Kinetics of Natural Detoxification of Hydrogen Cyanide Contained In Retted Cassava Roots

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Abstract
This work presents the kinetics of natural detoxification of hydrogen cyanide contained in retted cassava roots. Retting is traditional fermentation of cassava, performed to soften the roots. During retting, cyanide diffuses into water used for the retting. The fresh cassava roots (bitter and sweet varieties) used for this experiment were separately retted at ambient 0 temperature of 30 C. The cyanide content and pH were monitored daily. From the analysis of the experimental results, a first order consecutive rate equation is an adequate tool for explaining the mechanism of HCN reduction (or decay) in retted cassava roots. The detoxification constants for the bound cyanide in the bitter and sweet cassava roots were 0.378/day and
0.438/day respectively, while that of the free hydrogen cyanide were 0.63/day and 0.74/day for the bitter and sweet varieties respectively. Cassava tubers from different species cannot be fermented with the same retting condition unless they have same or close functional properties.

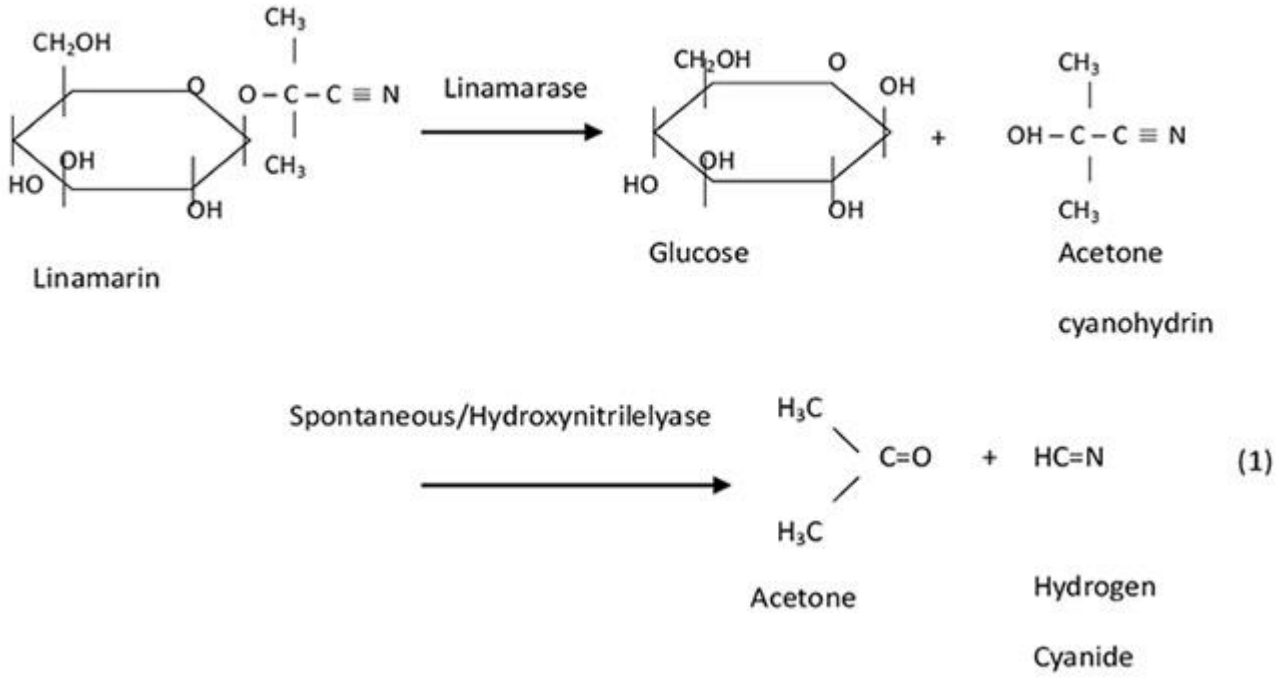
Keywords: Kinetics, Detoxification, Hydrogen Cyanide, Retting, Cassava

