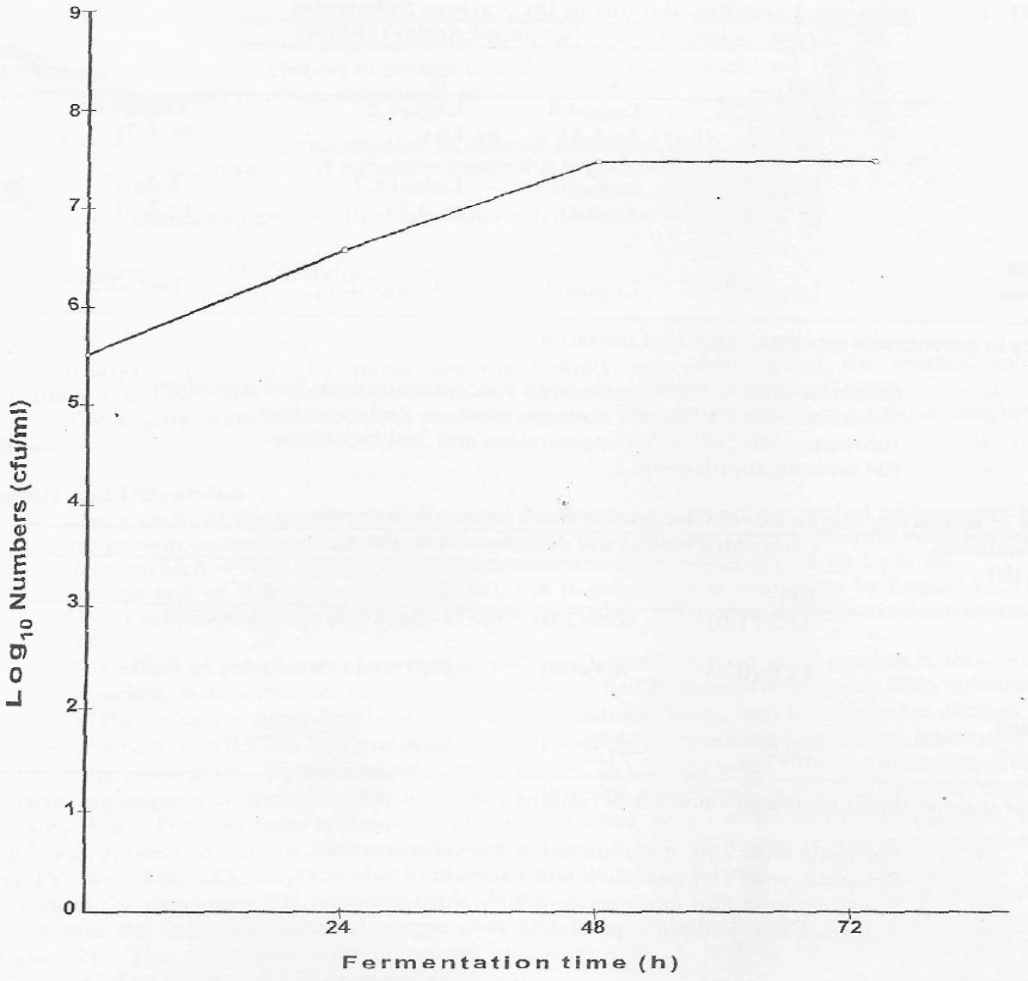
EH - Substrate with 6.7% w/v substrate concentration and 2ml inoculum
V = Substrate with 13.3% Wv concentration, an 2ml inoculum
W = substrate with 20% w/v concentration and 2ml inoculum
X = EH without supplement.

Table 2: Reducing Sugar Level Changes in the Various Substrates

Fermentation Time (h)	Concentrations in the different substrates (g/l)			
	EH	V	W	X
0	33.5(1.0)	63.0(2.0)	118.0(10.0)	33.0(0.3)
72	13.0(0.5)	36.0(00)	88(2.5)	15.5(1.5)
Utilised Amount	20.5	27.0	30.0	17.0

Values in parenthesis represent standard deviations

EH = Substrate with 2ml inoculum and 6.7% substrate cone.
V = Substrate with 2ml inoculum and 13.3% substrate cone.
W = Substrate with 2ml inoculum and 20% substrate cone.
X = Substrate EH, without supplement.



