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pH Dependence of Cyanogen Potentials of Local Wines (*Buruku and Pito*) Using Cyanomethemoglobin Complex

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ABSTRACT

The cyanogen potentials of local wines (burukutu, pito,) and the effect of pH (5.6-9.0) on the samples was determined using cyanomethemoglobin complex method. A total of 108 test samples were analyzed and the hydrogen cyanide content was found to vary considerably from 0.0313±0.0052 mg/L to 0.2444±0.0010 mg/L. pito was found to have the highest cyanogen potential with mean concentration of 0.2444±0.0010 mg/L at pH of 8.4 and burukutu found to have the lowest cyanogen potential with mean concentration of 0.0313±0.0052 mg/L at pH of 8.2. The highest mean concentration of cyanogen potential found in this study (0.2444±0.0010 mg/L) is lower than the recommended WHO and ISO standard (10mg/kg; 0.5-3.5 mg/kg body weight) and thus the samples may be consumed without the risk of cyanide poisoning.

KEYWORDS: Cyanogen, local wines, Cyanomehemoglobin, pH - dependance

I. INTRODUCTION

Cyanides comprise a wide range of compounds of varying degrees of chemical complexity, all of which contain a CN^- moiety. This group known as the cyano group consists of carbon atom triply-bonded to a nitrogen atom (C≡N). Humans are exposed to gaseous, liquid and solid forms of cyanides from a broad range of natural and anthropogenic sources. Many chemical forms of cyanide are used in industrial applications or are present in the environment. However, the cyanide anion CN^- is the primary toxic agent, regardless of origin [1]. The analysis of cyanide has become increasingly important because of its high toxicity both in its organic and inorganic compounds. Hydrogen cyanide is a very weak acid, with an acid dissociation constant of pK_a = 9.22 at 25 °C. It is soluble in water and alcohol. Hydrogen cyanide is commercially available as a gas or as a technical-grade liquid in concentrations of 5 % (v/v), 10 % (v/v) and 96-99.5 % (v/v). Phosphoric acid is added to liquid hydrogen cyanide as a stabilizer to prevent decomposition and explosion [2].

Alcoholic beverages have been used since the landing of the Pilgrims and they are non-therapeutic drugs that occupy a distinct place in our society [3]. There are three main varieties of alcohol, methyl alcohol (wood alcohol), isopropyl alcohol (rubbing alcohol) and ethanol (grain alcohol) [4]. Ethanol is the only type of alcohol that can be consumed. Commercial ethanol for consumption is prepared from various forms of starches and sugar by fermentation (palm wine is an exception, being obtained naturally from the sap of palm tree). The percentage content of ethanol in different alcoholic beverages varies, ranging from 2-4 % (v/v) in *burukutu*, 28 % (v/v) in beer and *palm wine*, and 30-60 % (v/v) in *ogogoro*, gin and brandy [5]. Alcoholic beverages have almost no food value except calories. It is a clear, colorless, mobile volatile liquid that undergoes reaction like oxidation, dehydration, esterification, reaction with metals, halogen acids or inorganic halides [5].

Ethanol affects the central nervous system, gastro-intestinal tract, cardiovascular system, endocrine, liver, lipid metabolism, foetal development and has immune suppression activity. Alcohol is absorbed into blood via the stomach and/or intestine depending on the amount consumed. About 90-98 % is metabolised in the liver to acetic acid and 2-10 % is excreted unchanged [6]. Effects of blood ethanol levels range from none to mild euphoria in 10-50 mg/dL blood concentrations to deep, possibly coma in 250-400 mg/dL blood concentrations [6]. The alcoholic content of palm wine varies depending on the degree of its fermentation. In Africa, palm wine is reported that to be consumed by over 10 million people [7]. The saps of raffia and oil palm have been widely reported to contain sugars mostly glucose and sucrose which are excellent substrates for yeast and bacteria fermentation. [7].

