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ORGINAL ARTICLE

Assessment of Some Fermentation Processes of Cassava Tubers

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ABSTRACT

Accelerating the fermentation process of cassava tubers was investigated by monitoring the pH, time and the addition of mild steel, leading and how it affects the rate of hydrocyanic acid (HCN) removal. The medium was monitored between the pH of 6.9 to 3.4 for peeled soaked tuber and pH between 6.7 to 3.3 for grated tubers. The analysis was carried out at constant temperature of $30^0C \pm 2$. The investigation of the hydrocyanic acid content and its removal was carried out in different media. The natural fermentation (control) retting time was 96 hours having 80.16HCN Mg/Kg with 78.80% HCN reduction for soaked tubers and 76.78HCN Mg/Kg with 79% detoxification for grated tubers respectively. Secondly, the inoculation with 2% w/w acetic acid with a retting time of 72 hours having 80.16HCN Mg/Kg with 83.15% detoxification and 76.78HCN Mg/Kg with 84.38% detoxification for soaked tubers and grated tubers respectively. Thirdly, the addition of 10g/L mild steel (nails) reduced the retting time to 48 hours having 80.16 HCN Mg/Kg with 81.90% detoxification for soaked tubers and 76.78HCN Mg/Kg with 85.68% reduction for grated tubers respectively lastly, the leaching process with the mother-liquor at 60 hours retting time having 80.16HCN Mg/Kg with 76.93% reduction for soaked tubers and 76.78HCN Mg/Kg with 78.52% detoxification for grated tubers. The rates of detoxification in all the processes were efficient when compared with the tolerance level recommended by food association organization/world health organization (FAO/WHO). The Fisher' Least Significant Difference(F-LSD) was employed at 5% least significant difference to compare the means of the result.The result shows no significant difference in their means.

KEYWORDS: *Cassava, fermentation, detoxification, hydrocyanic acid.*

INTRODUCTION

Cassava (Manihot esculenta Crantz) is an important root crop in Africa, Asia, South Africa and India, providing energy for about 500 million people[1]. Cassava roots are potentially toxic due to the presence of cyanogenic glycosides especially Linamarin[2]. Physiological deterioration occurs in cassava roots, 2-5 days after harvesting followed by microbial deterioration 3-5 days later[3]. Cassava farming population have empirically developed several processing methods for stabilizing cassava and reducing its toxicity[4]. Additionally the roots contain large quantities of the antI-nutrient factor cyanide and it changes in cassava into cyanogenic glycosides, linamarin and lotaustralin [4]. Fermentation is part of almost all these processes which transform its low technology and energy requirement to a final product [5]. During the consequent fermentation roots are softened. Disintegration of the tissue structure results in contact of linamarin with linamarase which is located in the cell walls [6] and subsequent hydrolysis to glucose and cyanohydrins, which easily breakdown to ketone and HCN [6]. One potential problem in processed cassava is the flavour of the product which may be undesirable to many people. However, any process that ruptures the cell walls will bring the enzymes into contact with the glycosides and will thus release cyanide and reduce the glycosides content of the final point [7]. The need to accelerate the fermentation process of cassava has arisen because of a rapidly increasing population in developing countries [8]. This work is aimed at assessing four methods of fermentation all of which involve monitoring the pH, time of the fermented cassava, addition of 2% w/w acetic acid and leaching with the mother-liquor (soaked and grated) with the rate of HCN removal as basis for the analysis.

MATERIALS AND METHODS

Twenty kilograms(20kg) of the tubers of 12- month old tubers of New Research (NR) 8082 cassava cultivar was harvested from a farm at the national Roots Crops Research Institute, (NRCRI) Umudike,

Umuahia, Nigeria. The roots were peeled, washed and immersed in water in a clean plastic container and another was grated and put in a muslin bag and submerged in water in a clean plastic container. The temperature of both containers was maintained constantly at of 30 ^{o}C.

Processing of Cassava Roots

Four (4) different procedures corresponding to the methods traditionally used in Nigeria were employed. The fermentation experiment for each technique studied was prepared in triplicate for soaked and grated tubers.

Procedure 1 (Standard): The fresh, peeled tubers were suspended in water and those of the grated category and were allowed to ferment for 96hours by the natural micro flora (control).

Procedure 2: The cassava tubers of both soaked and grated were peeled, washed and soaked in a sterile container with the inoculation of 2% w/w acetic acid and time reduced to 72hours. Equally the grated tubers were put into a clean Muslim bag and left to ferment for the same 72hours with the inoculation of 2% w/w acetic acid in the bag.