INTRODUCTION
Cassava (Mannihot esculenta) is a tuberous, woody perennial plant of the spurge family (Euphorbiaceae). Annual cassava production in Africa is about 84 million tones, with Nigeria having the highest- 30 million tones, Tanzania 5.7 million tones, Democratic Republic of Congo 16.8 million tones, Mozambique 5.3 million tones and Madagascar 2.4 million tones (Ehiagbonare et al 2009; Nweke, 1992). Most developing countries depend on cassava as their primary source of food (Lancaster et al, 1982). Cassava tubers are traditionally processed by a wide range of methods, which reduce their toxicity, improve palatability and convert the perishable fresh root into stable products. These methods consist of different combination of peeling, chopping, grating, soaking, drying, boiling and fermenting. Fermentation of cassava peel reduces toxicity. It converts enzymeresistant ligno-cellulose material into a more digestible substrate. Retting is traditional fermentation of cassava, a major step in the preparation of most indigenous cassava-base foods. It is performed to soften the roots, yield specific flavour by the production of organic acids and decreasing the pH, and degrade the endogenous cyanogenic compounds (Ogunsa 1980; Ayernor, 1985). During retting, cyanide diffuses into water used for the retting/fermentation (Nweke, 1992). According toEl Tinay et al (1984), peeling the rootsbefore retting decreases the amount of cyanide in the mash. The toxicity of a cyanogenic plant depends primarily on the potential concentration of hydrogen cyanide that may be released upon consumption.

The cyanogenic glycosides may be defined chemically as glycosides of the á-hydroxynitriles and belong to the secondary
metabolites of plants. They are amino acid-derived constituents of plants. The biosynthetic precursors of the cyanogenic glycosides are different L-amino acids, which are hydroxylated, then the Nhydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to á-hydroxynitriles and then glycosylated to Cyanogenic glycosides (Vetter, 2000).

Hydrogen cyanide is commercially available as a gas or as a technicalgrade liquid in concentrations of 5, 10, and 96–99.5%. It has 27.03 0 and 25.70 C relative molecular mass and boiling point respectively.
The conversion factors for hydrogen cyanide in air (at 20 °C and 101.3 3 3 kPa) are as follows: 1 ppm = 1.12 mg/m , 1 mg/m = 0.890 ppm (WHO, 2004).

Chemistry of the Detoxification
Catalyzed by linamarase, linamarin is rapidly hydrolysed to glucose and acetone cyanohydrin. Under neutral conditions, acetone cyanohydrin
decomposes to acetone and hydrogen cyanide. By storing the cassava waste water in open container, most HCN will evaporate and be rendered
innocuous.



The pH is one of the most reliable indicators of rate of fermentation. Total concentration of hydrogen ion, CH is related to pH by:

$$C_H = e^{-pH} \quad (2)$$

Varied works have been conducted on cassava fermentation/retting. Onabowale (1988) developed a combined acid hydrolysis and
fermentation processand achieved about 98% reduction in total cyanide after dehydration of the cassava flour for use in the feeding of chickens.

Ofuya et al (1989) used data from analysis of fermentation of cassava slurry to derive kinetic constants for rate of hydrolysis of bound cyanide,
formation of free cyanide, pH change and fermentation. Brahman et al (1991) studied the physico-chemistry, the biochemistry and the
microbiology of retting. To meet up with ever increasing demand for cassava based food, effort
should be geared towards viable cassava food processing. Generation of data for detoxification kinetics of cassava will encouragemechanization
that will enhance food safety and security.

MATERIALS AND METHODS

Equipment and Materials

Conical flask, test tube, ultraviolet spectrophotometer,electronic weighing balance (Model JT 502N), pipettes, screw capped bottles,beaker, glass containers, stop watch, thermometer, pH meter, bitter and sweet varieties of cassava rootswere used in this experiment.The chemical reagents employed were of analytical grade, and they include: alkaline picrate, sodium carbonate, distilled water, HCl and KCN solution.

Experimental Procedure

The fresh cassava roots (bitter and sweet varieties) were obtained from Akpugo in Enugu State, Nigeria. The processing of the cassava roots started the same day the cassava roots were harvested. The cassava roots were hand peeled, washed and sliced
into appropriate sizes. Then, the cassava roots (bitter and sweet varieties) were separately placed in different fermentation/retting
containers, adequately filled with clean water for the retting process. 0 The experiment was conducted under ambient temperature of 30 C.
The initial values of cyanide content and the pH of cassava samples were taken. Subsequent values of the properties were observed at 24
hours intervals for a period of four days. The cyanide in the roots and the retting water were recorded.

Measurement of Cyanide Content Cyanide Content

The alkaline picrate method (Williams, 1979) was used to determine the concentration of cyanide. According to the Wang and Filled Method
(alkaline picrate method), cyanide extraction with distilled water was allowed to stay overnight in a corked conical flask. The extract was filtered
and the filtrate was used for the cyanide determination in conformity of AOAC (1990).

Preparation of alkali picrate solution

1g of picrate and 5g of sodium carbonate was dissolved in a volume of minimally warm water. The volume was increased to 200ml with distilled
water.