Palm wine contains yeast, predominantly *Saccharomyces cervisiae*, which is responsible for the fermentation process. Since palm wine has a high alcoholic content, excessive

consumption of it has both health and socio-economic implications. *Burukutu* and *pito* are some of the indigenous alcoholic beverages in Nigeria. Both are produced mainly from the grains of guinea corn (*Sorghum vulgare* and *Sorghum bicolor).*

The mechanism of human toxicity for cyanide is by absorption and depends primarily upon its potency as a respiratory poison. The cyanide forms a highly stable complex with the cytochrome oxidase of aerobic organisms and thus deactivates the enzyme and breaks the electron transport chain. That is, the cell can no longer use the oxygen which is available to it. Tissues that depend highly on aerobic respiration such as the central nervous and the heart are affected.

Meanwhile, cyanide can be more drawn to methemoglobin than to cytochrome oxidase of the cells. Once cyanide bonds to methemoglobin, cyanomethemoglobin will be formed. The increasing global pollution of the environment requires a systematic monitoring of all kinds of food and drinks including wines (both local and industrially made wines) because there may be accumulation of toxic substances such as cyanide in them. Analysis of cyanide has become increasingly important because of its toxicity in both organic and inorganic cyanide compounds. It is one of the most lethal poisons known. This research therefore, determine the cyanogen potentials of local wines (*burukutu*, *pito*, fermented and unfermented *palm wine*) and to study the effect of pH on the cyanogen potentials of the samples using methemoglobin complex.

II. MATERIALS AND METHODS

A. *Collection of Samples*

1. ***Local Wines***): The samples used in this work are *burukutu*, *pito*. The samples were purchased in Angwan Kwara, Dadin Kowa, Keffi, Nasarawa State, Nigeria.
2. ***Blood Sample):*** Blood sample of healthy chicken was collected into anticoagulant (Acid-Citrate-Dextrose; ACD) at the Chemistry Laboratory I of Nasarawa State University, Keffi, Nigeria.

B. *Sample Preparation*

1. *Preparation of Hemoglobin:* Hemoglobin was prepared using the method in the laboratory manual of the biophysical laboratory of the Department of Chemistry, University of Ibadan, Nigeria. And [8].

The blood sample collected into anticoagulant (ACD) was centrifuged at the maximum speed of 4000 - 5000 revolutions per minutes (r.p.m), the centrifuge for 20 minutes at 5 ^{o}C in order to separate the plasma from the red blood cells and the supernatant was discarded. The red blood cell undergoes v/v wash with isotonic saline (11.5 g/L NaCl) at 5 ^{o}C three times. The centrifuge was set and washing was done at maximum speed of 4000 - 5000 revolutions per minutes (r.p.m) for 20 minutes per washing.

1. ***Preparation of Methemoglobin:*** To the red blood cell obtained in section 3.2.1, v/v ice-cold distilled water was added to lyse the cells, shaken vigorously for 10 minutes and the supernatant was decanted into a clean vessel, while the cell membrane settles as residue. NaCl (5 % w/v) was then added to the supernatant and this was refrigerated for 10 minutes. It was then centrifuged for 10 minutes and the supernatant (oxyhemoglobin) was decanted.

Then, 0.33g of $K_3Fe(CN)_6$ was dissolved in 250 mL normal saline and added to the oxyhemoglobin in ratio of 2 ($K_3Fe(CN)_6$) to 1 (oxyhemoglobin) by volume. The bright red colour of heamoglobin then change to dirty brown of methemoglobin.

To the prepared methemoglobin, a few crystals of KCN was added to clear the intense colouration. The absorbance was then run using uv-visible spectrophotometer at 570 nm and the concentration was determined using Beer-Lambert's Law (states that the amount of energy absorbed or transmitted by a solution is proportional to the solutions molar absorptivity and the concentration of the solute i.e the concentration of an analyte is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the transmitted light) with some modifications (volume of solution).

