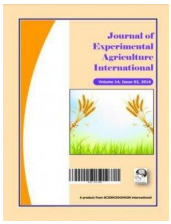

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Kunyima Procedure to Study Enzymatic Mechanism in Cassava Cyanide Removal: Case of Sassou Variety (Democratic Republic of Congo)

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABK designed the study, wrote the protocol and the first draft of the manuscript. Author PMK was the experimentalist of the results of this paper and managed the literature searches. He was the Master student in the laboratory (LACOPA). Author SNL managed the discussion and performed the statistical analysis. Author MBK managed the proof reading and correction of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Despite many publications on cyanide elimination in cassava by soaking in water, it still remains our preoccupation because the techniques elaborations of cyanide removal depend on a good uptake of phenomena. The traditional methods of cassava processing (steeping, peeling, drying) permit to eliminate a large proportion of cyanhydric acid, but the technology of the cassava treatment is still nowadays to be improved. That suggests the importance to better its processing and its production

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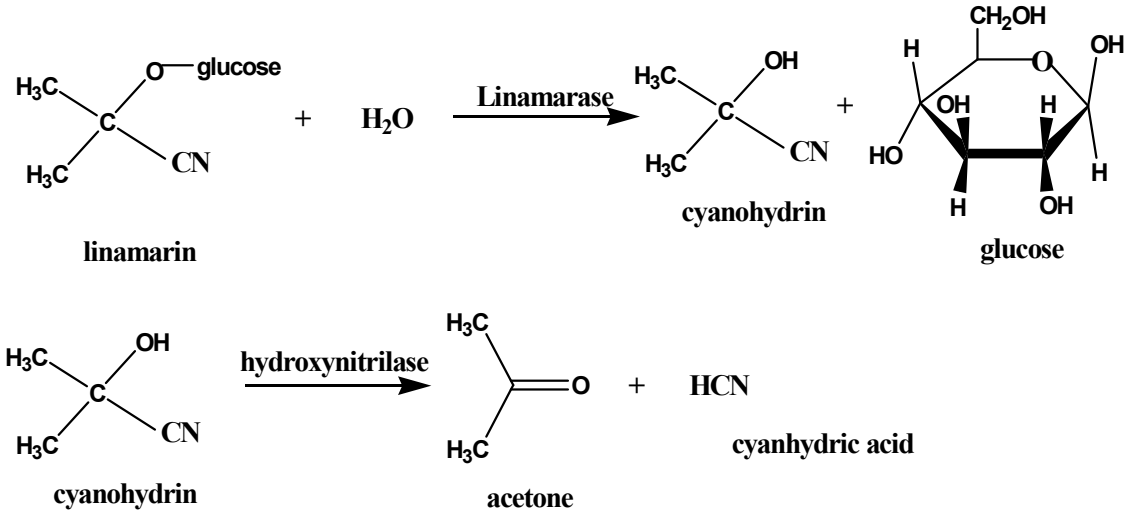
on the technological level by a suitable and reliable fast method (a few minutes) in view to eliminate the cyanide completely. So enzymatic kinetic study of a cassava variety, Sassou, in Democratic Republic of Congo, has been undertaken in this paper at temperature of 25°C by means of only pH-meter. The procedure needs to be called KUNYIMA Procedure for the rising scientific generations in order to remember the cute idea framed by Dr. Anaclet KUNYIMA B., Ordinary professor, university of Kinshasa Democratic Republic of Congo. It is needful to size a cassava reactor which will be a fast procedure of elimination of cyanide, economically payable and efficient with the production of cassava without cyanide according to the order of magnitude of the kinetic constants values found. In this paper cassava hydroxynitrilase has been successfully investigated and its K_M has been found 1.5 x 10^{-2} M; V_{max} = 0.0072 Mh^{-1}, R^2 = 0.972.

Keywords: KUNYIMA procedure; michaelis constant; cassava; hydroxynitrilase; pH-meter.

1. INTRODUCTION

The cassava which constitutes the basic food for numerous population of DRC is toxic because it contains the cyanide whose toxicity lead to the diseases such as legs paralysis (KONZO), goitre, mental cretinism, dwarfishness [1-3]. This is due to the formation hindering of T_4 (T_3), essential thyroidean hormones for the differentiation and the maturation of foetal tissues needful for the growth of the skeleton and of almost all the organs and also for the development of the central nervous system. A thyroid insufficiency started during the foetal life or at the birth entails a regression of the cortical neurons [2,4-6], that leads to the insufficiency of the brain development and its definitive lesions. Theses hormones monitor metabolism of glucids, lipids, protids, and regulate the speed of the enzymatic reactions and the temperature of the human body [5-7]. The essential toxic principle which exists in variable quantities in all the parts of the plant of cassava is a chemical compound called linamarin (93%-97%). It often coexists with its methylic homologous called methyllinamarin or lotaustralin (3%-7%) [8-12]. The linamarin is a cyanogénétic glucoside which is transformed into hydrocyanic acid (toxic) as it is shown in Scheme 1.

