Reduction of Hydrogen Cyanide Content from Cassava (*ManihotesculentaCrantz*)

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Abstract

Cassava (*ManihotesculentaCrantz*) is the most important food source in the tropics after rice and maize. The root is one-third of starch, 60% to 70% of water and 5% to10% of bark, peel and fibrous central cords. Cassava has three major deficiencies, poor shelf-life, low content of protein and free amino acids and high content of the poisonous cyanogenic glycoside. In cassava, the main cyanogenic glycoside is linamarin, while a little amount of lotaustralin is also present, as well as an enzyme linamarinase. These enzymes decompose to hydrogen cyanide. Traditional food processing methods such as boiling, soaking, fermentation and oven-drying reduce the hydrogen cyanide content. The major objective is to reduce the natural toxic compound (hydrogen cyanide) from harvestedcassava (*ManihotesculentaCrantz*) for food consumptions. In the present research work, harvested cassava was purchased from Inntakaw Township, Bago Region. The cassava was boiled, soaked with water and dried in an oven to reduce the HCN content. The harvested cassava was also fermented with salt (NaCl) and water to reduce HCN content. The hydrogen cyanide (HCN) content was determined by picrate method (UV- spectrophotometer) for quantitative test. The phytochemical examination and nutritional values of raw cassava and prepared cassava after processing methods were also studied. The factors affecting the reduction of HCN content of harvested cassava such as volume of water, boiling time, soaking time, fermentation time, drying time, and drying temperature were studied. The maximum content of HCN from cassava (300g) was reduced to 85.1±8.4ppm with 1000ml of water volume for 7days of fermentation time by using fermentation method.

Keyword: Cassava, Cyanogenic Glycosides, Hydrogen Cyanide, Traditional Processing Methods

Introduction

Fresh fruits and vegetables are an important section of healthy diets, but several fruits and vegetables contain small amounts of natural toxic compounds. (CFIA,2017) Food plants contain many compounds that can pose potential risks to consumers and one of these types of substances is cyanogenic glycosides. (Islamiyat, 2016) The fruits and vegetables themselves are not toxic. The presence of cynanogenic glycoside alone is not dangerous. Cyanogenic glycosides are chemical compounds contained in foods that release hydrogen cyanide, which is poisonous to humans. (CFIA, 2017) The toxicity of cyanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide. The toxicity of cyanogenic glycosides is associated with their ability to be hydrolyzed either spontaneously or in the presence of enzyme to produce cyanide as end products of their hydrolysis. Cyanogenic glycosides or cyanoglycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. (Islamiyat, 2016)Hydrogen cyanide may be toxic to humans and animals, and the severity of the toxicity depends on the quantity consumed. (CAC/RCP 73, 2013) In general, plants having cyanogen content above 20mg/100g of fresh plant material are considered harmful for human health. (Kanchan,2016) Cyanide can be lethal to humans and the acute dose is in the region of 1mg/1kg body weight. (Speijers,1993) Some journal stated that the lethal dose of cyanide ranges from 0.5 to 3.0mg per kilogram of body weight.(CFIA,2017) The level of cyanogenic glycosides produced is generally depending upon the age, the variety of the plant, plant part, environmental factors and climatic conditions and degree of processing. (Speijers,1993)Symptoms of cyanide toxicity in humans have been reported to include vomiting, stomach ache, diarrhea, convulsion and in severe case death.(Islamiyat, 2016)

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Literature Review

Cassava (*ManihotesculentaCrantz*)

Cassava roots and leaves cannot be consumed as they contain two cyanogenic glycosides linamarin (96%) and lotaustralin (4%).(Priya Kali Dhas) The major cyanogenic glycoside is linamarin in cassava, as well as an enzyme linamarinase. Linamarin is rapidly hydrolysed andcatalyzed bylinamarinase, to glucose and acetone cyanohydrin.Lotaustralinis hydrolysed to a related cyanohydrin and glucose. Under neutral conditions, acetone cyanohydrin decomposes to acetone and hydrogen cyanide.(FSANZ,2004)

