

DEGRADATION OF CYANOGENIC GLYCOSIDES DURING THE PROCESSING OF HIGH QUALITY CASSAVA FLOUR (HQCF)

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Abstract

Cyanide degradation during HQCF processing was investigated. Two unit operations, namely; size reduction and drying were considered as the major factors in detoxifying cassava during processing. Six cassava varieties were processed into flour, either by grating or slicing, followed by drying by solar or mechanical means. Cyanide content of fresh, grated and sliced cassava, as well as the final HQCF was determined by alkali titration. Cyanogen content varied widely among varieties and ranged from 5.24 to 12.01 mgHCN/kg with a mean of 7.79 mgHCN/kg. A general trend of higher reduction in cyanide content was observed in grating as compared to slicing. Mean cyanide content were 2.65 and 3.49 mgHCN/kg for grated and sliced cassava respectively. Most of the change in amount of cyanide for HQCF under slicing occurred during drying, while most it was expelled after pressing, when grating was used. A 2-way interaction of the main factors also showed a marked improvement in cyanide reduction. Better detoxification was established in solar drying as opposed to mechanical drying, since flours from the latter were generally higher in cyanide and with a mean of 3.28 mgHCN/kg compared to the former with a mean of 2.87 mgHCN/kg. Findings of this study highlight the need for better disintegration and longer drying for better detoxification of the cyanogenic glycosides

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1. INTRODUCTION

Cassava (*Manihot esculenta*) is undoubtedly one of the most important root tubers in Africa and serves as a food security crop for millions of people on the continent. In terms of crop area and yield, it ranks first among root crops in Ghana, with over 14 million MT produced in the year 2012 (MOFA 2013). Cassava is predominantly starch and contains little amounts of proteins, fats, vitamins and minerals. Customarily, the crop is largely consumed locally and does not return considerable amounts of foreign exchange. About 50% of the roots are consumed fresh at household level, while the remaining is processed into other forms. Only 1% of the cassava produced is used for industrial purposes.

In recent times attempts have been made to add more value to cassava and also make it more useful as a raw material for industrial applications. One of the approaches has been to process the roots into unfermented flour for

domestic and industrial uses. This flour, also called High Quality Cassava Flour (HQCF) is useful for bakery products, production of glucose syrup and starch as well as use as a glue extender for the plywood industry. HQCF can also serve as a source of starch for the textile industry. A drawback to optimal utilization of HQCF for food purposes, however, is attributed to its cyanogenic potential. This results from the hydrolysis of cyanogenic glycosides contained in cassava, and act as defense compounds against predatory attack (Heldt and Piechulla, 2011; Vetter, 2000). HCN, which is produced from the breakdown of the glycosides, has a long-term damaging effect on the nervous system and thyroid gland (Anhwange et al., 2011).

Several attempts have been made to reduce cyanide contents in cassava products. Methods such as size reduction, exposure to heat, retting and fermentation have been suggested to efficiently lower the cyanogenic potential of

products made from cassava (Amoa-Awua et al., 1996; Ampe and Brauman, 1995; Essers et al., 1996; Hidayat et al., 2002). However, the effectiveness of these unit operations or their combination in cyanide removal also depends on processing techniques adopted. By their mode and design, some unit operations involved in processing cassava into HQCF, such as size reduction and drying, have the potential of reducing cyanide content in the finished product considerably. As the popularity of domestic and industrial applications of HQCF continues to rise, there is the need to establish the role of unit operations in cyanide detoxification and how these processing steps combine to reduce cyanide in HQCF. The aim of this study was therefore to compare the effects of different techniques employed in two major processing steps on cyanide detoxification, during the production of HQCF.

Materials and Methods

Cassava roots

Six varieties of cassava obtained from experimental fields at the CSIR Crops Research Institute, Ghana were used in the study. Cassava roots were harvested 10 months after planting and processed into HQCF. The varieties were *Agbelifia*, *Ampong*, *Bankye hema*, *Doku duade*, *Essam bankye*, and *Sika bankye*.

