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Effect of Some Stimulants (Trona, Nail and Kerosene) on the Fermentation Kinetics of Some Selected Variety of Cassava Roots - *Manihot esculenta*

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Abstract: Different people, most often use different stimulants to enhance the fermentation process of different varieties of cassava (*Manihot esculenta*), unknown to them of the health and otherwise implication of such practice. This research work is aim at determining the effects of using varying amount of some stimulants (Trona, Kerosene and Nail) in the anaerobic fermentation of fixed amount of various variety (TMS 30572, TMS 4(2)1425, NR.8082)of peeled, washed cassava. The parameters used to monitor these include oxalic acid volume, cyanogenic glucoside of the fermented products, the amount of gas evolved during the fermentation period and the P^H of the fermented products. The results obtained showed that the smaller the amount of stimulant (Trona& Nail) added, the faster the fermentation rate for TMS 30572 followed by TMS 4 (2) 1425. This supports the literature on the fact that catalyst acts best in small quantities. But for kerosene, there was no remarkably effect both in small and increased volume. The level of toxicants or anti-nutritive factors like cyanogenic glucosides (HCN) and oxalic acid in the fermented cassava was very low (within the allowable dose) when stimulants used were. Among the stimulant that performed better is Trona followed by Nail and Kerosene.However, for N.R 8082, this species of cassava does not response to stimulant. Based on this, I therefore recommend that the use of Trona to aid fermentation of TMS 30572 variety of cassava be continued since the fermented product obtain is low in antinutritional factors.

Key words: Stimulants · Fermentation · *Manihot esculenta* · Cassava and Anti-nutritional factors

INTRODUCTION

Fermentation is an anaerobic or partial anaerobic process of oxidation of carbohydrates brought about by the activities of microorganism (like Enzymes) and extract prepared from them to produce substance /compound simpler than the starting substrate/compound [1]. Fermentation does not only denote anaerobic dissimilation process like the formation of alcohol, butanol–acetone. Lactic acid etc; but also entail the industrial production of vinegar, citric, acid, enzymes penicillium and other antibiotics and vitamins since they result from microbial process. Since the process of fermentation involves growth and proliferation of microorganism in foods, several changes may simultaneously occur in the organic food material [2-5]. These changes may lead to flavour enhancement of some foods, improved physical characteristics of food, alteration of the parent foodstuff to new and different product with a longer shelf life than parent raw – materials. Prolongation of the shelve life food. Fermentation is used industrially for the production of organic acids, aldehydes and ketones, example acetic acid, lactic acid (a food preservative) citric acid, ethanol, in the production of beverages like wines, spirit, palm wines, burukutus, yoghurt, butter, sour creams as well as production of antibiotics. Fermentation has a complex chemistry because of the involvement of many reaction which include redox, rearrangement, phosphorylation, dehydrogenation and decarboxylation reaction [6]. The following factors can affect the fermentation process of any food material. There are presence of stimulant (inhibitors), Temperature, Oxygen supply, P^H level and Micro – organisms

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2\,C_2H_5OH + 2C_{02}$$

The biochemical pathyway of fermentation is known as glycolysis. The mechanism starts with the conversion of polysaccharide (starch) to disaccharide (sucrose) into monosaccharides (glucose and fructose) by the enzyme invertase.

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Table 1: Characteristics of Various Varieties Used in Experiment

ATTRIBUTES	TMS-30572	TMS 4 (2) 1425	NR.8082
Branching habit	Profuse	Moderate	Profuse
Canopy development	Wide	Sparse	Profuse
Ecological adaptation	Moderate	Savannah	Wide
Pest and disease Tolerance	Moderate	Moderate	High
HCN of root (mg. 100g)	750	34	High
Starch yield	24	22	19
Fresh root yield (heap)	27	26	32
Gari yield (%)	25	25	22

$$C_{22}\ H_{42}O_{22} + H_20 \rightarrow (C_{12}H_{22}H_{11})_n$$

$$(C_{12}\ H_{22}O_{11})_n + H_20 \xrightarrow[\text{From yeast}]{\text{Invertase}} C_6\ H_{12}O_{11} + \underset{\text{Glucose Fructose}}{C_6\ H_{12}O_6}$$