The elimination of the cyanide from cassava has been realized many times and continues to be realized in Democratic Republic of Congo (DRC). In certain villages the cassava is soaked in flowing river water and a great quantity of cyanide is eliminated but the processing is not economically viable because it is not rapid (time is money). In the others villages the cassava is soaked in the butts water or exposed to the sun, the result is never satisfactory. Neither simple cooking nor addition of hot water to cassava produces totally satisfaction.



Scheme 1. Mechanism of transformation of linamarin into cyanhydric acid

2. MATERIALS AND METHODS

2.1 Materials

Sassou with the following characteristics has been used [13]:

Family: *Euphorbiaceae*
Order: *Euphorbiales*
Class: Dicotyledones
Branching: Angiospermes
Gender: *Manihot esculenta crantz*
Variety: Sassou

Fig. 1. Sassou Variety, DRC (Picture taken on the sample of experimentation after one week)

pH-meter (Hanna, HI98127), a balance KERN (EMR 500-1), distillate water and Cryothermostat Julobo have been used.

The figures have been plotted by means of origin 8, Matlab 7.12.0 (R 2011a) and IBM SPSS statistic viewer 19 programs.

2.2 Methods

2.2.1 Calculations

Cassava roots were peeled and sliced. The slices of 10g of fresh Sassou have been soaked into 70 ml distillate water; the acidity of solution has been followed as function of time at 25°C. The concentrations of HCN and CN^- have been calculated as follow [14].

pH = $\frac{1}{2}\ pKa - \frac{1}{2}\log\ Ca$

$$\boldsymbol{Ca = [HCN] = 10^{(pKa-2pH)}}$$

$$\mathbf{HCN} \underset{}{\overset{\mathbf{Ka}}{\rightleftharpoons}} \mathbf{H^+ + CN^-}$$

$[HCN]$= Ca

$$K_a = \frac{[H^+][CN^-]}{[HCN]} = \frac{[H^+]^2}{Ca}$$

$$[CN^-]=[H^+] = \sqrt{Ca.Ka}$$

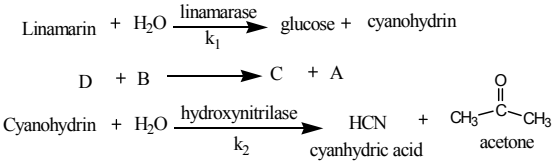
$$[CN^-]=[H^+] = \sqrt{10^{(pKa-2pH)}.10^{-pKa}}$$

$$[CN^-] = \sqrt{10^{-2pH}}$$

$$\boxed{[\mathrm{CN^-}] = 10^{-\mathrm{pH}}}$$

pKa (HCN) = 9.31 at 25°C [14,15].

- The velocity of HCN formation (v_a) and of CN^- formation (v_x) was calculated using ($v = \frac{\Delta C}{\Delta t}$)
- Enzymatic kinetic of Michaelis-Menten and Lineweaver-Burk method have been used [16,17].
- The two following reactions

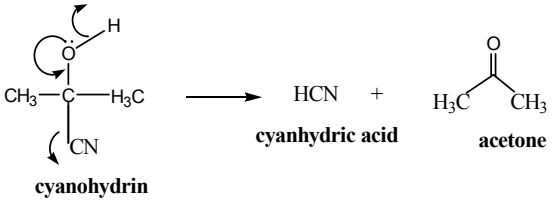


being consecutives, the last one is the limiting reaction ($k_1 > k_2$).

It has been observed that the errors on the calculated parameters are of the order of magnitude of 10^{-7}, so decision has been taken to neglect them in the tables.

2.2.2 Approximation

The contribution of lotaustralin (3%-7%) has been neglected for two reasons: the first is its presence in very small quantity and the second one is that the ethylic group on it makes it less competitive in the production of HCN. The reaction of production of HCN and acetone from only linamarin is the uppermost and promoted. Hence the large quantity of HCN, more than 90%, comes from linamarin [18].



As it can be seen stoechiometrically the [HCN] = [cyanohydrin] = [S] = [Substratum] = Ca. Globally it can be said that the quantity of HCN produced is proportional to the quantity of extractive substratum (cyanohydrin).

2.2.3 Michaelis-Menten Kinetic for a Reaction with one Substratum has been Reported

The kinetic model is based on the complex formation ES according to the following steps [16,17]:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E \quad (1)$$

A relation between the reaction velocity, both the substratum and the enzyme concentrations and individual step velocities is established. At the onset the reaction velocity is equal to $[ES]$ and the kinetic constant k_3 :

$$v = \frac{dP}{dt} = k_3[ES] \quad (2)$$

The complex formation velocity and its dissociation velocity are given by:

Formation velocity of ES = k_1[E] [S] (3)

Dissociation velocity of ES = (k_2+ k_3) [ES] (4)

Under stable state conditions:

$$k_1[E][S] = (k_2 + k_3)[ES] \quad (5)$$

By rearrangement of equation 5, one obtains:

$$[ES] = \frac{[E][S]}{(k_2+k_3)/k_1} \quad (6)$$

Michaelis constant has been defined (K_M)

$$K_M = \frac{k_2+k_3}{k_1} \quad (7)$$

(7) and (6) give rise to $[ES] = \frac{[E][S]}{K_M}$ (8)

