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EFFECTS OF pH AND PROCESSING ON THE CYANOGEN POTENTIALS OF IRISH POTATO (*Solanum tuberosum*) USING METHAEMOGLOBIN COMPLEX

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ABSTRACT

This study was aimed at investigating the effects of pH and processing on the cyanogen potentials of raw, cooked, parboiled, roasted and baked Irish potatoes (Solanum tuberosum) using methaemoglobin. The investigation was carried out at pH values ranging from 5.6 to 9.0 at intervals of 0.2 using a colorimeter. The results showed that the cyanide concentrations fell within the range of 1.18 ± 0.2316 mg/kg to 0.26 ± 0.034 mg/kg with raw potato having the highest concentration(1.18 ± 0.2316 mg/kg) at pH 8.6 and boiled potato having the lowest cyanide concentration (0.26 ± 0.034 mg/kg) at pH of 6.2. Among the processed samples, the roasted Irish potato recorded the highest value of cyanide concentration (1.12 ± 0.0289 mg/kg) while boiled potato maintained its

Introduction:

Irish Potato (*Solanum tuberosum*) is a crop that is widely grown in temperate regions' warmer area. It is an important food identified long ago for many communities (Breithaupt & Bamedi, 2002). Investigations have shown that Irish Potatoes are ranked as the fourth largest producing crop plant in the world, with the production capacity of over 300 million metric tons of tubers per annum (Breithaupt & Bamedi, 2002; Okonkwo *et al.*, 2002; Wuyep, 2012; Ambrose *et al.*, 2013).However, in Nigeria, research revealed

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position with the lowest value of cyanide concentration (0.26 ± 0.034 mg/kg) at pHs of 8.6 and 6.2 respectively. These values are low when compared with the maximum accepted value of 10 mg of HCN /1 kg body weight recommended by World Health Organization (WHO) and International Standard Organization (ISO). However, the study, provides information on the toxicity level of cyanide in raw and processed potatoes and therefore, suggests that the Irish potatoes may be safe for consumption using any of the above processing methods. It also shows that the processing methods can further reduce the cyanogen potentials of Irish potatoes which is in line with other literatures.

***Key words**: Irish potato, Processing, pH, Cyanide, Methemoglobin.*

That Plateau State is the major producer and marketer of Irish potatoes and that it has become an integral functions of the rural economy (Ambrose *et al*., 2013). Furthermore, Irish Potato is one of those crops that plays a vital role in nutrition diet of human since they provide the body with important vitamins and minerals. Cyanide is a chemical compound in nature that is capable of releasing cyanide ions upon reactions. Cyanide occurs naturally in plant foods in form of cyanogenic glycosides which releases hydrogen cyanide upon hydrolysis. Additionally, cyanogenic glucosides are a group of widely occurring natural substances which upon enzyme hydrolysis, produce hydrogen cyanide, glucose, ketones, or benzaldehyde (Berngner *et al.,* 2002; Onyesom *et al.,* 2008).According to *Vetter* (2000), over 2600 species of plants produce cyanogenic glucosides. Thus, the toxicity of cyanide is caused by the CN^-, which halts cellular respiration by acting as a non competitive inhibitor for an enzyme in mitochondria called cytochrome c oxidase(Ajaelu *et al*., 2008). Moreover, it has been reported that high exposure to this potent poison in humans may cause nausea, vomiting, diarrhea, dizziness, weakness, mental confusion, and convulsions followed by terminal coma and literally death (Cipollone *et al.,* 2005; Ussie *et al.,* 2007).Another studies have shown that most cases of cyanide poisoning in humans are caused by the consumption of unprocessed or partially processed foods

that contain cyanide. However, Many research works revealed that processing methods play a significant in reducing the level of cyanide contents in foods(Bradbury *et al.,* 1991; Iglesias *et al.,* 2002; Komolafe & Arawande, 2011). Simillar studies recommend that Irish Potatoes should not be consumed without processing because the active biochemical contents which includes cyanogenic glycosides in a raw potato can cause ingestion of bacteria or food borne illness that could be detrimental to human health (Nestel *et al.,* 2007).Therefore, this article investigated the effects of pH and various processing methods on the cyanogen potential of Irish Potatoes using cyanomethaemoglobin complex.