2. Preparation of Reagents

1. *Preparation of Phosphate Buffer:* Phosphate buffer (PB) with ionic strength (I) of 0.05 mol/dm^3 was prepared using *0.4 M of NaH_2PO_4 (168 g NaH_2PO_4 / L), 0.4 M of $NaHPO_4$ (62.4 g $NaHPO_4$ / L), 3 M of NaCl, and 0.4 M of NaOH (16 g NaOH / L) in 1 L of distilled water and its pH was adjusted to (5.6 - 7.8) by addition of small volumes of solutions of NaH_2PO_4 and Na_2HPO_4. The quantities of the various reagents required for preparation of the buffers at pH of 5.6 – 7.8 is as shown in Table 1.*
2. *Preparation of Borate Buffer*

   Borate buffer (BB) with ionic strength (I) of 0.05 mol/dm^3 (pH 8.0 - 9.0) was prepared using 0.3 M of NaOH (10 g of NaOH / L), 0.3 M of H_3BO_3 (18.55 g of H_3BO_3), 0.2 M NaOH (8 g of NaOH / L), 0.2 M H_3BO_3 (12.37 g of H_3BO_3 / L), and 3 M NaCl in 1L of distilled water and the pH was adjusted to 8.0 - 9.0 by addition of small volumes of solutions of NaH_2PO_4 and Na_2HPO_4. The quantities of the various reagents required for the preparation of the buffers at pH of 8.0 – 9.0 is as shown in Table 2.
3. Preparation of Isotonic Saline: To prepare 0.05 M isotonic saline, it was prepared by dissolving 11.50 g of NaCl in 1 L of distilled water.
4. Preparation of Potassium Ferricyanide K_3Fe (CN)

   To prepare 0.004 M Potassium Ferricyanide, it was prepared by dissolving 0.33 g of K_3 [Fe (CN) $_6$] in 250 mL isotonic saline.

5. *Preparation of Anticoagulant (Acid- Citrate-Dextrose; (ACD)*

   Anticoagulant was prepared by dissolving 5.1 g of trisodium citrate dihydrate (TCD), 1.6 g of citric acid monohydrate (CAM), and 2.4 g anhydrous dextrose (AD) in a 200 mL standard flask and was made up to 200 mL with distilled water.

Table I: Preparation of Phosphate Buffers with Ionic Strength of 0.05 M

Ph	0.4 M NaOH (mL)	0.4 M NaH_2PO_4 (mL)	3 M NaCl (mL)
5.6	1.00	250	13.0
5.8	1.83	250	12.6
6.0	2.82	250	12.6
6.2	4.28	250	12.2
6.4	6.30	250	11.6
6.6	8.87	250	11.0
6.8	11.80	250	10.2
7.0	14.80	250	9.4
7.2	17.50	250	8.7
7.4	19.70	250	8.1
7.6	21.40	250	7.7
7.8	22.60	250	7.4

Table II: Preparation of Borate Buffer with Ionic Strength of 0.05 M

pH	0.3 M NaOH (mL)	0.3 M H_3BO_3 (mL)	0.3 M NaCL (mL)
8.0	20.0	250	14.70
8.2	29.5	250	13.70
8.4	42.7	250	12.41
8.6	60.0	250	10.41
8.8	82.0	250	8.46
9.0	107.0	250	6.56

Experimental Procedure

The concentration of the stock methemoglobin prepared was determined using Beer-Lambert's law with some modifications. The prepared methemoglobin (1.5 mL) was added to 3 mL of phosphate buffer (pH 5.6 - 7.8) and borate buffer (8.0 - 9.0) to create a working medium for cyanogen measurement. 1 mL of sample was introduced into the medium above and was allowed to stand for 24 hours to equilibrate. The sample was centrifuged at highest speed (4000-5000 r.p.m.) and the absorbance of the supernatant was recorded at 540 nm.

$$A = ElC \quad \text{----------3.1}$$

Where

A = absorbance maximum of solute

C = concentration

L = path length of cuvette

E = extinction coefficient

Beer-Lambert law with modification

$$C = \frac{A_{540}}{11.5 \times 10^4} \times \frac{V + v_1}{v_1} \quad \text{(3.2)} \leftarrow \text{Modification}$$