Moreover $[S] = [S]_{total}$ if $[E] \ll [S]$

$$[E] = [E]_{total} - [ES] \quad (9)$$

$$[ES] = \frac{([E]_{total} - [ES])[S]}{K_M} \quad (10)$$

Rearrangement of equation 10 gives

$$[ES] = [E]_{total}\frac{[S]}{[S] + K_M} \quad (11)$$

(11) and (2) give rise to $V = k_3[E]_{total}\frac{[S]}{[S] + K_M}$ (12)

The maximum velocity, V_{max} , is reached when all the enzymatic sites are saturated of substratum, that means when $[S] \gg K_M$ and so $\frac{[S]}{[S] + K_M}$ tends tawards 1.

Hence $V_{max} = k_3[E]_{total}$ (13)

(13) and (12) give $V = V_{max}\frac{[S]}{[S] + K_M}$ (14)

Michaelis-Menten equation describes the kinetic curve of $V_a - [S]$ [4,16,17].

- For the weak concentrations of substratum, when $[S] \ll K_M$, $V = [S]\frac{V_{max}}{K_M}$ and the velocity is directly proportion to substratum concentration
- When $[S] \gg K_M$, $V = V_{max}$ and the velocity is independent on the substratum concentration
- The meaning of K_M is evident. When $[S] = K_M$, $V = \frac{V_{max}}{2}$. K_M is the needful substratum concentration to allow enzyme to reach $\frac{V_{max}}{2}$.

2.2.4 Solid-liquid phase extraction thermodynamics

The extraction of HCN in solid phase by means of water obeys a distribution of this HCN between two phases (solid phase and water phase) characterized by an equilibrium constant [19,20,21].

$$HCN_{solid\ phase} \rightleftharpoons HCN_{liquid\ phase}$$

In this case of extraction the equilibrium is however displaced towards the liquid phase (water). For each phase the balance equation can be written:

a) Liquid phase

$$C_{HCN_{liq.\ phase}} = X_{HCN_{free\ liq.\ phase}}$$

Where C_{HCN} is the formality of HCN and X_{HCN} is the current concentration of HCN. Given the HCN acidity constant (5.10^{-10}) is weak its dissociated fraction can be neglected.

b) Solid phase

When the extractant solvent (water) is put in contact with the solid phase two phenomena are bred:

1. Solubilisation

During this step one part of HCN remains unsolubilized that means under the bound form and one part of HCN is solubilized that means under free form near to migrate in water phase because it is known that the solubilisation is time depend phenomenon. The solubilized form has been called free species, that means detached from its stub.

2. Transfer (diffusion)

The solubilized form (free species) is transferred to water. Thus the extraction implies the solubilisation and the transfer. The balance equation in this solid phase can be written:

$$C_{HCN_{solid\ phase}} = X_{HCN\ bound\ solid\ phase} + X_{HCN_{free\ solid\ phase}}$$

There is difference between apparent equilibrium constant (K_{app}) and the true equilibrium constant (K_{true}). It should be noted that in solid phase there is unsolubilized HCN (bound) and solubilized HCN not yet transferred. So it can be imagined a relationship between K_{app} which is the ratio of the no identical species and K_{true} which is the ratio of the free species. Note that the process is working in dilute medium.

Indeed

$$K_{true} = \frac{X_{HCN\ free\ liq.\ phase}}{X_{HCN_{free\ solid\ phase}}}$$

$$K_{app} = \frac{C_{HCN_{liq.\ phase}}}{C_{HCN_{solid\ phase}}} = \frac{Ca}{C_o - Ca}$$

Where

Ca = $C_{HCN_{liq.\ phase}}$

C_o is the initial concentration obtained from the tray of the curve giving $C_{HCN_{liq.\ phase}}$ versus time.

$$K_{app} = \frac{X_{HCN\ free\ liq.\ phase}}{X_{HCN\ bound\ solid\ phase} + X_{HCN\ free\ solid\ phase}}$$

$$\frac{1}{K_{app}} = \frac{X_{HCN\ bound\ solid\ phase}}{X_{HCN\ free\ liq.\ phase}} + \frac{X_{HCN\ free\ solid\ phase}}{X_{HCN\ free\ liq.\ phase}}$$

$$\frac{1}{K_{app}} = \frac{1}{K_{true}} + X_{HCN\ bound\ solid\ phase}\ \frac{1}{X_{HCN\ free\ liq.\ phase}}$$

$$\frac{1}{K_{app}} = \frac{1}{K_{true}} + X_{HCN\ bound\ solid\ phase}\ \frac{1}{X_{HCN\ free\ liq.\ phase}}$$

$$\frac{C_o - Ca}{Ca} = \frac{1}{K_{true}} + X_{HCN\ bound\ solid\ phase}\ \frac{1}{Ca}$$

$$\frac{C_o}{Ca} = 1 + \frac{1}{K_{true}} + X_{HCN\ bound\ solid\ phase}\ \frac{1}{Ca}$$

The intercept of $\frac{C_o}{Ca}$ versus $\frac{1}{Ca}$ gives $\frac{1}{K_{true}} + 1$.

$$K_{true} = \frac{1}{b-1}$$

Where

b is the intercept

$K_{true} = e^{-\Delta G°/RT}$

$\ln K_{true} = -\frac{\Delta G°}{RT}$

$\Delta G° = -RT \ln K_{true}$

3. RESULTS AND DISCUSSION

The cassava roots generally contain dried materials (35%), glucides (89%), lipides (1%), protides (2.5%), fibres (4.5%), ash (3%), calcium (0.1%), phosphorus (0.1%), iron (0.003%), natrium (0.003%), potassium (1%), starch and linamarin [22]. The percentage of each compound varies with the nature of the soil and Cassava species.