Plate 1.1: Irish potatoes (*Solanum tuberosum*)

MATERIALS AND METHODS

Analytical grade chemicals used for the research work include Citric acid monohydrate, anhydrous dextrose, Potassium ferricyanide, Sodium hydroxide, monosodium dihydrogen phosphate, Disodium Hydrogen phosphate, Boric acid and trisodium citrate decahydrate which were purchased from chemical dealers in Jos main market, Plateau State. Samples of irish potatoes and the Blood of a healthy Rabbit were purchased from Keffi main market, Nasarawa State.

Sample Preparation

Raw potato mash

The raw potatoes were washed with clean water in order to get it free of sand and other earthy materials. They were peeled and grated using a manual grater and a mash of the raw samples was developed. It was mixed with deionized water for cyanide extraction.

Roasted potato mash

Irish potatoes that have undergone dry heating via hot charcoal flame were peeled and grated using a manual grater and a mash of the roasted potatoes was developed. It was mixed with deionized water for cyanide extraction.

Cooked potato mash

Peeled potatoes that were completely cooked were grated with the aid of a manual grater and a mash of it was developed. It was mixed with deionized water for cyanide extraction.

Parboiled potato mash

Peeled potatoes that were partially boiled were grated using manual grater and a mash of the parboiled samples was developed. It was mixed with deionized water for cyanide extraction.

Baked potatoes mash

Irish potato that had undergone prolong dry heat at a very high temperature with the aid of an oven was grated using a manual grater and a mash of the baked samples was developed. It was then mixed with deionized water for cyanide extraction.

Extraction of Liquor

The grated sample (5 g) of the raw potato that was steeped into a deionized water was kept for 24 hours and its liquor was extracted. Similar

procedure was carried out on the cooked, baked, parboiled, and roasted samples and their respective liquor was extracted (Ajaelu *et al.,* 2008).

Experimental Procedure

Preparation of Buffer Solutions

Phosphate and Borate buffers at pH of 5.6 – 7.8 with ionic strength 0.05 mol/dm^3 and pH of 8.0 – 9.0 with ionic strength 0.05 mol /dm^3 were prepared respectively using the method of Howard and Denton (2014).

Preparation of Hemoglobin

The blood sample collected in anticoagulant (Acid Citrate Dextrose) went through centrifugation at 1000 rpm for 20 minutes at 5 ^{o}C. This was carried out to separate the plasma from the red blood cells. The plasma (supernatant) was discarded leaving behind the red blood cells. The red blood cell produced were washed using v/v isotonic saline (9.0 g/L NaCl) at 5 ^{o}C, a process that was repeated in two more times. Centrifugation activities were set at maximum frequency between 4000-5000 revolutions per minute (r.p.m.) for 20 minutes per washing (Beetlestone & Irvine, 1964).

Preparation of Methemoglobin (1.690 X 10 $^{-3}$ M)

Ice-cold distilled water was added to the prepared red blood cells in order to lyse the cells. The mixture was shaken vigorously for 10 minutes and the supernatant was decanted into a clean vessel, leaving the (cell membrane) residue behind. Sodium chloride salt (5 % w/v) was added to supernatant and the mixture was refrigerated for 10 minutes. It was centrifuged for 10 minutes and the supernatant (oxyhemoglobin) was decanted to a clean vessel (Beetlestone & Irvine, 1964), $K_3Fe(CN)_6$ 0.004 mol/dm^3 was added to the oxyhemoglobin in ratio of 2:1 by volume. The bright red colour of hemoglobin at this point was changed to dirty brown colour of methemoglobin. To the prepared methemoglobin, a few crystals of KCN was added to clear the intense coloration. In order to determine the concentration of the methemoglobin, 0.1 cm^3 of the raw methemoglobin was added to 3 cm^3 of distilled water and the absorbance of the solution

was taken at maximum wavelength of 570 nm and its corresponding concentration was determined using a modification of Beer- Lambert's law (Ajaelu *et al.,* 2008).