The glucose which is a 6 – carbon sugar, is broken down to two molecules of pyruvic acid (CH_3CO COOH), a 3 – carbon compound. The pyruvic acid then may be reduced to form lactic acid or it may lose carbon dioxide to form acetal aldehyde which is subsequently reduced to form ethyl alcohol respectively [7, 8].

Cassava belongs to the family *Euphorbiaceae*. It is a perennial tuberous roots and variously branch. The height, spread and other characteristics vary among cultivars between 1 to 5meters'.The stem of the cassava is tall, thin and straight and has a number of nodes. The color of the stem usually varies from brown to gray. Cassava is grown by planting cuttings taken from the woody part of the stem during germination one or more of the auxiliary buds sprouts and the roots grow principally from the base of the cuttings. When the main shoot becomes reproductive, the buds at the distal nodes being to elongate and some develop as shoots. The growth cycle of a typical cassava is between 7 to13 months [9-11].

Classification of Cassava: Cassava varieties are often classified according to the levels of cyanogenic glycosides (hydro cyanic acid HCN) in the tuber and leaves.The major groups are: SWEET CASSAVA: - (*Manihot utilisima*) contains low concentration of HCN. And the HCN are concentrated in the peel. Example include Tms 4 (2) 1425, Trns 30572 Tms 30555 etc. BITTER CASSAVA: - Botanically called (*Manihot palmate*) contains high HCN level are when taken unfermented is very poisonous. Example includeTms 50385.

Many cassava varieties under cultivation can be distinguished by such morphological characteristics as leaf size, color, shape, branching habits plant height color of stem and petiole tuber shape and color, time – to – maturity and yield.

Nutritive Composition of Cassava: Cassava – whether sweet or bitter variety contains some chemical substances like carbohydrates (starch, from which glucose is produced) (2-30%) water (75 -81%), fat (0.1%), fiber (1.0- 1.6%), Ash (1-1.5%) and mineral and vitamins.

Uses of Cassava and Starch: Very importantly, cassava has contributed significantly to meeting the food security needs of the country. The potential of cassava is far when compared to other staple food. Domestically, it is eaten asFufu, garri, alibo, flour in bakeries. *The root*: The cassava root when peeled, grated, fermented and fried produces garri. The root can be chipped or sliced for livestock. Cooked, boiled or baked for table use. Root peel as livestock feed.The starch is used for the following purpose.-In confectionery (moulding of cast sweets),Thickening agent, Fermented beverages (beer), Laundry starch, Gums, Dextrins (bonding pigment to paper) Adhesives, Glues and pastes, Blended with peanut flour, Nonfat milk solids, Oil well drilling and Acetone etc.

Toxicant in Cassava Root: Cassava tuber contains toxic substances both in the sweet and bitter variety which is believed to be poisonous to the body of mammal when ingested. The toxicants include cyanogenic glycoside, oxalic acid, tannins, phytic acid and saponins etc.

Factors Affecting Fermentation of Cassava: Oxygen Supply: Since fermentation is an anaerobic process, oxygen should be avoided to enhance the process rate.

Temperature: Increase or decrease in temperature may destroy or retard the activities of microorganism. For fermentation of cassava, optimum temperature of 35°C – 40°C is required for both enzymatic action and growth.

pH: Depending on the microorganism involved, pH may be lowered or increased to suit the process. For the fermentation of cassava, a slightly acidic medium will favor the fermentation of cassava. (At pH range of 5.5 - 6.0).