Procedure 3: The cassava tubers were peeled, washed and soaked in a clean water and 10g/l mild steel (nails) were added to the container, and this was also done for the grated tubers and the fermentation process was allowed to proceed for 45hours.

Procedure 4: The cassava tubers were peeled, washed and soaked in already fermented liquor (mother-liquor), and it was also carried out in the grated tubers. The retting time was 60 hours.

Chemical Analysis

Different samples of fermented cassava tubers (soaked and grated) were carried out in 100 ml of distilled water and the pH monitored with a pH meter. The sample homogenate was titrated to pH of 8.0 with 0.01 M $AgNO_3$ using iodine starch iodide as indicator [9].

The titratable acidity was expressed as lactic acid equivalents on a wet basis. The cyanide content was determined as described by Oyewole and Ogundele [10].

Statistical Analysis

Each treatment was replicated three times. Data obtained were analyzed using the F-SLD method at 5 % least significant difference to determine the differences in their means.

RESULTS

Quantitative determination of HCN content as a function of pH variation and addition of mild steel (nails) during the retting of cassava tubers carried out in four different procedures are shown as follows:

Procedure 1: For (soaked and grated tubers) which was allowed to ferment for 96 hours by the natural micropores and it was used as a control yielded the 80.16 HCN mg/kg for both soaked and grated tubers and thus released a free HCN of 78.80 % for soaked tubers and 79.00 % for grated tuber detoxification.

Procedure 2: For (soaked and grated tubers) the fermentation process involved inoculation of the system by introducing 2 % w/w acetic acid into the medium and the fermentation process became faster at 72 hours and yielded 80.16 HCN mg/kg initially for soaked tubers and 76.80 HCN mg/kg for grated tubers with 83.15 % and 84.34 detoxification respectively.

Procedure 3: Cassava tubers (soaked and grated) occurred with addition of 10g/l mild steel (nails) and the retting time reduced drastically to 48 hours yielded 80.16 HCN mg/kg for soaked tubers with 81.90 % detoxification and 76.80 HCN mg/kg for grated tubers with 85.68 % detoxification.

Procedure 4: The cassava tubers (soaked and grated) occurred at 60 hours and a leaching technique of the soaked and grated tubers into the mother-liquor was employed and this procedure yielded 80.16 HCN mg/kg for soaked tubers with 76.93 % detoxification and 76.78 HCN mg/kg with 78.52 % detoxification for grated tubers.

TABLE -1: Changes In The Fermented Cassava Using Different Accelerating Methods

For soaked tubers

Sample I –Natural fermentation (control)

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	6.40	80.16	0.00	0.000
12	6.10	72.63	9.39	0.001
24	6.90	49.28	38.59	0.033
36	6.40	34.22	57.31	0.001
48	6.40	31.54	60.66	0.025
60	6.10	30.20	62.33	0.001
72	5.50	26.40	67.07	0.016
84	4.80	22.15	72.37	0.006
96	4.70	17.00	78.80	0.124
				$0.207/_9$

MS_E = 0.023 LSD = 0.15

Sample II – Inoculation of 2 % w/w acetic acid

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	5.60	80.16	0.00	0.000
12	5.30	50.63	36.84	0.093
24	5.00	20.62	74.28	0.001
36	4.80	16.45	79.48	0.010
48	4.70	15.42	80.77	0.003
60	4.60	15.00	81.29	0.015
72	4.60	13.51	83.15	0.010
				$0.132/_7$

MS_E = 0.019 LSD = 0.15

Sample III - Addition of 10 g/l Mild Steel

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	3.80	80.16	0.00	0.000
12	3.40	46.52	41.97	0.001
24	3.60	33.75	57.90	0.001
36	3.50	15.00	81.29	0.008
48	3.40	14.50	81.90	0.002
				$0.11/_5$

MS_E = 0.002 LSD = 0.06

Sample IV – Leaching with the Mother-liquor

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	6.90	80.16	0.00	0.000
12	6.50	65.80	17.91	0.791
24	6.40	55.64	30.59	0.025
36	6.30	30.35	62.14	0.004
48	6.20	27.00	66.32	0.003
60	6.20	18.50	76.93	0.004
				$0.827/_6$

MS_E = 0.138 LSD = 0.45

FOR GRATED TUBERS

Sample I - Natural fermentation (control)

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	6.20	76.78	0.00	0.000
12	6.00	60.21	21.58	0.073
24	6.70	45.50	40.74	0.017
36	6.40	32.00	58.33	0.004
48	6.40	28.75	62.56	0.003
60	6.00	28.75	65.74	0.003
72	5.20	26.31	71.21	0.001
84	4.50	22.11	73.76	0.003
96	4.50	20.15	79.00	0.023
				$0.125/_9$