Procedure for cyanide determination

(i) To 1ml of the sample filtrate in a corked test tube 4ml alkaline picrate was added and incubated in a water bath for 5 minutes.
(ii) After colour development the absorbance of the corked test tube was read in spectrophotometer.
(iii) Also the absorbance of the blank containing 1ml distilled water and 4ml alkaline picrate solution was read.
(iv) The cyanide content was extrapolated from a cyanide standard curve.

Preparation of cyanide standard curve
(i) Different concentrations of KCN solution containing 5 to 50ìg cyanide were prepared in a 500ml conical flask.
(ii) 25ml of 1N HCl was added.
(iii) Using the different concentrations, the cyanide standard curve was Prepared.

Empirical Modelling and Measurement of Rate Constants for cyanide reduction
a. Reaction of Linamarin with Linamarase in the cassava roots According to Conn (1979), the conversion process of Equation (1) can
be represented as:

Bound cyanide Intermediate
Free Cyanide (HCN) (3)
Where k1 and k2 are rate constants for cyanide removal.
The concentrations of cyanide (CBCN, CINT and CHCN) are related to time, t as follows:
Considering Equation (3) above, the rate of the detoxification process can
be expressed as shown in Equations (4), (5) and (6) below

(Octave, 2003):

$$BCNBCN\ CkdtdC1/_{K_1} = \quad (4) \qquad \xrightarrow{K_2}$$

$$dC_{..}/dt = k_{.}C_{BCN} - k_{.}C_{..} \quad (5)$$

INTHCN CkdtdC2/= = (6)
Where CBCNo is the initial concentration of bound cyanide and CHCN is the concentration of free cyanide at time (t), k1 and k2 are the
detoxification constants and t is time. The computer expressions for the concentration of bound cyanide, intermediate cyanide and free HCN obtained from solving Equations (4), (5) and (6) are stated below.

$$C_{BCN} = C_{BCN0} * \exp(-k_1 * t) \quad (7)$$

$$C_{INT} = C_{BCN0} k_1 * ((\exp(-k_1 * t))/(k_2 - k_1) + (\exp(-k_2 * t))/(k_1 - k_2)) \quad (8)$$

$$C_{HCN} = C_{BCN0} * (1 + (k_2/(k_1 k_2)) * \exp(-k_1 * t) + (k_1/(k_2 k_1)) * \exp(-k_2 * t)) \quad (9)$$

Trend line function in Microsoft excel aided the simulation curves that displayed the reduction constant, k1. The first order reaction of
the bound cyanide reduction was tested using the graph of Figure 3. With the consideration of the cyanide levels in the cassava roots and
retting water, the values of the k2 for both cassava varieties were obtained through iteration method. For the parameter estimation,
the known parameters are fitted into Equation (9) to obtain the unknown variables using Microsoft Excel Template. That is, with the

values of k1 and k2, the concentration of free HCN (CHCN) is obtained.If k2 is greater than k1, the rate is determine by k1 (the slowest step) and if
k1 is greater than k2, the rate is determine by k2 (the slowest step). That is, the slowest step has the greatest influence on the overall reaction
rate (Octave, 2003).

EXPERIMENTAL RESULTS AND DISCUSSION

Experimental Results

The experimental Results are presented in Table 1 and Figures 1, 2, 3, 4 and 5 below. The values of the detoxification constant (k1) are
0.378/day and 0.438/day for bitter and sweet cassava respectively. With the consideration of the cyanide level in the cassava roots and
retting water, the values of the k2(0.63/day and 0.74/day for bitter and sweet varieties respectively) were obtained through iteration
method.

Table 1 Level of cyanide content and pH for retted cassava at an average ambient temperature of 30ºC.

Time (Day)	Bitter Cassava BCN; tuber Cyanide; (Mg/L)	used water (mg/L)	pH	Sweet Cassava BCN; tuber Cyanide; (mg/L)	used water (mg/L)	pH
0	29.0	6.5		5.35	8.0	
1	20.0	8.0		4.50	5.0	4.53
2*	12.8	7.2		4.79	3.5	5.40 4.82
3	11.0	6.0		4.60	2.3	5.20

ing copious form surface scum.