Neither the proteins hydrolysis such as albumines, globulines, glutelines, prolamines and protamines which are all of them the globular proteins solubles in water nor starch hydrolysis giving rise to amylose (soluble) and amylopectine (unsoluble) can not be foreseen, because the above mentioned proteins are absents in cassava roots and the starch hydrolysis happens only when the hot water is used [23].Recall that in this case however the distillate water has been used at 25°C on fresh cassava and during experimentation all the solutions have been covered to avoid the influence of the air CO_2.

The phosphoric acid formation under our experimental conditions is improbable. The minerals elements present in cassava roots do not give the contribution to the formation of the observed acidity, otherwise the increase of pH would have been observed. Hence the evolution of the acidity in water with time is exclusively due to the specific enzymatic hydrolysis of the linamarin, compound giving the specific reaction with linamarase and hydroxynitrilase [22,23,24].

Table 1 shows the decrease of pH with time.

Table 1. Measured and calculated parameters as a function of time at 25°C

Time	p^H	$C_{HCN} = S =$ Ca	C_{CN^-} =X	1/S	v_a	v_x	$1/v_a$ for Ca
h	-	M	M	M^{-1}	Mh^{-1}	Mh^{-1}	$M^{-1}h$
0	6.89 ± 0.001	-	-	-	-	-	-
2	6.63 ± 0.001	0.00011	2.34E-07	9090.90909	5.5E-05	1.17E-07	0.182 E+05
4	6.35 ± 0.002	0.00041	4.46E-07	2439.02439	15.0E-05	1.06E-07	0.067 E+05
6	6.19 ± 0.001	0.00085	6.45 E-07	1176.47059	22.0E-05	0.99E-07	0.045E+05
8	5.89 ± 0.001	0.00339	12.88E-07	294.98525	127.0E-05	3.22E-07	0.008E+05
10	5.71 ± 0.001	0.00776	19.49E-07	128.86598	218.5E-05	3.31E-07	0.005E+05
12	5.56 ± 0.001	0.01549	27.54E-07	64.55779	386.5E-05	4.03E-07	0.003E+05
14	5.32 ± 0.002	0.04677	47.86E-07	21.38123	1564.0E-05	10.16E-07	0.001E+05
16	5.09 ± 0.001	0.13489	81.28E-07	7.41345	4406E-05	16.71E-07	0.00023E+05
18	4.83 ± 0.001	0.44668	148.00E-07	2.23874	15589.5E-05	33.36E-07	0.000064E+05
20	4.58 ± 0.002	1.41254	263.02E-07	0.70794	48293.0E-05	57.51 E-07	0.00002E+05
22	4.35 ± 0.001	4.07380	446.68E-07	0.24547	1.33063	91.83E-07	0.752
24	4.23 ± 0.002	7.07945	588.84E-07	0.14125	1.50283	71.08E-07	0.665
26	4.13 ± 0.001	11.22018	741.31E-07	0.08913	2.07037	76.24E-07	0.483
28	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
30	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
32	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
34	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
36	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
38	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
40	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
42	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
44	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
46	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305
48	4.03 ± 0.001	17.78279	933.25E-07	0.05623	3.28131	95.97E-07	0.305

In Table 1 the time is expressed in hours (h), the mean values of pH are used, M is the molarity of HCN and CN^- , $\mathcal{v}_a$ is the velocity of HCN formation ($\frac{\Delta C_{HCN}}{\Delta t}$) and $\mathcal{v}_x$ is the velocity of HCN dissociation ($\frac{\Delta C_{CN^-}}{\Delta t}$) or the velocity of CN^- formation

The Table 1 gives the values of pH of solution as a function of time and the calculated values of [HCN], $[CN^-]$, $\frac{1}{Ca}$, the velocity of HCN formation (v_a), velocity of CN^- formation (v_x) are indicated. When velocity of HCN formation (v_a) is compared to velocity of CN^- formation which is also the velocity of HCN dissociation (v_x) it can be seen that v_a is higher than v_x as it is plotted in Fig. 2.

This shows that HCN is effectively a weak acid, it is weakly dissociated. This behavior can also be observed in Fig. 3 where C_{CN^-} is plotted versus C_{HCN}.

Also both the velocity of HCN formation (v_a) and the velocity of its dissociation (v_x) increase with time and afterwards (28h) remain constant showing the roll of hydroxynitrilase as it can be seen in Fig. 4.