Traditional Processing Methods of Hydrogen Cyanide Reduction

Food processing procedures will reduce the levels of hydrogen cyanide before consumption through the action of plant enzymes. (Speijers,1993) Cyanide is volatile, during processing techniques will volatilize the remaining cyanide to low level. (Islamiyat, 2016)Boiling or cooking, cell walls rupture which permit leakage of cell content including antinutrients and toxic substances.(Kanchan,2016). The solubilization of cyanogenic glycoside from the small chips into the large volume of water seemed to better explain the cyanogen removal than enzymatic degradation. (Julie,2008,2009)Soaking method is quite effective in eliminating cyanogens particularly which have low content. The decrease in cyanogen also depends on some factors like temperature, time and soaking medium in which the materials is soaked.(Kanchan,2016)Drying is also an appropriate processing method for removal of cyanogenic glycosides in food plants up to the permissible limits. (Kanchan,2016) The efficiency of cyanide removal during drying is depending on moisture content of the roots.(Islamiyat, 2016)The cyanogen removal process can be improved by increasing the soaking and fermentation times.(Julie,20082009) Fermentation is allowed to progress beyond this time, the food mash becomes acidic (sour taste) and the acidity retards the spontaneous dissociation of the cyanohydrins and fixes them in the food. (CAC/RCP 73,2013)

Materials and Methods

Raw Materials

Harvested cassava was purchased from Inntakaw Township, Bago Region. Picric acid (BDH chemicals Ltd. England), sodium bicarbonate (J.T.BAKER CHEMICAL CO., PHILLIPSBURG, N.J. 08865), phosphatebuffer solution(pH-7) and chloroform (JDH chemicals Ltd. China) are analar grade.

Methods

Traditional processing methods (Boiling, soaking, oven-drying and fermentation)were used to reduce the hydrogen cyanide (HCN) content.The determination of HCN content was analyzed by picrate method for quantitative test.

Reduction of Hydrogen Cyanide Content from Cassava by Using Boiling Method

The cassava was peeled and washed with water. The washed raw material was cut into small size (0.5cm thick) and 100g of material was mixed with optimum volume of distilled water, into 2L of pot. The pot was put onto the electric stove, and boiled at 100ºC (±2ºC) for optimum boiling time, to reduce hydrogen cyanide content from harvested cassava. The boiling time was changed at every 15minutes and the volume of water was changed with interval of 200ml. HCN content was measured at every 15minutes of boiling time and with interval of 200ml of water volume. The reduced amount of HCN content in processed materials was recorded.

Reduction of Hydrogen Cyanide Content from Cassava by Using Soaking Method

The small size cassava(100g) was mixed with optimum volume of distilled water, into plastic container and soaked at room temperature for optimum soaking time, to reduce hydrogen cyanide content from harvested cassava. The soaking time was changed at every 12 hours. The water was changed with interval of 200ml. HCN content was measured at every soaking time and with interval of 200ml of water volume. The reduced amount of HCN content in processed materials was recorded.

Reduction of Hydrogen Cyanide Content from Cassava by Using Oven--Drying Method

The small size cassava(200g)was spread on the steel tray covered with aluminum foil. These trays were put into the hot-air oven at optimum temperature and dried for optimum drying time, to reduce hydrogen cyanide content from harvested raw materials. The drying temperature was changed with interval of 10ºC and drying time was changed at every 1hr. HCN content was measured at every drying time and with interval of 10ºC of drying temperature. The reduced amount of HCN content in processed materials was recorded.

Reduction of Hydrogen Cyanide Content from Cassava by Using Fermentation Method

The small size cassava(300g)was mixed with 10g of salt and optimum volume of distilled water into crock. And, the mixture was fermented at room temperature for optimum fermentation time, to reduce hydrogen cyanide content from harvested raw materials. The fermentation time was changed at every 1day and the water volume was changed with interval of 200ml. HCN content was measured at every fermentation times and with interval of 200ml of water volume. The reduced amount of HCN content in processed materials was recorded.

Results

Table (1) Effect of Volume of Water on Reduction of HCN from Cassava by Boiling with Water, Soaking in Water and Fermentation with Salt Solution

Weight of cassava for boiling and soaking method=100g
Weight of cassava for fermentation method=300g
Weight of salt (NaCl)=10g
Boiling time=30min, Soaking time=6hr
Fermentation time=3days, Boiling Temperature=100°C±2
Soaking and Fermentation Temperature=room temperature