MS_E = 0.0.14 LSD = 0.12

Sample II – Innoculation of 2 % w/w acetic acid

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	5.40	76.78	0.00	0.000
12	5.10	44.71	41.77	0.471
24	4.80	18.59	75.79	0.169
36	4.60	15.00	80.47	0.017
48	4.50	14.10	81.64	0.304
60	4.40	12.60	83.59	0.010
72	4.40	12.60	84.38	0.024
				$6.99/_7$

MS_E = 0.990 LSD = 1.11

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	5.40	76.78	0.00	0.000
12	5.10	44.71	41.77	0.471
24	4.80	18.59	75.79	0.169
36	4.60	15.00	80.47	0.017
48	4.50	14.10	81.64	0.304
60	4.40	12.60	83.59	0.010
72	4.40	12.60	84.38	0.024
				$6.99/_7$

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	5.40	76.78	0.00	0.000
12	5.10	44.71	41.77	0.471
24	4.80	18.59	75.79	0.169
36	4.60	15.00	80.47	0.017
48	4.50	14.10	81.64	0.304
60	4.40	12.60	83.59	0.010
72	4.40	12.60	84.38	0.024
				$6.99/_7$

Sample III - Addition of 10 g/l Mild Steel

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	3.80	76.78	0.00	0.000
12	3.40	42.40	44.78	0.001
24	3.60	30.61	60.10	0.001
36	3.50	14.40	81.25	0.008
48	3.50	11.00	85.68	0.002
				$0.12/_5$

MS_E = 0.002 LSD = 0.06

Sample IV – Leaching with the Mother-liquor

Time (hours)	pH	HCN (mg/kg)	Free HCN %	$MS_E = \frac{SS_E}{n}$
0	6.70	76.78	0.00	0.000
12	6.40	62.50	18.60	0.791
24	6.30	50.83	33.80	0.025
36	6.20	27.42	64.29	0.004
48	6.10	24.00	68.75	0.003
60	6.10	16.50	78.52	0.004
				$0.827/_6$

MS_E = 0.138 LSD = 0.45

- MS_E = Mean Square
- SS_E = Sum of Squares due to error
- n = Sample Size
- LSD = Least Significant Difference

$$LSD = 2.086\sqrt{\frac{2MS_E}{n}}$$

Note: The HCN retention can also be determined by subtracting the % free HCN (reduction) from 100 %.

DISCUSSION

The fermentation of cassava tubers called retting shows softening of tubers and further reduction of potentially toxic cyanogenic glycosides present in tubers[9]. The fermentation processes took place in an acid environment (6.9 – 3.4) which confirms the work of Ogbo[11]. However, this type of fermentation process although inexpensive and simple, cassava retting involves a complex microbial process and its durability. The population and composition of the microorganisms as well as the reduction of cyanogenic glycosides at various stages of the present study is similar to that reported in studies of cassava fermentation [11,5]. In the procedures employed, the pH decreased with increase in the production of organic acids by lactic acid bacteria which contribute the most dominant microflora [5]. In procedure I, natural fermentation of cassava tubers may involve undesirable microorganisms resulting in a decrease in pH and the production of offensive odours. However, the accelerating process was higher in grated tubers than in soaked tubers. This indicates that the loss in structural integrity caused by grating is of great importance for cell wall degradation, which perhaps enhances contact with endogenous enzymes, therefore increasing the surface area. The grating may have improved the contact between microbial enzymes and linamarin thereby reducing the effects of innocuous microorganisms [3,5,12].

Okafor and Ejiofor [13] observed a slow drop in pH during cassava retting but results confirm rapid drop in pH obtained when the cassava is grated prior to fermentation[14,15]. The addition of mild steel reduced the retting time drastically from 96 hours to 48 hours and produced a drop in pH. There were decreases in the retting times of the three (3) introduced methods as compared to the control but

the one with the addition of mild steel (nails) shows maximum reduction.The presence of mild steel (nails) may have served as catalyst which speeds up the fermentation process thereby reducing the retting time. A blackish colouration was also observed on the fermented tubers (soaked and grated). This could be the reduction of iron (III) oxide to iron (II) oxide which is more readily utilized by bacteria. However, the offensive odour was significantly higher in grated tubers than in soaked tuber. This may be due to the disintegration of the tissue structure which exposes it to more enzymatic attack than the soaked tubers. However, according to FAO/WHO, further work needs to be done on the detoxification level of the cassava tubers as to reduce or eliminate the cyanic poisoning to a tolerable level.

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