$$C = \frac{A_{570}\, X 10^{-4}}{11.5} X \frac{V + V_i}{L\, X\, V_i} \quad \text{---} \quad 1.1$$

Where C = concentration of methemoglobin in mole heme per litre, A_{570} = Absorbance maxima of methemoglobin,V = Volume of distilled water,V_i = Volume of methemoglobin,

L = Path length of cuvette and 11.5 x 10^4 = extinction coefficient at that wavelength.

The stoichiometry of the reaction is $Hb^{+}OH_2 + CN \qquad HbCN + H_2O - - - 1.2$

From the equation (1.2), one mole of cyanide ligand reacts with one mole of heme iron. This relationship was used to calculate the concentration of cyanide in mg/kg. The positive charges on the species Hb^{+} OH_2 refer to the net positive charge which the iron atom caries in methemoglobin (Ajaelu *et al*., 2008).

Determination of Cyanogen Contents of the Samples

In the determination of cyanide contents in the samples prepared, 1.0 cm^3 of the liquor from each sample was introduced into 0.5 cm^3 of MetHb plus 3 cm^3 each of phosphate buffers (pH 5.6 – 7.8), and borate buffers (pH 8.0 – 9.0) in a cuvette. The mixture was allowed to stand for 24 hours to establish equilibrium.

The mixture was centrifuged and the absorbance of the supernatant was determined colorimeter. This was carried out for each sample at various pH in triplicates (Ajaelu *et al*., 2008).The absorbance was carried out in triplicate each at maximum wavelength of 520 nm. The concentration of the cyanomethemoglobin complex formed by each of the sample was determined using Beer-Lambert's law and its modifications (Ajaelu *et al*., 2008).

$$C = \frac{A_{520}\, X 10^{-4}}{11.5} X \frac{V + V_i}{L\, X\, V_i} \quad \text{---} \quad 1.3$$

Where C = concentration of cyanogen in mg/kg, A_{520} = Absorbance maxima of cyanide,
V = Volume of buffer solution, V_i = Volume of liquor and methemoglobin, L = Path length of cuvette and 11.5 x 10^4 = extinction coefficient at that wavelength

RESULT AND DISCUSSION

In this research work, the complex of cyanomethemoglobin formed was used to evaluate the cyanogen potentials of raw and the processed Irish potatoes.Tables 3.1-3.5 show the variations in the cyanide concentrations of each sample at different pHs while table 3.6 shows the summary of the mean cyanide concentration for the entire samples at a particular pH and the mean cyanide concentration of each sample at the pH range(5.6-9.0). The investigation was carried out with pH varying from 5.6 to 9.0 at 0.2 intervals.The variations in the concentrations as estimated and recorded in the tables(3.1-3.6) were likely to be accounted for by the differences in the degrees of temperature which varied in direct proportion to the sources, medium and the quantity of heat energy gained by the system and the pH (Adindu *et al.,* 2003). From the results display in Table 3.6, highest mean cyanide concentration was recorded in raw potato to be 0.83 mg/kg and the lowest mean concentration was recorded in boiled potato to be 0.53 mg/kg. The mean concentration value discovered for parboiled potato was 0.81 mg/kg which is closed to 0.83 mg/kg recorded for raw potatoes (Table 3.6). The mean cyanide concentrations for the boiled potato was the lowest at 0.53 mg/kg followed by that of the baked Irish potatoes and then roasted potato with their mean cyanide concentrations measured as 0.56 mg/kg and 0.80 mg/kg respectively (Table 3.6). Generally, the mean cyanide concentrations with respect to pH variations appeared relatively low at the acidic medium and high at the basic medium (Table 3.6). This outcome is due to the fact that at lower pH, the solution tends to yield un-dissociated hydrogen cyanide (HCN) form which resulted into less free cyanide ions (Koenig, 2014). The major differences observed in the mean cyanide concentrations estimated for the raw Potatoes (0.83 mg/kg) and in the boiled sample (0.53 mg/kg) (Table 3.6) is probably due to the