Processing roots into HQCF

HQCF produced by grating

Cassava roots were processed into HQCF following the method described by Dziedzoave et al., (2006), at the Root and Tuber Products Development Unit of the CSIR-Food Research Institute (FRI). The roots were washed with potable water and hand-peeled with stainless steel knives and washed twice in potable water before further processing. Peeled cassava roots were then grated in a motorized grater. The grated mash was immediately loaded into polypropylene woven sacks and dewatered in a manual single screw press. The pressed cake was disintegrated manually and spread thinly on trays for drying in either a solar tent dryer or

a mechanical dryer. Solar drying took place over a period of 14 hours during which the thinly spread granules were turned several times. Mechanical drying also took place for 8 hrs at 60 °C in a diesel-operated walk-in cabinet dryer. The dried granules were ground in a hammer mill and sieved (250 µm) to obtain a smooth and free-flowing flour.

HQCF produced by slicing/chipping

Cassava roots were washed and peeled as described in the previous section. Peeled cassava roots were quickly chipped (< 5 mm thickness) in a motorized slicer. The freshly chipped roots were immediately spread thinly on drying trays and dried either in a solar tent dryer or a mechanical dryer. Solar drying took place over a period of 14 hours during which the thinly spread chips were turned several times. Mechanical drying also took place for 8 hrs at 60 °C in a diesel-operated walk-in cabinet dryer. The dried chips were ground in a disc attrition mill and sieved (250 µm) to obtain a smooth and free-flowing flour. All the equipment used to process the HQCF were fabricated by the CSIR-FRI, Accra.

Cyanide determination

Cyanide content in cassava roots and HQCF was determined using the alkaline titration method (ISO, 1975). Twenty grams (20 g) of sample was weighed into a 1 L distillation flask and 200 mL distilled water added for steam distillation. The distillate (150 mL) was collected in 20 mL of 1 N NaOH. The apparatus was adjusted in order for the tip of the condenser to dip below the surface of the NaOH solution in the receiver. The distillate in NaOH solution was transferred into a 250 mL volumetric flask made up to the mark and 100 mL of this solution titrated against 0.02 N AgNO₃ solution to a permanently turbid end point. The results were calculated using the relation 1 mL 0.02 N AgNO₃ = 1.08 mg HCN, and reported as mean ± standard deviation.

Experimental design and statistical analysis

The experiment was setup in a 6x2x2 factorial and the principal factors were:

1. Cassava variety: *Agbelifia*, *Ampong*, *Bankye hema*, *Doku duade*, *Essam bankye*, *Sika bankye*
2. Method of size reduction: Slicing and Grating
3. Method of drying: Solar drying and Mechanical drying

The data obtained were analyzed for differences using Univariate GLM (SPSS 17.0.1) and means considered significantly different at $p \leq 0.05$. Significantly different means were separated by Duncan's Multiple Range Test.

Results and discussions

Fresh cassava roots

Table 1 presents the cyanide content of the varieties used in the study, which ranged between 5.24 and 12.01 ppm of HCN on dry matter basis. The mean cyanide content (7.79 ppm) obtained for these varieties were less than the recommended value of <10 ppm (FAO/WHO, 1991) and the range of 10 to 500 ppm given in previous studies by Siritunga and Sayre (2003) and 160-700 ppm by Burns et al., (2012). The cyanide content in fresh cassava is an indicator of its toxicity and partly correlates with taste of the root crop, hence its use in categorizing the crop into sweet and bitter varieties (Chiwona-Karlton et al., 2004).

As previously established (Nambisan, 2011) and was therefore expected, the cyanogen potential was markedly different ($p < 0.0001$) among different varieties since they showed wide variations in HCN content. Differences in cyanogenic potential in cassava have also been attributed to edaphic factors, rainfall pattern as well age (Cardoso et al., 2005; Charles et al., 2005). On fresh weight basis cyanide content of this magnitude, as observed among these varieties, are classified as harmless (<50ppm HCN) (Brito et al., 2013).

Unit operations in processing

Adequate processing drastically reduces the cyanide content of cassava to limits that do not pose a health risk to consumers. In this study degradation of the cyanogen was observed to occur in two major unit operations, namely disintegration (size reduction) and drying. The reduction in cyanogen content which occurred in these stages of processing are significant as far as the safety of foods products from cassava are concerned.