Note that the change of pH is not observed after 28hours for this variety of cassava (Sassou) where the extraction of HCN at 25°C is fast. The enzymes have selectivity and proficiency to accelerate the velocity of the formation reaction of a complex enzyme-substratum [4,17].

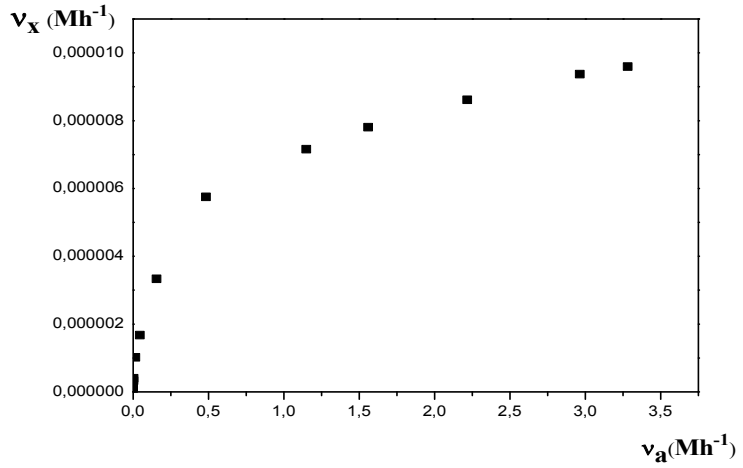


Fig. 2a. $\mathcal{v}_x$ versus $\mathcal{v}_a$

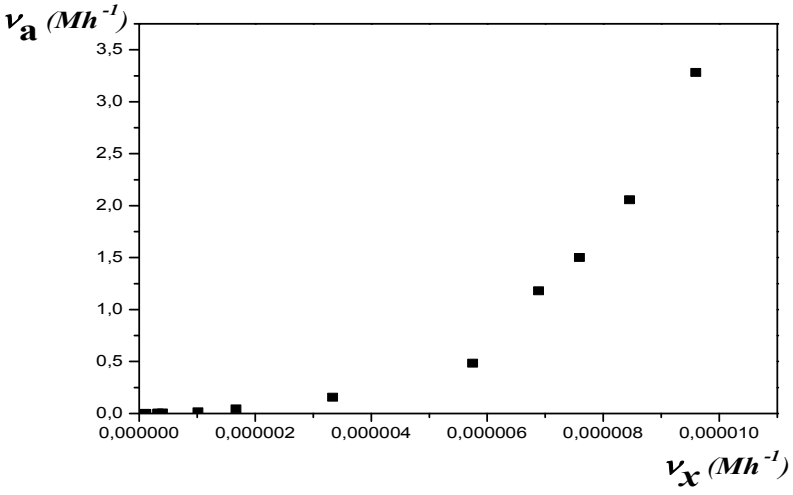


Fig. 2b. $\mathcal{v}_a$ versus $\mathcal{v}_x$

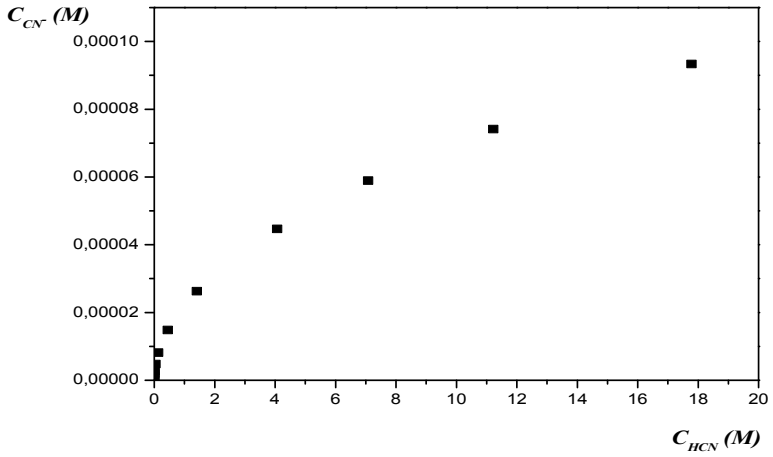


Fig. 3. C_{CN^-} versus C_{HCN}

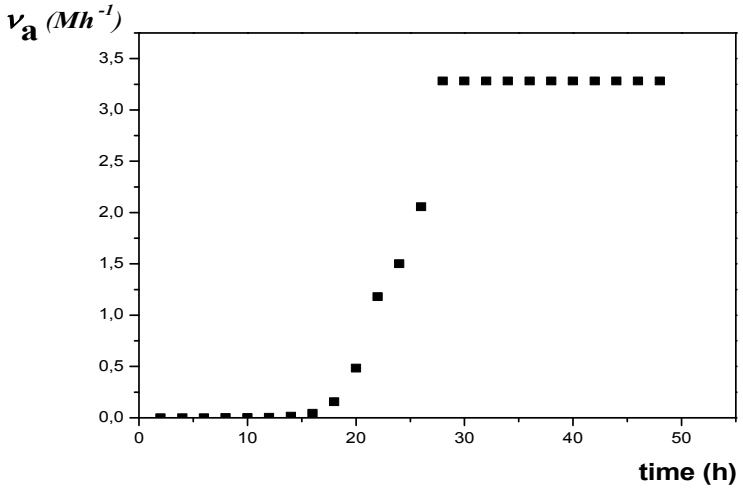


Fig. 4a. v_a versus time

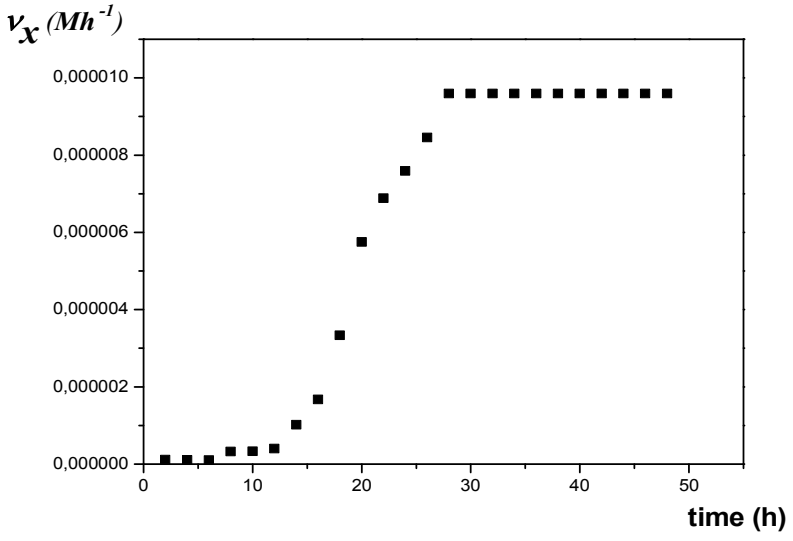


Fig. 4b. v_x versus time

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} P + E$$

Where E is enzyme and S is substratum, ES is a complex enzyme-substratum and P is the product of reaction. The formation of the complex ES gives rise to a transition state for the reaction having a low activation energetic barrier. The reaction needs less external energy contribution to happen. The critical first stage in a reaction with enzymatic catalysis is the complex formation ES. The complex ES is the reactant in the conversion step of the substratum in product, its concentration determines the reaction velocity which is maximum when all enzyme molecules are complexed by substratum.

This situation corresponds to the substratum high concentrations where the enzyme is so called saturated with the substratum. For an enzyme given concentration, the figure of initial velocity of reaction (V_a) versus substratum initial concentration $[S]$ shows a typical curve of saturation kinetic where a tray is observed at high $[S]$ when velocity V= V_{max} .

