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Varietal diversity and processing effects on the biochemical composition, cyanogenic glucoside potential (HCNp) and appearance of cassava flours from South-Eastern African region

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Abstract

Changes in biochemical quality and cyanogenic safety in flours from different cassava varieties grown within the South-eastern African region as influenced by processing technique were investigated. Two local (Mweulu and Tanganyika) and four improved Cassava Mosaic Disease (CMD)-tolerant cassava varieties (Chila A, Chila B, Bangweulu and Kampolombo) were processed using different processing techniques (chipping, grating and soaking). Flours obtained from these products were studied for changes in their starch content, total, reducing and non-reducing sugars, colour and cyanogenic potential (HCNp) using standard analytical methods. The results showed that the different processing techniques had only minimal but significant ($p < 0.05$) effects on the starch and sugar content of the different cassava varieties. Flour made from chips from all six cassava varieties had relatively high cyanogenic potentials with values ranging between 30.1 mg HCN/kg in Chila A to 64.3 mg HCN/kg in Bangweulu. Grating and soaking of the roots, however resulted in drastic reductions in the levels of the cyanogenic glucosides in all the varieties. The high HCNp levels in the chips from Chila A and Bangweulu were reduced to 16.2 and 13.5 mg HCN/kg by the grating and soaking treatments respectively. Similar reductions in HCNp levels were noted for all the other varieties. Likewise, grating and soaking also caused significant ($p < 0.05$) increase in the L^*-values of the cassava flours compared to the cassava chip flours. Soaked Kampolombo had the highest L^*-value of 78.40 suggesting that the soaked Kampolombo cassava sample was whiter than the rest of the samples. These findings have implications for the preference and adoption of cassava varieties where the colour of the flour is deemed to be important for the preparation of preferred dishes.

Introduction

Cassava (*Manihot esculenta* Crantz), a dicotyledonous perennial woody shrub with an edible starchy root, belongs to the family of Euphorbiaceae and is the most widely distributed and cultivated crop in different parts of the low land tropics (Amsalu, 2006; Montagnac *et al*., 2009; Afoakwa *et al*., 2012). The crop is widely grown as staple food and animal feed with a total cultivated area of over 18 million ha (Baguma *et al*., 2008) and according to Aryee *et al*. (2006), over half a billion of the world's population depend on cassava as their major staple. Africa is the largest production center of cassava with 53% of world production (Teka *et al*., 2013). Cassava is grown predominantly by small-scale farmers with limited resources (El-Sharkawy, 2003). While the roots are predominantly used for household food consumption, cassava products are also used in international trade in the form of dried chips,

pellets, flour, starch and fermented products such as *gari* (fermented and roasted cassava grits) thus contributing to the economy of exporting countries (Eke *et al*., 2007; Mweta *et al*., 2008). Cassava has several advantages over other crops due to its outstanding ecological adaptation, low labor requirement and high tolerance to extreme stress conditions such as drought and poor soils.

Cassava a starchy staple, whose roots are very rich in carbohydrates, is a major source of food energy. Raw cassava roots have more carbohydrate than potatoes but less carbohydrate than wheat, rice, yellow corn, and sorghum on a 100 g basis (Afoakwa *et al*., 2012). The carbohydrate content in cassava roots is reported to range from 32% to 35% on a fresh weight (FW) basis, and from 80% to 90% on a dry matter (DM) basis (Montagnac *et al*., 2009). Eighty percent of the carbohydrates produced in cassava roots are starch (Gil and Buitrago, 2002); around 83% is in the form of amylopectin and 17%

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is amylose (Rawel and Kroll, 2003). Cassava roots contain small quantities of sucrose, glucose, fructose, and maltose (Tewe and Lutaladio, 2004; Tewe, 2013). These sugars are only present in minute quantities with ranges between 1.57% to 2.89% dry weight bases (Aryee *et al.*, 2006).

Cassava roots are low in protein content, highly perishable postharvest, and some varities have high content of cyanogenic glycosides: linamarin and lotaustralin (methyl-linamarin) (Enidiok *et al.*, 2008). When ingested without proper processing, substantial quantities of cyanogenic glycoside, i.e linamarin and a small amount of lotaustralin (Burns *et al.*, 2012; Kalenga and Nyirenda, 2012) present in cassava present a possible hazard. Both compounds are hydrolyzed by the plant's endogenous enzyme, linamarase to release free cyanide (Yeoh and Sun, 2001). Cyanide inhibits cellular respiration of all aerobic organisms by blocking mitochondrial electron transport and preventing oxygen uptake (Yeoh and Sun, 2001). High exposure to cyanide in humans causes nausea, vomiting, diarrhoea, dizziness, weakness and sometimes death (Merck, 2008).

Some studies have shown that medical conditions caused by degeneration of the nervous system, konzo and tropical ataxic neuropathy (TAN), may affect people who consume a monotonous diet of bitter (high cyanogenic glucoside) cassava (McKey *et al.*, 2010; Burns *et al.*, 2010). Cyanogenic glucoside content in cassava may range from 10 to 500 mg HCN equivalents/kg DW in the root parenchyma (Dufour, 1988; Chiwona-Karltun *et al.*, 2004). Bitter varieties of cassava have cyanogenic glucoside levels exceeding the Food and Agriculture Organization/ World Health Organization (1991) recommendation of 50 mg HCN equivalents /kg FW, which makes cassava acutely toxic for humans (CAC, 2013). Consumption of 50 to 100 mg of cyanide has been associated with acute poisoning which is lethal to humans.