The effect of the two size reduction methods on cyanide during HQCF processing is illustrated in Figure 1. Cyanide content of cassava roots processed using these two methods showed considerable differences ($p < 0.05$), with those grated having lower HCN levels and showing higher reduction in cyanide content, compared to the sliced samples. Reduction in cyanide content was highest in grated *Agbelifia* (70.0%) followed by *Essam bankye* (61.4%) and *Doku duade* (44.8%). Changes in amount of cyanide after slicing ranged from 8.7% (*Sika bankye*) to 34.8% (*Essam bankye*) and *Ampong*, correspondingly showing the highest and lowest percentage loss. *Sika bankye*, was observed to have the least amount of cyanide (3.01 ppm) after size reduction. The role of size reduction is to bring enzyme and substrate into contact with each other. This operation disrupts the cellular structure of cassava roots and causes the glycosides to come into contact into contact with endogenous linamarase and in the process initiates the hydrolysis of these compounds (Essers et al., 1996). The products of the hydrolysis are unstable and decompose spontaneously at temperatures greater than 35°C into HCN which is volatile.

Among the two approaches mainly used, grating effects a more efficient breakdown of tissues into a fine mass which enhances enzyme-substrate contact, compared to slicing. Aside of being a better size reduction technique, grating involves pressing, which results in further loss of cyanide as the HCN produced is extracted with the water (Kemdirim et al, 1995). Differences in cellular

structure and morphology, as well as differences in amount of linamarase, may well account for the different effects observed between varieties. Linamarase has been found to be stored in different compartments in different varieties of cassava (Santana et al., 2002) and therefore the same extent of size reduction may have different effects as far as detoxification of cyanide in cassava roots is concerned

Effect of drying method used

The cyanide content (mg HCN/kg) in HQCF produced by different size reduction and drying methods is summarized in Table 2. Cyanide in grated or sliced ranged between 1.55 ppm and 4.48 ppm for mechanical drying and 1.31 ppm and 3.81 ppm for solar drying. Generally, the cyanide content of cassava flour from this study were well below the 41 ppm, 43 ppm 50 ppm and 56-57 ppm independently reported in previous studies by Cardoso et al., (2005), Cumbana et al., (2007), Jones et al., (1996) and Dolodolotawake and Aalbersberg (2011).

Analysis of Variance (ANOVA) conducted on the results in Table 2 indicate that all three factors (variety, size reduction method and drying method) had significant effects ($p < 0.05$) on cyanide in HQCF (Table 3). This therefore confirms the widely-held assertion that the cyanide content of cassava flour is dependent on the variety of cassava and processing methods employed.

A general trend of an effective degradation was noticed with solar drying as opposed to mechanical drying, since the solar dried flour had relatively lower cyanide content (Table 2). The degradation of cassava cyanogens is a somewhat complex phenomenon that is also influenced by moisture content, rate of moisture removal and probably enzyme activity. Indeed previous reports have indicated that enzymatic hydrolysis of glycosides halt at moisture contents lower than 12-18% (Essers, 1996; Mlingiet al., 1995), and this results in accumulation of potentially harmful cyanogenic compounds. Moisture removal is

by far slower in solar drying compared to mechanical drying and this favors detoxification (Essers, 1996; Mlingi et al., 1995). Faster rates of moisture removal are likely to render linamarine molecules immobile thereby limiting its interaction with hydrolysis enzymes.

In addition to slower drying rates, solar drying provides more exposure time for enzyme-substrate interaction, which is central to degradation of cyanogenic glycosides. Contrary to this mode, relatively higher drying rates are encountered in mechanical drying and contact time is shorter for enzyme substrate interaction. Additionally, in mechanical drying, temperature is relatively higher and may affect the stability of linamarase enzyme which is needed for hydrolysis of cyanogens. Activity of the enzyme decreases with increasing temperature, with maximum stability activity cited to range between 40-50 °C (Nwokoro and Anya, 2011), even though some authors have reported about 55°C (Petruccioli et al., 1999).

Degradation during processing

Figure 2 presents a general overview of cyanide degradation during size reduction and drying (both solar and mechanical). A close look at the result reveals that subtle differences exists in the dynamics of cyanogen breakdown when different tissue breakdown techniques are applied to process HQCF. Generally there were a 65.9 and 55.2 percentage reductions in cyanide content of the starting material for size reduction by grating and slicing respectively.