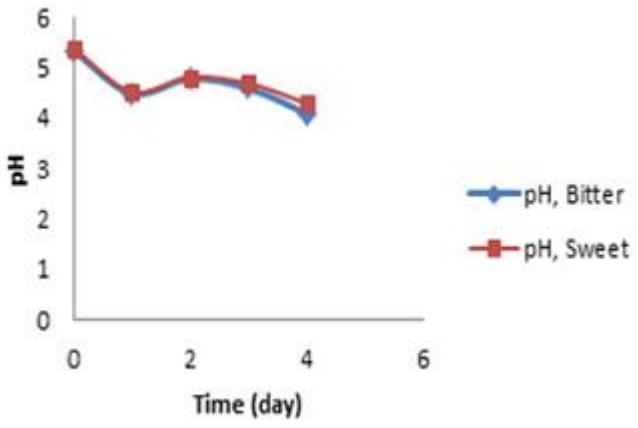


Figure 1. The pH of the bitter and sweet cassava varieties against time

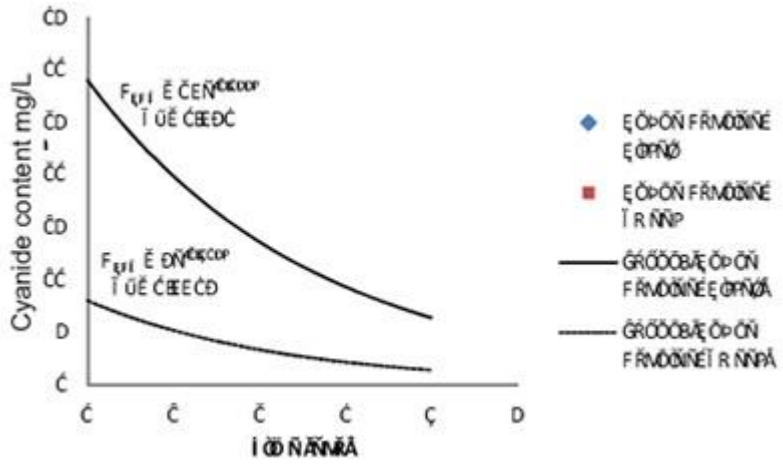


Figure 2, Graph bound cyanide of the bitter and sweet cassava varieties

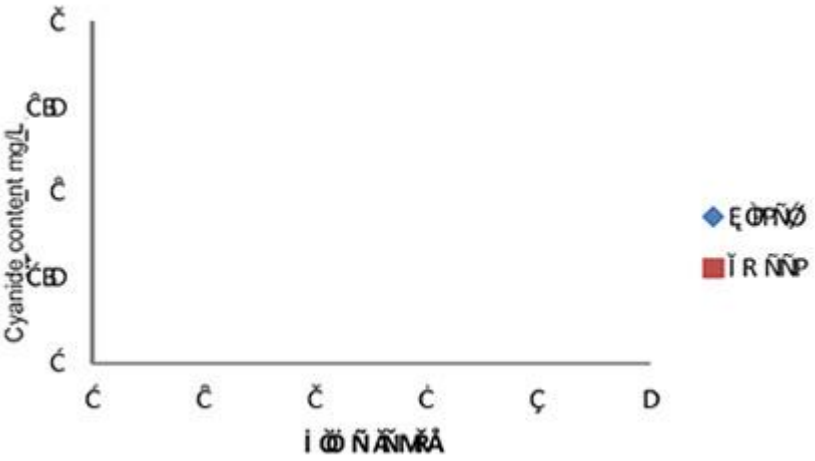


Figure 3, Test for first order reaction of the bound cyanide reduction

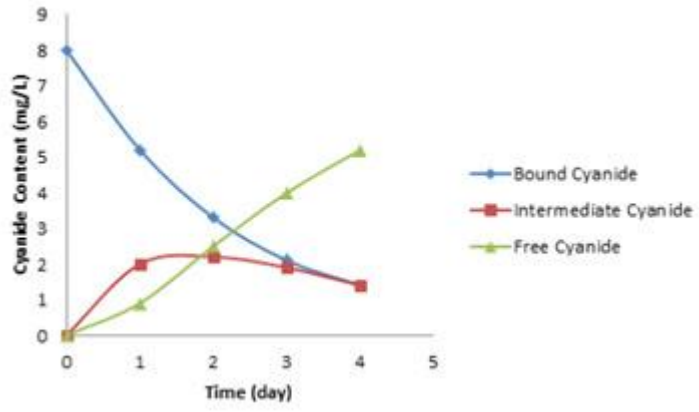


Figure 4, Variation of cyanide content (bound, intermediate and free cyanide) in the bitter cassava variety with time.