Where

C = concentration of cyanomethemoglobin in mole of heme per litre

A_{540} = absorbance maximum of cyanide

V = volume of buffer

v_1 = volume of cyanomethemoglobin

l = path length of cuvette

11.5×10^{-4} = extinction coefficient at that wavelength

The concentration of the cyanomethemoglobin complex formed by each sample and at each pH was then determined using a modification of Beer-Lambert's law as written in the laboratory manual of the Biophysical Laboratory of the Department of Chemistry, University of Ibadan, Nigeria. & [8].

Beer-Lambert law

The stoichiometry of the reaction:

$$Hb\ ^{+}OH_2 + CN^{-} \longrightarrow HbCN + H_2O \quad \text{(3.3)}$$

Equation (3.2) indicates that one mole of cyanide ligand will react with one mole of the heme iron. This relationship will be used to calculate the concentration of cyanide in mg/kg. The positive charge on the species $Hb\ ^{+}OH_2$ refers to the net positive charge which the iron atom carries in methemoglobin.

Equation (3.2) indicates that one mole of cyanide ligand will react with one mole of the heme iron. This relationship will be used to calculate the concentration of cyanide in mg/kg. The positive charge on the species $Hb\ ^{+}OH_2$ refers to the net positive charge which the iron atom carries in methemoglobin.

Determination of Cyanogen Contents

Using uv-visible spectrophotometer, 3 mL of deionized water was used as blank to calibrate the spectrophotometer in order to determine the absorbance of the methemoglobin at 570nm.

In the determination of the cyanogen content, 3 mL of phosphate buffers and borate buffers was used as blank.

1.5 mL of methemoglobin and 1.0 mL of sample was added to 3.0 mL of each phosphate buffer (pH 5.6 - 7.8) and borate buffer (pH 8.0 - 9.0) in a cuvette and allowed to stand for 24 hours and then centrifuged at maximum speed (4000-5000 r.p.m.) of centrifuge for ten minutes. The absorbance of the supernatant of the resulting solution was recorded at maximum wavelength of 540 nm. This was done in triplicate and the average determined. The concentration of

cyanomethemoglobin in each case was calculated using equation (3.2).

III. RESULTS AND DISCUSSION

A. Results

Table III: Concentration of Stock Methemoglobin

Absorbance I	Absorbance II	Absorbance III	Mean Concentration mg/L x 10^{-4}
0.87	0.85	0.84	0.2226

Table 4: indicates the cyanogen potentials of *pito* in mg/L with respect to pH rang (5.6-8.98).

There was no regular pattern in the mean concentration of cyanomethemoglobin with increase in pH but however, it was noticed that at pH of 8.4, under the optimal working condition of 24 hours, the highest mean concentration of cyanomethemoglobin was (0.2444±0.0010 mg/L), and there was a decrease to less than half the concentration at pH 7.8 (0.0435±0.0040 mg/L) at which the lowest cyanogen potential was recorded.

Table IV: Cyanogen Potential of *Pito* (mg/L) at pH 5.6-9.0.

pH	Mean concentration (mg/L) x 10^{-4}	S.D x 10^{-6}	% C.V	S.E x 10^{-6}
5.6	0.1870	0.6590	0.3520	0.4367
5.8	0.1835	0.3564	1.9420	0.2176
6.0	0.1053	0.1503	0.1430	0.0887
6.2	0.0548	0.0000	0.0000	0.0000
6.4	0.1261	0.1503	0.1190	0.0867
6.6	0.0565	0.8358	1.4790	0.5200
6.8	0.1713	0.4057	2.2880	0.2693
7.0	0.0800	0.2635	3.2950	0.1560
7.2	0.0583	0.1503	0.2580	0.0867
7.4	0.9820	0.1503	0.1530	0.0867
7.6	0.1088	0.1562	0.1440	0.0933
7.8	0.0435	0.4026	0.9260	0.2633
8.0	0.1983	0.5200	0.2620	0.3467
8.2	0.2148	0.8415	0.3920	0.5233
8.4	0.2444	1.0566	0.4320	0.6100
8.6	0.1513	0.2600	0.1720	0.1733
8.8	0.1626	0.1533	0.0940	0.0900
8.98	0.1522	0.1503	0.0990	0.0867

S.D = Standard deviation, S.E = Standard errors, C.V (%) = Coefficient of variation

Table V: indicates the cyanogen potentials of *burukutu* in mg/L with respect to pH range (5.6-8.98).