As shown in Fig 2 **a** and **b**, whereas the elbow of the scree takes a relatively gentle descent (towards drying) in the case of grating (followed by pressing) and depicts a lower loss at the drying stage, it was much steeper with slicing. For grating (Figure 2a), which proved to be the better of the two disintegration methods, detoxification rapidly occurred to a greater extent after size reduction and the degraded compounds expelled with the water during pressing. Slicing (Fig 2b) on the other hand showed a sharp contrast to this

observation. With this, the magnitude of cyanide loss during drying was greater (37.1%) and therefore suggests that most of the hydrolysis of the cyanogens takes place during this period.

Conclusion

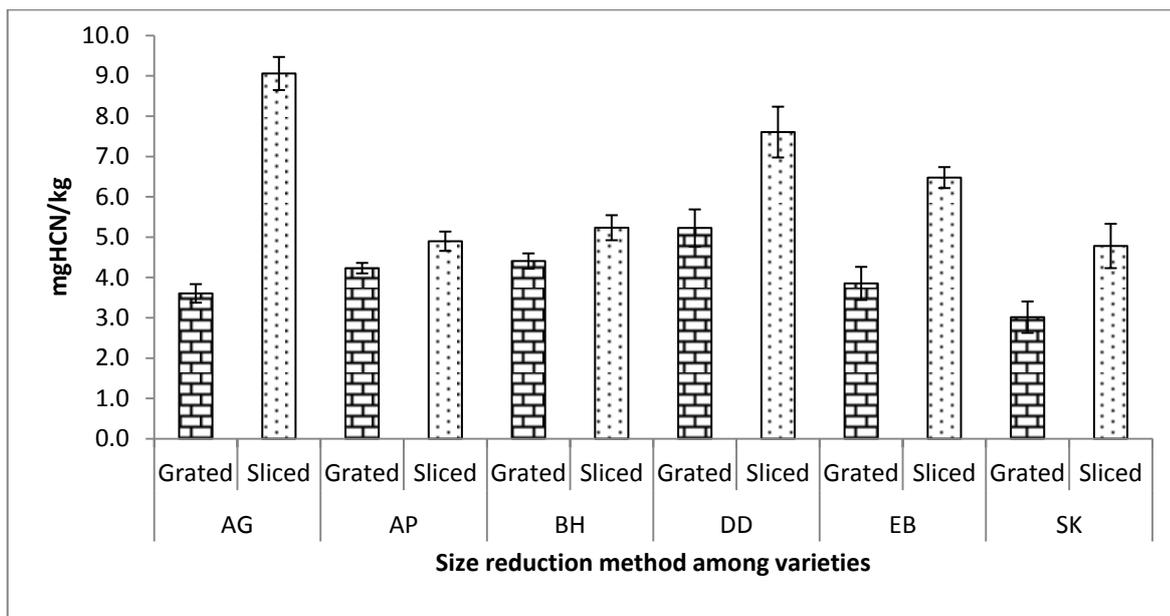
Variety of cassava and processing techniques as well as a combination of these factors affects the extent of cyanide degradation differently during HQCF production. Cyanide content of fresh cassava roots was reduced considerably after disintegration and drying. Amount of cyanide saw an overall reduction by 65.9% for grating and 55.2% for slicing. Generally solar drying resulted in HQCF with lower cyanide

than mechanical drying. These observations further emphasize the need for finer cell disruption and prolonged drying in order to allow for extensive degradation and efficient detoxification of cassava cyanogens

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Figures and Tables



AG-Agbelifia, AP-Ampong, BH-Bankye hema, DD-Doku duade, EB-Essam bankye, SK-Sika bankye