residual concentration of alkaloid in the unprocessed sample and considerably low in boiled potatoes due to the fact that boiling (which usually requires more water, greater heat and longer time for the entire operations) is one of the traditional ways of reducing toxicity in alkaloid dominant foods (Abiona *et al*., 2002). Thus, from the results obtained (Table 3.1), it could be inferred that the variations in the mean cyanide concentrations estimated for the raw Potatoes and from one processed sample to the other are due to several parameters which include temperature, concentration and pH most especially the temperature (Akon *et al.,* 2010).This result also agrees with the work done by Ajaelu *et al*. (2008) on the use of methaemoglobin complex in estimating the cyanogenic potentials of cassava and cassava products to ascertain the effects of different processing and at different pHs on the cyanogenic potentials. The cyanide content in mg/kg recorded in cassava mash, pre-fufu, fufu and gari was estimated to be in the range of 14.68 – 9.87 mg/kg respectively. These concentrations are much higher when compared to the values obtained in the raw and processed potatoes (Raw potato, Roasted potato, Parboiled, Baked, Cooked) with cyanogen concentrations ranging from 0.85- 0.53 mg/kg.

Table 3.1: Cyanogen potentials (mg/kg) of raw *Solanum tuberosum* at pH 5.6- 9.0

pH	Mean Concentration x 10^{-4}	S.D x 10^{-6}	C.V	S.E
5.6	0.48	0.0959	0.2011	0.0610
5.8	0.76	0.2975	0.3924	0.0830
6.0	0.64	0.1382	0.2161	0.0870
6.2	0.71	0.2850	0.4022	0.0890
6.4	0.81	0.2272	0.2792	0.0830
6.6	1.07	0.0556	0.0519	0.0370
6.8	0.86	0.0791	0.0915	0.0530
7.0	0.78	0.1679	0.2165	0.1120
7.2	0.79	0.3291	0.4185	0.0630
7.4	0.81	0.0586	0.0734	0.0390
7.6	0.82	0.5259	0.6445	0.3270

7.8	0.85	0.3166	0.3709	0.1010
8.0	0.74	0.0965	0.1304	0.0630
8.2	0.76	0.1246	0.1633	0.0831
8.4	1.11	0.1250	0.1137	0.0832
8.6	1.18	0.2316	0.1967	0.1501
8.8	1.00	0.3799	0.4176	0.2312
9.0	0.79	0.05007	0.6294	0.0104

S.E = Standard error C.V= Coefficient of variation S.D= Standard deviation

Table 3.2: Cyanogen potentials (mg/kg) of boiled *Solanum tuberosum* at pH 5.6-9.0

pH	Mean Concentration x 10^{-4}	S.D x10^{-6}	C.V	S.E
5.6	0.45	0.0034	0.0077	0.0200
5.8	0.46	0.1790	0.3870	0.0530
6.0	0.48	0.0468	0.0970	0.3060
6.2	0.26	0.0034	0.0135	0.0200
6.4	0.45	0.0947	0.2102	0.0400
6.6	0.77	0.1160	0.1503	0.0700
6.8	0.52	0.0957	0.1844	0.0900
7.0	0.64	0.2929	0.4601	1.7660
7.2	0.65	0.1945	0.2970	1.2960
7.4	0.74	0.1186	0.1593	0.7900
7.6	0.61	0.6636	0.0940	0.0600
7.8	0.98	0.1510	0.1539	0.9331
8.0	0.83	0.1791	0.2163	0.0531
8.2	0.76	0.1392	0.1828	0.0731
8.4	0.94	0.1112	0.1770	0.6701
8.6	0.88	0.1751	0.1990	0.1330
8.8	0.90	0.0575	0.0637	0.4061
9.0	0.94	0.0386	0.0411	0.0231

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S.E = Standard error ,C.V= Coefficient of variation SD= Standard deviation