Micro-organism: Suitable microorganism should be chosen in order to produce a desire product(s). Since in most cases, the speed and completeness of a desired microbial action are proportional to the quantity of organisms present. The reproduction of microorganism is an essential part of almost every microbial process. Some microbial cells reproduce by fission (splitting the cells), some by forming bud or sprout that will grow until they reach the size and maturity of the mother cell and others both ways.

Stimulants and Their Effects on the Fermentation of Cassava: Stimulants otherwise called activators are substances, which in minute quantities will accelerate the microbiological process. Some examples of stimulants used in the fermentation of cassava tuber includes:

- Nail
- Kerosene
- Trona (Akawu in Igbo) (Na_2CO_a, $NaHCO_3$, $2H_2O$).

MATERIALS AND METHODS

An eleven-month old varieties of *Manihot utilisima* (sweet cassava) harvested from Federal Ministry of Agriculture farm settlement at Ugbo-oye off Trans-Ekulu, Enugu State was used (TMS 30572, TMS 4(4) 1425 and N.R. 8082) varieties.

Stimulants used: The volume and weight of the various stimulants used in the fermentation are

Kerosene: (0.1g, 0.2g, 0.3g), Trona:(0.1g, 0.2g, 0.3g), Nail:(0.1g, 0.2g, 0.3g).

Apparatus Used: Plastic bowls, distillation flask, plastic tubing (rubber tubing), beakers, measuring cylinder, thermometer, pH meter, clamps and retort stands, volumetric flasks, knife, conical flasks, Burette, Pipette, Bunsen Burner, Weighing balance, Grinding machine, filter papers, funnels etc.

Reagents Used: Potassium permanganate solution (KMnO4), Silver nitrate solution ($AgNO_3$)0.1M, Potassium iodide solution (KI), 0.01M Sulphuric acid (H_2SO4), Ammonium solution (NH_3) 0.2M, Distilled water (H_2O).

Method

Sample Preparation and Fermentation: The cassava tuber was peeled and washed with water to remove the thick brownish cover, which contain high cyanogenic acid. The washed cassava was cut into chips and 50gram each of various cassava samples weighed and introduced into the clean distillation flasks. This was followed by the addition of 100ml of distilled water and allowed to ferment for four (4) days at room temperature and pressure. This is the control. The control set was three (3) in number each containing one variety of cassava species. The above procedure was repeated but with the various stimulants; Trona, kerosene and Nail added to the fermentation flask.

Determination of Parameters Used in Monitoring the Fermentation

Cyanide Test: 50g each of various variety of peeled cassava was grinded separately and 50ml of deionized water added to it to make slurry. The slurry was then filtered.

To 25ml of the milky filtrates in a conical flask was added 1ml of 0.2M NH_3 solution and 1ml of 0.01M KI solution.The resulting solution was then titrated against 0.1M $AgNO_3$ solutions in the burette. The corresponding burette readings was taken and evaluated. This was also done for the fermenting cassava, with various concentrations of the stimulants.

Oxalic Acid Test: Determination of the Oxalic acid content of both fresh and fermented cassava was carried out.20ml of the cassava filtrates of various variety were obtain in the manner stated under cyanide test and was then transferred into a clean conical flask and 5ml of 2M solution of sulphuric acid was added and the mixture was immediately brought to 70°C by heating. The hot solution was titrated against the $KMnO_4$ solution in the burette until a pink coloration, which persisted for 20 seconds result. At this point, the titration was discontinued until there is a total disappearance of the pink color. The titration was continued on observation that the first pink coloration has disappeared. The end point is reached when a faint pink coloration, which persisted for 30 seconds upon shaking the flask, was again observed. At this point the burette reading was also noted.

P^H Determination: for the P^H determination, the digital P^H meter was used after being standardized using a buffer of known P^H value to obtain the $P^H$of the grinded flesh and fermented samples in slurry form and the value read from the screen.