There was no regular pattern in the mean concentration of cyanomethemoglobin with increase in pH but however, it was noticed that at pH of 8.8, under the optimal working condition of 24 hours, the highest mean concentration of cyanomethemoglobin was (0.1739±0.0033 mg/L), and there was a decrease to less than half the concentration at pH 8.2 (0.0313±0.0052 mg/L) at which the lowest cyanogen potential was recorded.

Table V: Cyanogen Potential of *Burukutu* (mg/L) at pH 5.6-9.0

P^H	Mean concentration (mg/L) x 10^{-4}	S.D x 10^{-6}	% C.V	S.E x 10^{-6}
5.6	0.0931	0.1285	0.1381	0.7833
5.8	0.0844	0.1502	0.0170	0.0867
6.0	0.1591	0.1139	0.0716	0.6967
6.2	0.1270	0.6590	0.0519	0.4367
6.4	0.1070	0.9416	0.0880	0.6100
6.6	0.0956	0.3003	0.0314	0.1733
6.8	0.0591	0.7506	0.1270	0.4333
7.0	0.1583	0.4016	0.0254	0.2633
7.2	0.1704	0.1234	0.0332	0.7833
7.4	0.0904	0.3003	0.0332	0.1733
7.6	0.0679	0.1739	0.1678	0.6967
7.8	0.0461	0.6596	0.1431	0.4367
8.0	0.0418	0.9430	0.2256	0.6100
8.2	0.0313	0.5251	0.1678	0.3500
8.4	0.1209	0.1088	0.0900	0.6967

S.D = Standard deviation, S.E = Standard errors, C.V (%) = Coefficient of variation

Table VI: Indicates the highest and lowest concentrations of cyanomethemoglobin in each of the two samples (*pito and burukutu*) and the respective pH where concentrations were obtained.

Table VI: Concentrations of Cyanomethemoglobin of the two Samples and their Respective pH.

Sample	pH	Highest Concentration (mg/L) x 10^{-4}	pH	Lowest Concentration (mg/L) x 10^{-4}
Pito	8.4	0.2444±0.0010	7.8	0.0435±0.0040
Burukutu	8.8	0.1739±0.0033	8.2	0.0313±0.0052

Table VII: shows the lowest and highest concentrations of cyanomethemoglobin found in the overall analysis at each

pH range (5.6-8.98) and the samples in which the concentrations were found.

Table VII: Comparation of Concentrations of cyanomethemoglobin of the Samples at pH (5.6-8.98).

pH	Sample with Highest Concentration	Highest Concentration in mg/L $x10^{-4}$	Sample with Lowest Concentration	Lowest Concentration in mg/L $x10^{-4}$
5.6			*Burukutu*	0.0931±0.0012
5.8	*Pito*	0.1835±0.0035	*Burukutu*	0.0844±0.0015
6.0	*Burukutu*	0.1591±0.0011		
6.2	*Burukutu*	0.1331±0.0065	*Pito*	0.0548±0.0000
6.4	*Pito*	0.1261±0.0015		
6.6			*Pito*	0.0565±0.0083
6.8			*Burukutu*	0.0591±0.0075
7.0			*Pito*	0.0800±0.0026
7.2	*Burukutu*	0.1704±0.0012	*Pito*	0.0583±0.0015
7.4				
7.6			*Burukutu*	0.0679±0.0017
7.8			*Pito*	0.0435±0.0040
8.0			*Burukutu*	0.0418±0.0094
8.2	*Pito*	0.2148±0.0084	*Burukutu*	0.0313±0.0052
8.4	*Pito*	0.2444±0.0010	*Burukutu*	0.1209±0.0010
8.6			*Burukutu*	0.0339±0.0026
8.8	*Burukutu*	0.1739±0.0033	*Pito*	0.1613±0.0052
8.9			*Burukutu*	0.1113±0.0065