Figure 1: Size reduction technique and its effect on cyanide detoxification

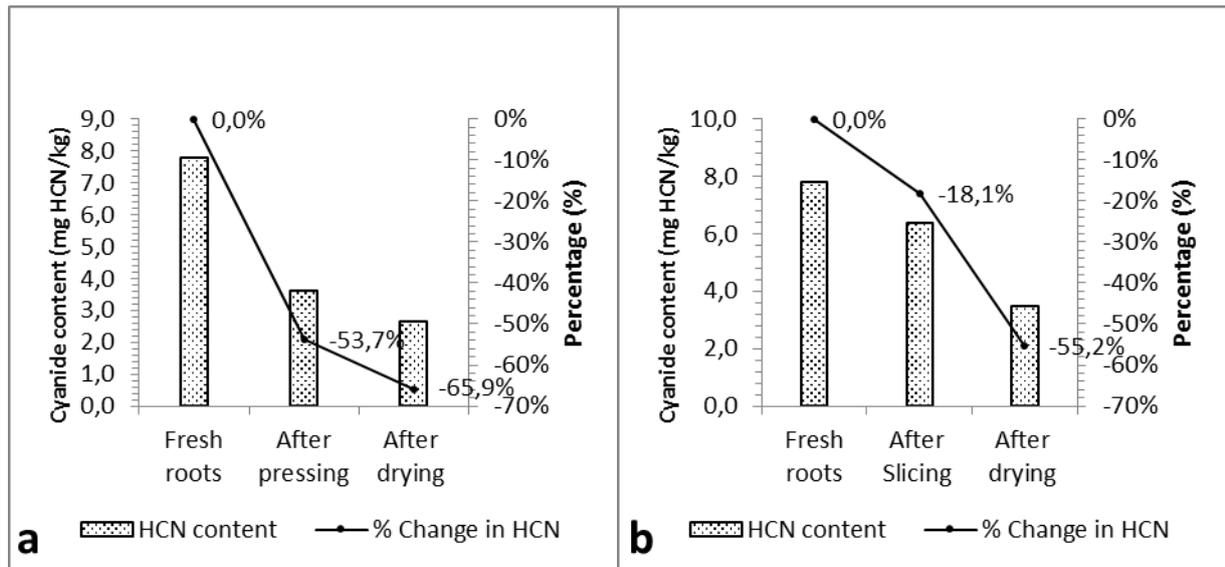


Figure 2: Cyanide loss at different stages of processing by: (a) grating and (b) slicing

Table 1: Moisture and cyanide content of six varieties of cassava

Variety	Moisture	mgHCN/kg (dm)
Agbelifia	67.08±0.18 ^d	12.01±0.09 ^e
Ampong	66.80±0.53 ^d	6.39±0.30 ^b
Bankye hema	58.29±0.20 ^a	6.40±0.21 ^b
Doku duade	58.24±0.04 ^a	9.47±0.32 ^d
Esam bankye	59.90±0.23 ^c	7.22±0.44 ^c
Sika bankye	59.37±0.12 ^b	5.24±0.56 ^a

Means within the same column with different superscripts are significantly different (p<0.05)

Table 2: Cyanide content (mg HCN/kg) of HQCF

Variety	Mechanical drying		Solar drying	
	Grating	Slicing	Grating	Slicing
Agbelifia	2.84±0.33 ^b	3.74±0.79 ^c	1.73±0.43 ^a	2.88±0.55 ^a
Ampong	2.82±0.21 ^b	3.51±0.35 ^b	2.46±0.19 ^b	3.59±0.43 ^{bc}
Bankye hema	3.88±0.22 ^c	2.46±0.56 ^a	3.61±0.73 ^c	3.81±0.84 ^c
Doku duade	3.76±0.54 ^c	4.48±0.44 ^d	3.01±0.81 ^d	3.45±0.52 ^b
Esam bankye	1.55±0.68 ^a	3.41±0.51 ^b	1.31±0.57 ^e	2.91±0.66 ^a
Sika bankye	2.79±0.29 ^b	4.16±0.60 ^c	2.10±0.73 ^f	3.54±0.74 ^b

Means bearing different superscripts are significantly different (p<0.05)

Table 3: ANOVA showing significant F-values of cyanide content in HQCF

Source of variation	F-ratio	p-value
Variety	39.42	<0.0001
Drying method	43.67	<0.0001
Size reduction method	176.27	<0.0001
Variety x drying method	13.30	0.0001
Variety x size reduction	27.57	<0.0001
Drying x size reduction	5.99	0.0207

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