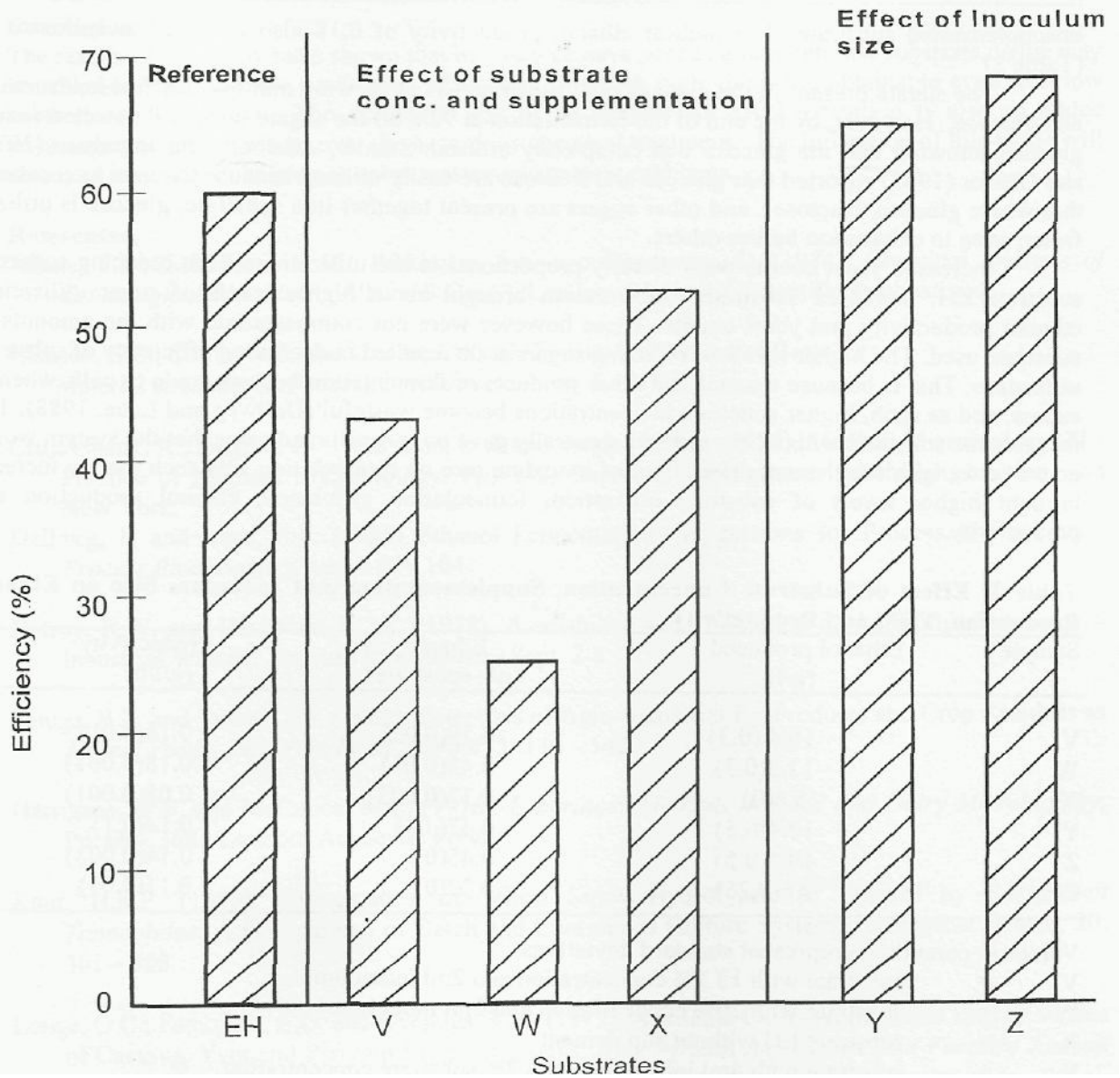


Figure 2: Effects of Substrate Concentration, Supplementation and Inoculum bize on btticiency ot Substrate Utilization.