Sr. No	Volume of Water, ml	Residual content of HCN from Cassava by Boiling Method	Volume of Water, ml	Residual content of HCN from Cassava by Soaking Method	Volume of Water, ml	Residual content of HCN from Cassava by Fermentation Method
		Content (ppm)		Content (ppm)		Content (ppm)
1.	0	728.6±5.6	0	1039.5±30.8	0	738.5±36.4
2.	400	388. ±5.6	400	629.6±22.	400	211.8±25.2
3.	600	356.4±11.2	600	554.4±50.4	600	176.2±19.6
4.	800	300.9±22.4	800	544.5±308	800	148.5±8.4
5.	1000	247.5±19.6	1000	469.2±47.6	1000*	118.8±5.6
6.	1200	201.9±16.8	1200*	362.3±25.2	1200	126.7±11.2
7.	1400*	176.2±14	1400	397.9±36.4	-	-
8.	1600	199.9±8.4	-	-	-	-

Table (2) Effect of Boiling, Soaking and Fermentation Time on Reduction of HCN from Cassava by Boiling with Water, Soaking in Water and Fermentation with Salt Solution (Picrate Method**)**

Weight of cassava for boiling and soaking method=100g
Weight of cassava for fermentation method=300g
Weight of salt (NaCl) =10g
Volume of Water for Soaking Method =1200ml
Volume of Water for Boiling Method =1400ml
Volume of Water for Fermentation Method =1000ml
Boiling Temperature =100°C±2
Soaking and Fermentation Temperature =room temperature

* the most suitable condition, ± SD of two replicates
These experiments were carried out at the Industrial Chemistry Department, Dagon University.

Table (3) Effect of Drying Time on Reduction of HCN Content from Harvested Cassava by Drying in an Oven

Weight of Cassava =200g
Drying Temperature=80ºC

Sr. No	Drying Time, hr	Residual content of HCN from Harvested Cassava
		Content (ppm)
1.	0	788±83.9
2.	4	405.9±53.2
3.	5	279.1±42
4.	6	199.9±14
5.	7*	124.7±19.6
6.	8	168.3±19.6

Table (4) Effect of Drying Temperature on Reduction of HCN Content from Harvested Cassava by Drying in an Oven

Weight of Cassava =200g
Drying Time =7hr

Sr. No	Drying Temperature, ºC	Residual content of HCN from Harvested Cassava
		Content (ppm)
1.	0	837.5±70
2.	60	239.5±14
3.	70	188.1±25.2
4.	80	152.4±30.8
5.	90	122.7±16.8
6.	100*	99±22.4
7.	110	118.8±5.6

* the most suitable condition, ± SD of two replicates
These experiments were carried out at the Industrial Chemistry Department, Dagon University.

Sr. No	Boiling Time (min)	Residual content of HCN from Cassava by Boiling Method	Soaking Time (hr)	Residual content of HCN from Cassava by Soaking Method	Fermentation Time (day)	Residual content of HCN from Cassava by Fermentation Method
		Content (ppm)		Content (ppm)		Content (ppm)
1.	0	766.2±14	0	896.9±42	0	817.7±47.6
2.	30	198±16.8	6	348.4±2.4	3	140.5±8.4
3.	45	170.2±11.2	12	281.1±5.6	4	134.6±11.2
4.	60	154.4±16.8	24	243.5±25.2	5	130.6±11.2
5.	75	120.7±8.4	36	170.2±22.4	6	116.8±14
6.	90*	99±16.8	48*	114.8±16.8	7*	85.1±8.4
7.	105	114.8±22.4	60	138.6±11.2	8	97.02±14

Table (5) Nutritional Values of Harvested and Prepared Cassava (50g)

Sr.No.		Harvested Cassava	Boiled and Dried Cassava
1.	Protein%	2.8	1.3
2.	Fat %	0.6	N.D
3.	Fiber%	4.7	4.2
4.	Carbohydrate%	78.4	34.3
5.	Moisture%	64	7.6
6.	Ash%	1.9	3.4

These experiments were carried out at the Department of Research and Innovation, Analysis Department, the Government of the Republic of the Union of Myanmar Ministry of Education

Table (6) The Results of Phytochemical Examination of Cassava (*Manihot esculenta*)

No.	Type of Compound	Observation of Unprocessed Raw Material	Results	Observation of Processed Material	Results
1.	Alkaloid	Cream, indigo, deep brown, yellow colour ppt.	+	No ppt.,deep blue color, yellowish brown, no ppt.	-
2.	Carbohydrate	Purple ring	+	Purple ring	+
3.	Glycoside	Turbid	Trace	White ppt.	+
4.	Phenol	Yellowish green colour	Trace	Greenish yellow color	Trace
5.	α- amino acid	Purple colour	+	Purple pink color	+
6.	Saponin	No persistent foam	-	No persistent foam	-
7.	Tannin	No ppt.	-	No ppt.	-
8.	Flavonoid	Pink colour	+	Pink colour	Trace
9.	Steriod	Bluish green	+	-	-
10.	Terpenoid	Pink	+	Pink	+
11.	Reducing sugar	Brick red ppt.	+	Orange ppt.	+
12.	Starch	Indigo colour	+	Deep blue color	+
13.	Cyanogenic glycoside	Pink colour	+	No color change	-

These experiments were carried out at the Pharmaceutical Research Department, Ministry of Industry.