Determination of Gas Evolved: The amount of CO_2 evolved is directly proportionalto thevolume of water displaced in the measuring cylinder of the fermentation setup. Since the fermentation lasted for four days, the volume of water displaced was taken in other to known the amount of gas evolved.

DISCUSSION

In Trona, it was observed that at quantity 0.1gram, the volume of gas evolved is highest which follows a gradual drop with increase in quantity of stimulant, which synchronizes with the extent of fermentation. This effect was also noticed when nail was used as a stimulant.

Table 2: Effect of Three Stimulants on the Extent of Fermentation of the Medium

Stimulant	Qty (g)	Tms4(2)1425	Tms30572	NR8082
TRONA	0.1	- - -	- - -	-
	0.2	- - -	- -	-
	0.3	-	- -	- - -
KEROSENE	0.1/L	- -	- - -	- -
	0.2/L	-	- -	- -
	0.3/L	-	- -	
NAIL	0.1	-	- - -	-
	0.2	-	- -	-
	0.3	- -	- -	- -
CONTROL		-	- -	- -

--- = Fermented very well
--- = Fermented well
--- = Not well

Table 3: Effect of Volume Co_2 Evolved During Fermentation of the Cassava Varieties: (Maximum Volume after Four Days).

Stimulant	Qty (g)	Tms4(2) 1425(CM^3)	Tms30572 (CM^3)	NR8082 (CM^3)
TRONA	0.1	435	485	10
	0.2	300	345	5
	0.3	11	375	60
KEROSENE	0.1	200	5	15
	0.2	170	30	10
	0.3	175	282	15
NAIL	0.1	160	335	5
	0.2	185	313	20
	0.3	175	255	80
CONTROL		20	125	65

The quantity of gas evolved is directly proportional to the extent of fermentation.

Table 4: Effects of Stimulants on Ph of the Medium

Stimulant	Qty (g)	Tms4(2)1425	Tms30572	NR8082
TRONA	0.1	5.7	6.3	5.5
	0.2	5.8	6.4	6.1
	0.3	5.9	6.8	5.8
KEROSENE	0.1	5.6	6.1	6.1
	0.2	5.2	5.8	6.2
	0.3	5.1	5.9	6.2
NAIL	0.1	5.7	5.1	6.8
	0.2	5.8	5.2	6.9
	0.3	6	4.8	6.9
CONTROL		5.3	5.5	5.9

Table 5: Effects of Three Stimulants on Oxalic Acid of the Medium

Stimulant	Qty (g)	Tms4(2)1425	Tms30572	NR8082
TRONA	0.1	8.75×10^{-2}	1.05×10^{-1}	8.1×10^{-2}
	0.2	7.7×10^{-2}	1.15×10^{-1}	8.57×10^{-2}
	0.3	6.0×10^{-2}	1.27×10^{-1}	9.75×10^{-2}
KEROSENE	0.1	8.37×10^{-2}	1.13×10^{-1}	7.6×10^{-2}
	0.2	8.5×10^{-2}	1.41×10^{-1}	1.0×10^{-1}
	0.3	6.8×10^{-2}	1.36×10^{-1}	1.15×10^{-1}
NAIL	0.1	7.83×10^{-2}	1.20×10^{-1}	1.24×10^{-1}
	0.2	7.4×10^{-2}	1.10×10^{-1}	1.28×10^{-1}
	0.3	7.75×10^{-2}	1.11×10^{-1}	1.25×10^{-1}
CONTROL		$7.3 \times x 10^{-2}$	1.25×10^{-1}	1.17×10^{-1}