B. Discussion

In this study, two samples of locally made wines (*pito,* and *burukutu*) were analyzed for their cyanogen potentials at varied pH (5.6-8.98) using methemoglobin complex method. The cyanomethemoglobin complex formation has been employed to evaluate the cyanogens potentials of the samples.

Each of the two (2) samples analyzed, were analyzed in eighteen (18) different pH range and the analysis were conducted in triplicate making it a total of one hundred and eight (108) test samples analysed.

Comparing the results of all the two samples, the hydrogen cyanide content varied considerably from 0.0313±0.0052 mg/L to 0.2444±0.0010 mg/L.

The highest cyanogen potentials are recorded for *pito* at pH of 8.4 (0.2444±0.0010 mg/L), for *burukutu* at pH 8.8 (0.1739±0.0033 mg/L). While the lowest cyanogens potential for *pito* at pH 7.8 (0.0435±0.0040 mg/L), and for *burukutu* at pH 8.2 (0.0313±0.0052 mg/L).

Comparing all the samples analyzed, *pito* was found to have the highest cyanogen potential with mean concentration of 0.2444±0.0010 mg/L at pH of 8.4 and *burukutu* found to have the lowest cyanogen potential with mean concentration of 0.0313±0.0052 mg/L at pH of 8.2.

At each of the pH of the buffer used for this analysis, the samples with the lowest and highest concentrations and their corresponding concentrations were determined and are as shown in Table 7.

Comparing the results with the submissions of the committee on toxicity of chemicals in food, consumer products and the environment in the UK which states that 10 mg/kg are accepted standard of cyanide in the food samples (htt//cot.food.gov.UK/pdfs/cotstatement apriot 2006), the highest mean concentration of cyanomethemoglobin found in this analysis is 0.2488±0.0015 mg/Land this is lower than the standard.

More so, the World Health Organization (WHO) states maximum accepted value for cyanogens potential to be 10mg/kg [9], and International Standard Organization (ISO 2164-1975 NT) standard relating to the determination of cyanogens in leguminous plant states a sample is regarded as free from hydrogen cyanide if the sample contains a value lower than 10 mg/kg [10].

Acute lethal dose of cyanide for mammals is 0.5 mg/kg of body weight and that of human is 0.5-35 mg/kg of body weight. [9].

Therefore, knowing fully that the concentration found in all the samples of this study is far lower than 10 mg/kg, it is therefore considered that all samples are not cyanogenic.

Variation in volatility rate of cyanogenic potentials can be expected because the processes of analysis can be easily affected by parameters like temperature, concentration and pH [10]. Meanwhile, beverages (alcohols) should not be stored under adverse conditions such as in the sun as most sellers do, and certainly the storage period should not be prolonged to avoid the conversion of cyanide to urethane which is possible with subsequent reactions overtime in the presence of heat [11].

IV. CONCLUSION

The study revealed the highest cyanogen potential for *pito* at pH 8.4 to be (0.2444±0.0010 mg/L) and the lowest for *burukutu* at pH 8.2 (0.0313±0.0052 mg/L). The study has also revealed a wide range of cyanide concentration at the pH range of study for local wines (*pito,* and *burukutu*) compared with accepted standards [12], which is 10 mg/kg and 0.5-3.5 mg/kg body weight respectively. Result of this research has been shown that the samples may be consumed without the risk of cyanide poisoning.

Conflicts of Interest

The authors whose names are listed below certified that they have NO affiliations with or involment in any organization or entity with any financial such as honoraria: education grants or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs)

in the subject matter or materials discussed in this manuscript.

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