Discussion

The HCN content in cassava was determined by picrate method.The determination of initial content of HCN in harvested cassava was carried out and the results are respectively shown in Tables(1) to Table (4).In Table (1), the harvested cassava was boiled by varying the water volume for 30min andthe maximum amount of HCN from cassava was reduced to 176.2±14 ppm by using 1400ml of water during 30min of boiling time.The solubilization of cyanogenic glycoside into a large volume of water is efficient for boiling method.In these studies, the volume of water was increased to reduce HCN content and the cyanogenic glycoside cannot be solubilized in these boiling conditions. The harvested cassava was soaked by varying the water volume for 6hr of soaking time for reduction of HCN content and the maximum content of HCN from cassava was reduced to 362.3±25.2ppm with 1200ml of water. The HCN content did not reduce when testing with 1400ml than 1200ml of water. Because it may depend on the room temperature during soaking time.The harvested cassava was also fermented with salt by varyingthe water volume for 3days of fermentation time. The maximum content of HCN was reduced to 118.8±5.6ppm with 1000ml of water volume.When the water volume was increased than optimum water volume, the HCN content cannot be reduced during the fermentation time.

In Table (2), the harvested cassava was boiled by varying the boiling time with optimum water volume to reduce the HCN content.The maximum content of HCN from cassava was reduced to 99±16.8ppm for 90min of boiling time. The results of other variable are shown in Table (2).The inefficiency of this processing method is due to the high temperature. When the boiling time was increased, the boiling temperature may also be increased therefore the enzyme cannot be hydrolyzed to hydrogen cyanide.In Table (2), it was shown that the cassava was soaked with 1200ml of water volume by varying the soaking time. The maximum content of HCN was reduced to 114.8±16.8ppm for 48hr of soaking time. The soaking time was increased to 60hr to reduce the HCN content from cassava and the reducible content of HCN was decreased than optimum soaking time.The room temperature may be increased during soaking time.The cassava was also fermented by varying the fermentation time with1000ml of water volume and the results are shown in Table (2). The maximum content of HCN was reduced to 85.1±8.4ppm for 7days of fermentation time. In 8days of fermentation time, the reducible content of HCNfrom fermented material was decreased.Because the materials were immersed for long periods can introduce fungi, mold spores and undesirable bacteria into the final products.

In Table(3), the harvested cassava was also dried by varying the drying time. It was shown that the maximum content of HCN was reduced to 124.7±19.6ppm at 80ºC for 7hr of drying time and the results are shown in Table (3). In Table (4), it has been seen that the maximum content of HCN was reduced to99±22.4ppm at 100ºC for 7hr of drying time. At 110ºC, the reducible HCN content was decreased and the appearance of dried cassava is dark brown color.At high temperature, the enzymes in the root may be denatured and cannot be hydrolyzed to hydrogen cyanide. During oven-drying, when drying temperature was increased and also increased cyanide retention in root. Therefore, the optimum condition is 7hr of drying time at 100ºC of drying temperature for reduction of HCN from cassava. The HCN content from cassava was reduced byusing boiling method and also dried in an oven to prepare the readymade food product.Thenutrition values of raw cassava and prepared food products were also studied and the results are shown in Table (5).The results of phytochemical examination for harvested cassava were shown in Table (6). By using

phytochemical test, the present of cyanogenic glycoside occurred in harvested cassava was appeared to pink color. After processing methods, any colorwas not changed in cassava and it is shown that the absent of cyanogenic glycoside in cassava.

Conclusion

Cassava tubers are traditionally processed by using many methods, which reduce their toxicity, improve palatability and convert the perishable fresh root into ready-made food products. For reduction of hydrogen cyanide level from cassava, the different traditional processing methods such as boiling, soaking, drying and fermentation were applied. In present research work, the maximum content of hydrogen cyanidefrom cassava was reduced to85.1±8.4ppm with 1000ml of water for 7days of fermentation timeby using fermentation method.

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