The approximation applied can be seen in Fig. 5; the curve shape predicted by the theory has been observed. The hydroxynitrilase obeys Michaelis relation.

Hans Lineweaver-Dean Burk method has been used $\frac{1}{V_a} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\frac{1}{[S]}$ as it is indicated in Fig. 5 where it can be observed that

K_M = 0.015 = 1.5 x 10^{-2} ; V_{max} = 0.0072;

$-\frac{1}{K_M}$ = - 67.457; R^2 = 0.972.

The ulterior experiments will show us the choice between the two-two sequential reaction mechanism, two-two ping-pong reaction mechanism or a reaction of enzyme with one substratum. The values of K_M is very variable, it depends on enzyme nature, substratum nature, temperature and pH.

K_M is not only a measure of the catalytic efficiency but also the enzyme affinity for its substratum. It has been reported [16] that the enzymes have properties sensitive to pH. Many proteins are active in narrow zone of pH generally comprised between 5 and 9 because pH is acting on different factors [15]:

1) Substratum-enzyme bond
2) Catalytic activity of enzyme
3) Ionisation of substratum
4) The variations of proteins structure (generally important only at utmost pH).

It has been reported that the initial velocities of many enzymatic reactions as a function of pH give rise to bell shaped curves. These curves are the reflex of the ionization state certain residues of amino acid which are in specific ionization state for the activity of the enzyme [15]. In the above mentioned case however (Fig. 6) this phenomenon is not entire because only the half bell shaped curve is observed in solid-liquid