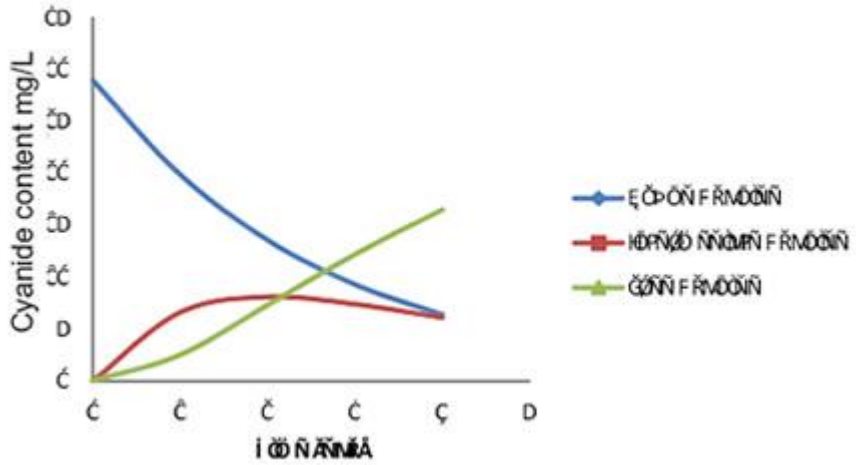


Figure 5, Variation of cyanide content (bound, intermediate and free cynide) in the sweet cassava variety with time.

DISCUSSION OF RESULTS

The result analyses are presented using table and figures. Table 1 shows the values of cyanide content and pH for retted cassava at an
average ambient temperature of 30 ºC. Frothing was noticed; cassava roots softening began on the second day of the fermentation. Retting
was considered to be over after 4 days; frothing copious formed surface scum on the fourth day. The values of the bound cyanide in the
cassava roots reduced from 29.0 mg/L and 8.0 mg/l at day 0 to 5.8 mg/L and 1.3mg/L at day 4 for bitter and sweet cassava respectively. It
shows that 80% and 83.75% of cyanide contents were reduced from the bitter and sweet cassava roots respectively.

Figure 1 presents the pH of the bitter and sweet cassava varieties against time.The pH is one of the most reliable indicators of rate of
fermentation. There was reduction in pH from day 0 to day 1. It reduced from 5.35 and 5.40 to 4.50 and 4.53 for the bitter and sweet
cassava varieties respectively. However from day 1 to day 2 there was increase in pH, which later falls to have undulating curve. This
phenomenon would be attributable to the microbial activities taking place in the retting process.

Figure 2 presents the graph of bound cyanide of the bitter and sweet cassava varieties versus time. It shows the exponential reduction of
bound cyanide with time for both cassava varieties. Trend line function in Microsoft excel aided the simulation curves that displayed
the reduction constant (k1) as 0.378/day and 0.438/day for bitter and sweet cassava varieties respectively. The first order reaction of the
bound cyanide reduction was tested using the graph of Figure 3. With the consideration of the cyanide levels in the cassava roots and retting
water, the values of the k2, 0.63/day and 0.74/day, for bitter and sweet varieties respectively) were obtained through iteration method. For
both varieties, k1, the slowest step, has the greatest influence on the overall reaction rate

(Octave, 2003). The difference in the
detoxification constants may be attributed to the difference in the cyanide content of the species. On mechanization, cassava tubers
from different species cannot be fermented with the same retting condition unless they have same or close functional properties. Bound
cyanide, intermediate cyanide and free cyanide (HCN) relationship are shown in Figure 4 (bitter variety) and Figure 5 (sweet variety). The
point at which the bound cyanide was equivalent to that of the free cyanide reflects the point of increase in microbial activities (Ofuyaet
al, 1989). Thus, the periods, 2-3 days are believed to be critical to the fermentation process for both bitter and sweet cassava varieties.

CONCLUSION

From the analysis of experimental results, the following conclusions can be drawn:

- The HCN content of cassava roots can be largely reduced by retting process at an average ambient temperature of 30ºC.
- A first order consecutive rate equation is an adequate tool for explaining the mechanism of HCN reduction (or decay) in retted cassava roots.
- The detoxification constants for the bound cyanide in the bitter and sweet cassava roots were 0.378/day and 0.438/day respectively, while that of the free hydrogen cyanide were 0.63/day and 0.74/day for the bitter and sweet varieties respectively.
- Cassava tubers from different species cannot be fermented with the same retting condition unless they have same or
close functional properties.

RECOMMENDATIONS

- The results of this work should be used to establish a basis for comparison with future studies, and with other systems similar to the hydrogen cyanide reduction.
- The detoxification constants will be useful in the design and fabrication of units for mechanization of cassava retting.

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