Table 3.3: Cyanogen potentials (mg/kg) of baked *Solanum tuberosum* at pH 5.6-9.0

pH	Mean Concentration x 10^{-4}	S.D	C.V	S.E
5.6	0.38	0.0317	0.0844	0.0183
5.8	0.38	0.0652	0.1702	0.0403
6.0	0.38	0.1759	0.4722	0.1163
6.2	0.38	0.0366	0.0943	0.0243
6.4	0.37	0.1160	0.3099	0.0730
6.6	0.56	0.1049	0.1856	0.0691
6.8	0.52	0.0920	0.1768	0.0547
7.0	0.53	0.0480	0.0916	0.0303
7.2	0.51	0.1368	0.2682	0.0893
7.4	0.63	0.5875	0.9558	0.0121
7.6	0.58	0.1236	0.2125	0.0793
7.8	0.79	0.1179	1.4885	0.1870
8.0	0.59	0.0035	0.0059	0.0020
8.2	0.53	0.0184	0.0346	0.0120
8.4	0.49	0.0314	0.0640	0.0123
8.6	0.63	0.2073	0.3290	0.1217
8.8	0.66	0.0164	0.0419	0.0183
9.0	0.66	0.0275	0.0612	0.0034

S.E = Standard error ,C.V= Coefficient of variationS.D= Standard deviation

Table 3.4: Cyanogen potentials (mg/kg) of roasted *Solanum tuberosum* at pH 5.6-9.0

pH	Mean Concentration x 10^{-4}	S.D x 10^{-6}	C.V	S.E
5.6	0.64	0.0455	0.0709	0.3030
6.0	0.65	0.3869	0.5856	0.2253
6.2	0.65	0.0387	0.0568	0.2253
6.4	0.66	0.5583	0.8508	0.2223
6.6	0.68	0.1976	0.2116	0.3223

6.8	0.93	0.2950	0.4692	0.1317
7.0	0.63	0.1256	0.1840	0.1703
7.2	0.69	0.6644	0.8412	0.4041
7.4	0.79	0.0563	0.0685	0.0343
7.6	0.82	0.0970	0.1033	0.0571
7.8	0.94	0.1304	0.1169	0.0753
8.0	1.12	0.1176	2.1145	0.2760
8.2	1.01	0.3113	0.2858	0.2027
8.4	1.11	0.1682	0.1446	0.1030
8.6	1.02	0.0289	0.0428	0.0183
8.8	0.61	0.2772	0.4541	0.1241
9.0	0.60	0.3017	0.5086	0.2011

S.E = Standard error,C.V= Coefficient of variation S.D= Standard deviation

Table 3.5: Cyanogen potentials (mg/kg) of parboiled *Solanum tuberosum* at pH 5.6-9.0

pH	Mean Concentration x 10^{-4}	S.D x 10^{-6}	C.V	S.E
5.6	0.74	0.0455	0.0709	0.303
5.8	0.71	0.0064	0.9031	0.0630
6.0	0.68	0.0003	0.5060	0.0020
6.2	0.77	0.3494	0.4535	0.2021
6.4	0.67	1.1026	0.5988	0.6490
6.6	0.93	0.1744	1.5988	0.1010
6.8	0.87	0.0826	0.1876	0.0524
7.0	0.86	0.0985	0.0948	0.0651
7.2	0.81	0.1141	0.1141	0.0671
7.4	0.95	0.2024	0.1406	0.1250
7.6	0.82	0.1443	0.2132	0.0831
7.8	0.86	0.1954	0.1559	0.1152
8.0	0.77	0.2620	0.2268	0.1561
8.2	0.94	0.2220	0.1678	0.1581
8.4	0.98	0.0357	0.3651	0.0224

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8.6	1.05	0.0600	0.0692	0.0441
8.8	0.84	0.3113	0.3722	0.1814
9.0	0.69	0.2161	0.3222	0.1270