EH: Enzymatically treated substrate (with 6.7% substrate, supplement and 2m! (3.3 x 10^4 cfu/ml) inoculum size.

V: EH with 13.3% substrate
W: EH with 20% substrate
X: EH without supplement
Y: El I with 4ml (9.2 x iO^7 cfu/ml) inoculum
Z: EH with 8ml (2.3 xIO^{14} cfu/ml) inoculum

substrates V,W,X,Y and Z produced 7.6, 10.4, 12.8, 5.6, 10.4 and 10.4g/l respectively. The highest ethanol yield of 0.47g/l occurred in Y with inoculum size of 4ml, and the least (.33g/g) occurred in the unsupplemented substrate. The highest ethanol productivity of 0.18 also occurred in substrate W (Table 3)

The sugars present in the cassava pee! substrate EH at Oh were maltose, Raffmose, Fructose, and glucose. However, by the end of the fermentation at 72h, all the sugars were still detected except glucose, showing that the glucose was completely utilized. Stanier, Adelberg and Ingraham (1979) and Okafor (1987) reported that glucose and fructose are easily utilized because they are hexoses and that where glucose, fructose and other sugars are present together in a substrate, glucose is utilized faster, even to exhaustion before others.

Increased yeast counts were directly proportional to the utilization of the reducing sugars in

substrate EH. Increased substrate concentration brought about higher levels of sugar utilization, ethanol productivity and yeast counts. These however were not commensurate with the amounts of substrate used. The higher levels of reducing sugars at Oh resulted in decreased efficiency of substrate utilization. This is because ethanol and other products of fermentation become toxic to cells when in excess, and as such, higher substrate concentrations become wasteful (Dellweg and Luca, 1988). The fact that the substrate without supplement generally gave poor results indicates that the system would go on better with supplement. The effect of inoculum size on fermentation was such that an increase brought higher levels of substrate utilization, fermentation efficiency, ethanol production and productivity.

Table 3: Effect of Substrate Concentration, Supplementation and Inoculum Size on Ethanol Production, Yield and Productivity

Sample	nol produced (g/D	Ethanol (g/g substrate)	Productivity (g/l/h)
V	10.4(0.3)	0.39(0.03)	0.14(0.01)
W	12.8(0.3)	0.43(0.01)	0.18(0.001)
X	5-6(0)	0.33(0.005)	0.08(0.001)
Y	10.4(0.5)	0.47(.03)	0.14(0.1).
Z	10.4(0.5)	0.45(0)	0.14(0.003)
EH	7.6(0.25)	0.37(0)	0.11(0.01)

Values in parenthesis represent standard deviations.

V = substrate with 13.3% concentration and 2mI inoculum
W = substrate with 20% concentration and 2ml inoculum
X = substrate EH without supplement
Y = substrate with 4ml inoculum and 6.7% substrate concentration
Z = substrate with 8ml inoculum and 6.7% substrate concentration
EH = substrate with 2ml inoculum and 6.7% concentration

Recommendations

In Nigeria today, cassava's economic value is increasing due to the new government's and private sector's focus on it as substrate for ethanol production. This results in increased utilization and therefore a large-scale availability of the peels. Industries producing cassava flour, paste or whey either for food, starch or ethanol production should be made to pre-treat and utilize the peels in a side plant for the production of ethanol. This will at the long run reduce the utilization of cassava for ethanol production and therefore increase food production.

Conclusion

The results of this study have shown that by using cassava peel as a fermentation substrate, value may be added to it through the production of alcohol for which high yields are obtainable even with low substrate concentrations. The successful use of an enzyme for saccharification is also an added advantage and improvement over the hazardous chemical treatment. The utilization of this waste will also go a long way in helping to solve waste management problems.

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