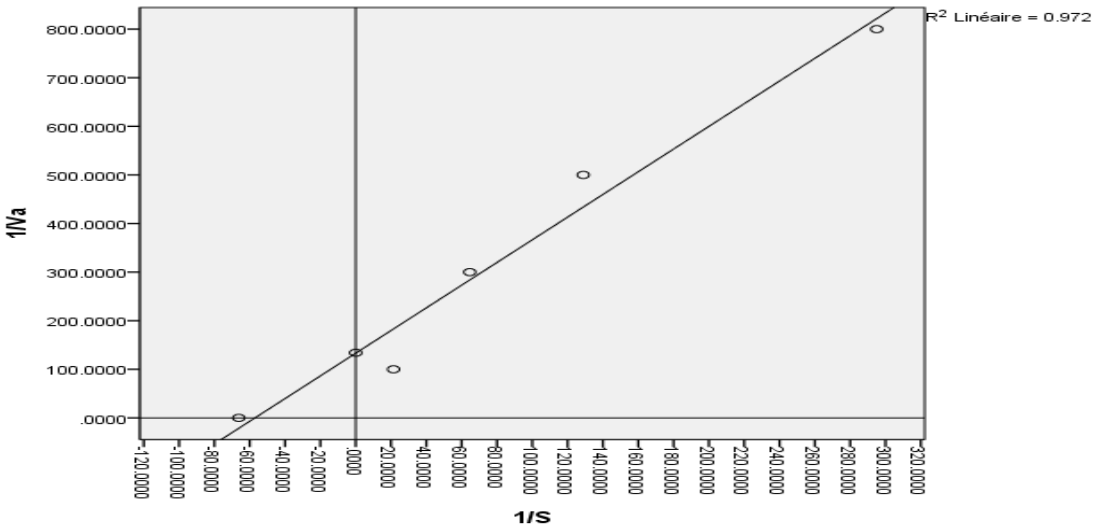


Fig. 5. $\frac{1}{V_a}$ versus $\frac{1}{[S]}$

phase extraction in the interval of the studied pH. It should be noted that the determination of enzymatic reactions mechanisms from only kinetic data are uncertains. It is necessary to resort to other experimental approaches should confirmation is needed. Experiments are continuing in laboratory and we are hopeful that in nearest future the real mechanism will be elucidated. When the HCN concentration is followed as a function of time a characteristic curve is obtained showing a tray corresponding to its extractive initial concentration (Fig. 7).

It is observed the same phenomenon for CN^- concentration as it is shown in Fig. 8.

Concerning the thermodynamics study, Table 2 gives the parameters used for the calculation of K_{true} and $\Delta G°$.

The values in Table 2 are used for the determination of the intercept, **b** as presented in Fig. 9.

According to the values of the intercept K_{true} and $\Delta G°$ are calculated.

Indeed:

$$K_{true} = \frac{1}{b-1} = \frac{1}{2.48245\ X\ 10^{-5} - 1} = 1.000025\ i^2$$

with *i* = imaginary number.