S.E = Standard error, C.V= Coefficient of variation S.D= Standard deviation

Table 3.6: Mean cyanide concentrations (mg/kg) of raw and processed *Solanum tuberosum* at pH 5.6-9.0

pH	Raw	Parboiled	Roasted	Baked	Boiled	Mean
5.6	0.78 ± 0.06	0.74 ± 0.10	0.64 ± 0.06	0.38 ± 0.11	0.45 ± 0.03	0.54
5.8	0.76 ± 0.02	0.71 ± 0.04	0.58 ± 0.01	0.38 ± 0.10	0.46 ± 0.01	0.58
6.0	0.64 ± 0.01	0.68 ± 0.03	0.65 ± 0.03	0.38 ± 0.02	0.48 ± 0.02	0.57
6.2	0.71 ± 0.06	0.77 ± 0.01	0.65 ± 0.01	0.38 ± 0.03	0.26 ± 0.03	0.55
6.4	0.81 ± 0.10	0.67 ± 0.02	0.66 ± 0.02	0.38 ± 0.01	0.45 ± 0.02	0.59
6.6	1.07 ± 0.12	0.93 ± 0.11	0.68 ± 0.01	0.56 ± 0.03	0.76 ± 0.04	0.80
6.8	0.86 ± 0.01	0.87 ± 0.10	0.93 ± 0.07	0.52 ± 0.01	0.52 ± 0.01	0.74
7.0	0.78 ± 0.04	0.86 ± 0.03	0.63 ± 0.04	0.53 ± 0.02	0.62 ± 0.04	0.69
7.2	0.79 ± 0.13	0.81 ± 0.01	0.69 ± 0.03	0.51 ± 0.03	0.61 ± 0.01	0.69
7.4	0.81 ± 0.08	0.95 ± 0.07	0.79 ± 0.01	0.63 ± 0.01	0.72 ± 0.06	0.78
7.6	0.82 ± 0.06	0.82 ± 0.10	0.82 ± 0.10	0.58 ± 0.06	0.61 ± 0.03	0.73
7.8	0.85 ± 0.03	0.86 ± 0.02	0.94 ± 0.12	0.79 ± 0.04	0.92 ± 0.02	0.88
8.0	0.74 ± 0.01	0.77 ± 0.01	1.12 ± 0.14	0.60 ± 0.01	0.83 ± 0.01	0.81
8.2	0.76 ± 0.02	0.94 ± 0.03	1.01 ± 0.01	0.53 ± 0.02	0.76 ± 0.05	0.60
8.4	1.11 ± 0.07	0.98 ± 0.04	1.11 ± 0.08	0.49 ± 0.01	0.94 ± 0.02	0.93
8.6	1.18 ± 0.23	1.05 ± 0.08	1.12 ± 0.03	0.65 ± 0.10	0.88 ± 0.07	0.97
8.8	1.00 ± 0.11	0.84 ± 0.06	0.61 ± 0.02	0.66 ± 0.02	0.90 ± 0.10	0.80
9.0	0.79 ± 0.04	0.67 ± 0.01	0.60 ± 0.10	0.66 ± 0.06	0.94 ± 0.04	0.73
MS	0.85 ± 0.06	0.83 ± 0.05	0.80 ± 0.04	0.56 ± 0.04	0.53 ± 0.03	

The results are expressed as the mean(M) ±standard deviation(S) of 3 determinations

Conclusion

This study has uncovered the variations in the concentrations of cyanide in different processed forms of Irish potatoes at different pHs. The raw and the processed potatoes were found to have different cyanide concentrations. Thus, highest cyanide concentration(1.18 ± 0.0231 mg/kg) was recorded in raw potato at pH 8.6 and lowest concentration (0.26 ± 0.034 mg/kg) was recorded in boiled potato at pH 6.20. From the result, it can be concluded that high concentrations appeared more frequently in basic medium of the solution than in the acidic medium. Also, the processing methods used in this study, play a vital role in reducing the level of the cyanide concentrations in the processed samples. However, when compared with the maximum accepted standard of 10 mg/kg recommended by WHO (2012), it can be inferred that the Irish Potatoes may be consumed through any of these processing methods without creating any risk associated with cyanide to human health.

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