Table 6: Effect of three stimulants on HCN content of the medium

Stimulant	Qty (g)	Tms4(2)1425	Tms30572	NR8082
TRONA	0.1	4.0×10^{-3}	8.0×10^{-3}	4.8×10^{-3}
	0.2	4.8×10^{-3}	6.4×10^{-3}	3.6×10^{-3}
	0.3	1.12×10^{-3}	1.68×10^{-2}	1.2×10^{-3}
KEROSENE	0.1/L	1.32×10^{-2}	6.0×10^{-3}	8.0×10^{-4}
	0.2/L	2.8×10^{-3}	2.0×10^{-3}	8.0×10^{-4}
	0.3/L	1.12×10^{-3}	4.0×10^{-3}	1.2×10^{-3}
NAIL	0.1	5.2×10^{-3}	1.16×10^{-2}	4.0×10^{-4}
	0.2	4.84×10^{-3}	8.4×10^{-3}	1.2×10^{-3}
	0.3	3.6×10^{-3}	8.0×10^{-3}	2.4×10^{-3}
CONTROL		9.6×10^{-3}	4.4×10^{-3}	2.8×10^{-3}

But there was a variation when kerosene was used as a stimulant. This can be attributed to the fact that using kerosene as a stimulant will tend to cover the surface of the water. That is why the volume of gas evolved is small when compared to other stimulant. On the variety of cassava that fermented well, it was observed that TMS 30572 performed better followed by TMS 4(2) 1425 and then N.R 8082, which was observed in the volume of gas evolved.

Analysis of Table 4: The result of the P^H shows that for Trona, as the quantity of stimulant increases the pH value increases for all the variety of cassava towards basicity. The trends was because of the alkalinity nature of Trona which is a sodium salt (Na_2CO_3. $N_aHCO_3.2H_2O$) since the extent of fermentation affects P^H.From Table 3, it shows that TMS 30572 ferments very well with respect to control followed by TMS 4(2) 1435 and then N.R. 8082.

For Kerosene, its effect on the pH was significant because kerosene affects the rate of fermentation although it performed well in TMS 30572.

For Nail, pH range for all the variety is within 5.2 – 6.9, an indication that a lot of the cyanogenicglycoside has been removed from the fermenting tuber leaving the fermented product with little HCN content because, high pH favors microbial activities. The remaining little HCN can further be reduced by boiling.

Generally, from the research, it shows that using small quantity of stimulant increases the rate of value of gas evolved A detail analysis from the table shows that all the stimulant are good for fermentation but Trona is most preferred because of its high performance in glycoside removal and pH of the product.

Analysis of Table 5: For TMS 4(2) 1425, the effect of stimulant on this is such that in the presence of stimulants, there is an increase in the quantity of oxalic acid being removed from the fermenting cassava, when compared with the value of control. it was also found that

the oxalic acid removal is dependent on the quantity of stimulant added i.e. to say that it (oxalic acid) removed increases with decrease in quantity of stimulants. Generally, oxalic acid is decreasing down the group with increase in quantity of stimulant for all the variety of cassava

Analysis of Table 6: The result of the research show that the presence of stimulant increase the quantity of Cyanogenic glycosidal being converted to hydrocyanic acid (which is a toxicant in cassava) being removed.A comparison of the result with respect to the control value shows that in the presence of stimulants more quantity of the toxicants is removed. Among the stimulants, it was observed that Trona does better than other followed by nail and then kerosene. Based on this, therefore I recommended the use of Trona during fermentation of cassava because the fermented product will contain very minute quantity of the toxicant far below the lethal dose per 100grams.

Conclusion and Recommendations: The fermentation of cassava (*Manihot esculenta*) was rapidly increased by the addition of small quantities of stimulant, thus, reducing the period of fermentation by accelerating rate of fermentation. TMS 30572 performed better followed by TMS4 (2) 1425 and N.R 8.82.The toxicant (HCN and $H_2 C_2 O_4$) level of were very low when stimulant is used. Among the stimulant that performed better is Trona followed by Nail and Kerosene.Based on this, I therefore wish to recommend that, the use of Trona to aid fermentation of TMS 30572 variety of cassava be continued since the fermented product obtained is low in antinutritionalfactors.Again, the terms of cost involvement, Trona when compared to other stimulants (Nail and Kerosene) is easy to come by.

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