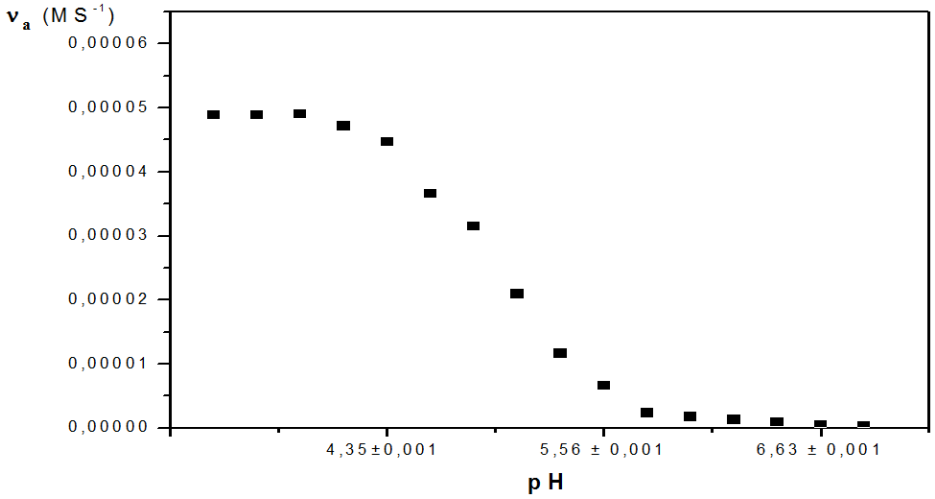


Fig. 6. V_a versus pH

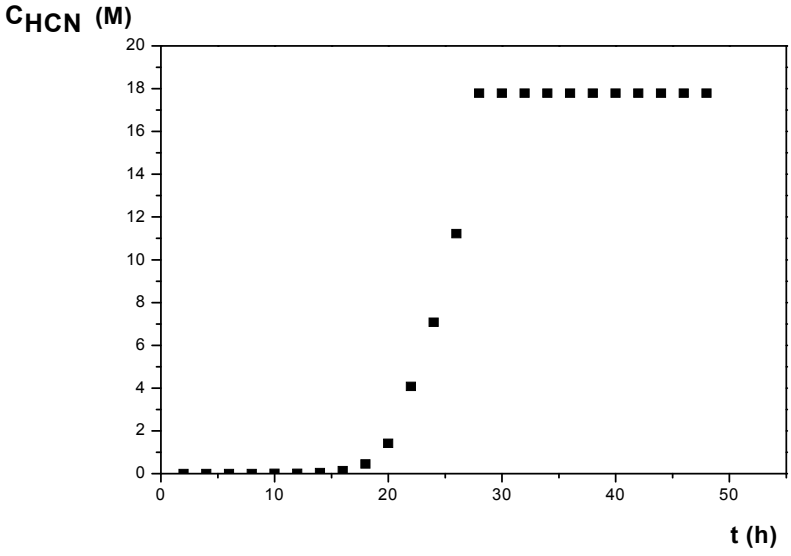


Fig. 7. C_{HCN} versus time

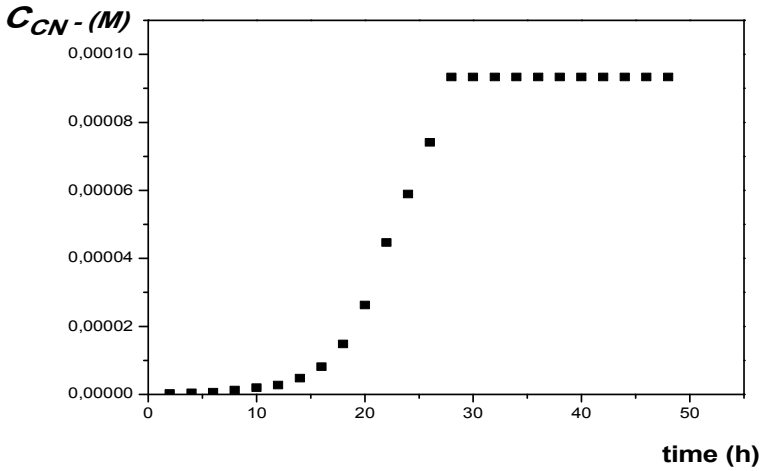


Fig. 8. C_{CN^-} versus time

Table 2. Values of C_0 (HCN), Ca, $\frac{C_0}{Ca}$, $\frac{1}{Ca}$

C_a	$\frac{1}{C_a}$	C_0	$\frac{C_0}{C_a}$
0	0	0	0
0.00011	9090.90909	17.78279	161661.72730
0.00041	2439.02439	17.78279	43372.65854
0.00085	1176.47059	17.78279	20920.92941
0.00339	294.98525	17.78279	5245.66077
0.00776	128.86598	17.78279	2291.59665
0.01549	64.55778	17.78279	1148.01743
0.04677	21.38123	17.78279	380.21788
0.13489	7.41343	17.78279	131.83179
0.44668	2.23874	17.78279	39.81103
1.41254	0.70794	17.78279	12.58923
4.07380	0.24547	17.78279	4.36516
7.07945	0.14125	17.78279	2.51189
11.22018	0.08913	17.78279	1.58489

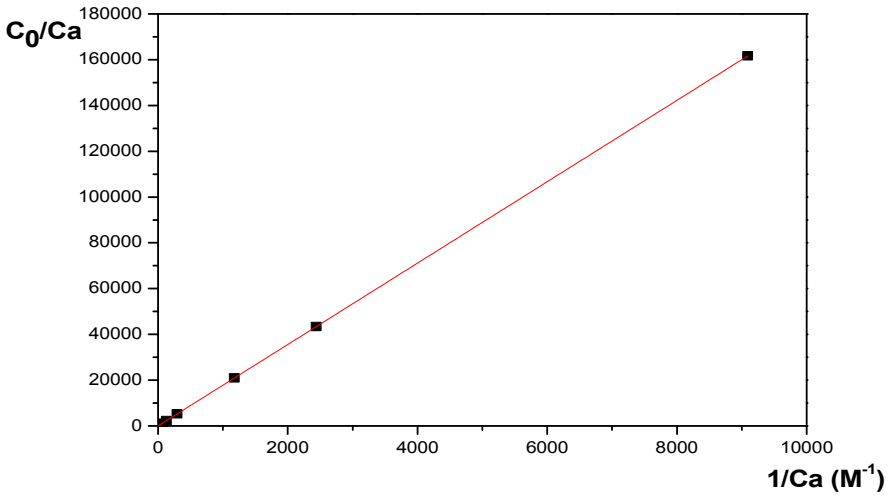


Fig. 9. C_0/Ca versus 1/Ca

$\Delta G° = - RT \ln K_{true}$
$= (- 62 \times 10^{-6} - 4.955 \ln i)$ kJ mol^{-1}.

It can be observed the existence of the negative equilibrium constant which is of course the imaginary constant meaning that the exchange of cyanide between the solid phase and water phase is so slow that no sharing equilibrium can be set up permanently between the content of those two phases.

The extraction of cassava cyanide by means of water is an irreversible phenomenon towards the water, it takes a few hours.

4. CONCLUSION

KUNYIMA procedure is a simple method proposed to deal with the enzymatic kinetic of the cyanide elimination in cassava using solely pH-meter in order to size a cassava processing reactor. It is aimed to build a fast and efficient method, economically payable (time is money) which will be a fast procedure for elimination of cyanide with the production of cassava without cyanide according to the order of magnitude of the kinetic constants values determined.

The conception of cassava cyanide removal reactor depends on kinetic parameters [25,26]. It is in this logic KUNYIMA procedure should be adopted.

This procedure will allow to distingwish the two-two sequential reaction mechanism from the two-two ping-pong reaction mechanism or the reaction of enzyme with one substratum [15].

This procedure needs to take into account thermodynamics information according which the HCN extraction from cassava roots to water at 25°C is an irreversible phenomenon towards the water. It will not be surprising to find the same observations on the other species at the other temperatures as it will be revealed in the futures papers.

Ultimately it can be said that linamarin and hydroxynitrilase are undoubtedly Michaelis enzymes [15].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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