The twin problems of perishability and the poisonous nature of the cyanogenic glucosides present in cassava can be overcome by thorough processing. According to McFarlane (1982), the extreme perishability of cassava roots has led to the development of a range of processing techniques even by the earliest Amerindians cultivators of the crop more than 4,000 years ago. The cyanogenic glycosides content can be reduced to safer levels during processing which generally involves soaking, peeling, grating, fermentation, drying and/or frying. These processes also improve the palatability and prolong the shelf life of the resulting products. This also leads to the production of diversified food products. However, the extent of cyanogenic glucoside reduction during processing depends on the processing method as well as the cassava variety (Chiwona-Karltun *et al.*, 2004). Again, both processing and varietal differences influence the production of diversified food products, among others due to inherent characteristics such as starch content, sugar content and levels of the endogenous enzymes i.e. linamarase. Due to the importance of cassava as a major source of food in south-eastern Africa, there is a need for various post harvest practices that can significantly reduce HCNp. Promoting the wider production and consumption of cassava in south-eastern Africa requires simultaneous focus on processing practices. Although varieties have been improved for root yield, their HCNp retained after various processing techniques is not well documented. Some new improved cassava varieties grown extensively across south-eastern Africa include Chila A, Chila B, Mweulu, Bangweulu, Tanganyika and Kampolombo. This study investigated the effects of processing technique (chipping, grating and fermentation by root soaking) and varietal variations on the biochemical composition, cyanogenic potential and appearance of cassava (*Manihot esculenta* Crantz) flours from the named varieties grown in south-eastern African region.

Materials and Methods

Cassava samples

Two local and four improved CMD-tolerant cassava varieties were obtained from the farmers' fields in the Chisamba Community located within the Chongwe District in Zambia. These were: Kampolombo, Bangweulu, Chila A and Chila B, Mweulu and Tanganyika. Out of these the two varieties, Mweulu and Tanganyika were local. The improved varieties came from the cassava breeding programme released varieties by the Zambian Agricultural Research Institute (ZARI) (Alene *et al.*, 2013).

Sample preparation

The roots were harvested and transported to the laboratory within three hours of harvest. At the laboratory, the root samples were cleaned, peeled and washed with room temperature water. The samples were then processed using three common processing techniques (chipping, grating and soaking). Soaking was done by dipping the peeled roots in plastic drums containing water for 48 hours after which the cassava roots were removed, cut into cubes and oven dried at 60°C for 48 hrs. Chipping was done by cutting parts

of the parenchyma from the distal, middle and apical sections of peeled tubers into cubes and oven dried at 60°C for 48 hrs. The grated samples were prepared by grating the cassava roots using a traditional grater into cassava meal, which was oven dried at 60°C for 48 hrs.

The oven dried cubes and grated samples from the three processing procedures were ground in a Hammer mill (Christy and Norris Ltd., Model 2A, Chelmsford, Surrey, UK) into flour to pass through a 250-μm sieve. The flour samples obtained were then packaged into polypropylene bags and kept at room temperature (25°C) for analyses. Triplicate samples were prepared for each variety and triplicate analyses were conducted on each of the replicates. The mean values of all analyses conducted and standard deviations were reported.

Starch extraction and analysis

The starch was extracted from the flour samples using the procedure outlined by Trim *et al*. (1993). About 400 g of the samples were weighed and milled with 800 ml of water using waring blender (Philips 8010G, New York, NY, USA). The slurry sample was filtered through a clean cheese cloth. The solids retained by the cloth were washed with 4000 ml of water and filtered through cheese cloth until there was little or no starch in the residue. The filtrate was allowed to sediment overnight and the liquid decanted and discarded. The starch was dried under room temperature until completely dry. The dried starch was weighed and expressed as a percentage of dry weight.

Determination of total sugars

Total sugars were determined by methods described by Lane and Eynon (Pearson, 1970). Ten grams of the sample was dissolved in 100 ml of distilled water. Ten (10) ml of concentrated HCl was added to the solution and heated in a water bath for 10 minutes. The solution was then neutralized with a base preferably NaOH. The solution was made up to 200 or 300 ml with distilled water and filtered. 10 or 25 ml of mixed Fehling's solution was pipetted into a conical flask followed by the addition of 15 ml of the solution from the burette. The solution was heated and on boiling three drops of methylene blue was added. Further quantities of the solution were added from the burette (1 ml at a time) at 10–15 seconds interval to the boiling liquid until the indicator was completely decolourised. The titre values obtained corresponded to mg of invert sugar per 100 ml.

Determination of reducing sugars and non-reducing sugars

Reducing sugars were determined by methods described by Lane and Eynon (Pearson, 1970). About 40–60 g of the samples was dissolved in 150 ml of distilled water. The solution was made up to 200 or 300 ml with distilled water and filtered. 10 or 25 ml of mixed Fehling solution was pipetted into a conical flask followed by the addition of 15 ml of the solution from the burette. The solution was heated and on boiling three drops of methylene blue was added. Further quantities of the solution were added from the burette (1 ml at a time) at 10–15 seconds interval to the boiling liquid until the indicator is completely decolourised. The titre values obtained correspond to mg of invert sugar per 100 ml. The amount of non-reducing sugars was obtained by subtracting the reducing sugars from that of the total sugars.

Colour determination

Colour of the samples were determined using a Hunter Lab Colour Difference meter (CDM) model CR-300 (Minolta camera co. Ltd; Tokyo, Japan) using a white porcelain plate with L = 98.34, a = -0.21 and b = 0.19 as reference. The results obtained were expressed in Hunter L^*, a^* and b^* values. L^* represent lightness (with 0= darkness/blackness to 100= perfect/brightness); a^* corresponds to the extent of green colour (in the range from negative= green to positive = redness); b^* represents blue in the range from negative=blue to positive=yellow. Triplicate readings were made and the average taken.

Cyanogenic glucoside determination

The acid titration method (AOAC, 1984) for the determination of hydrocyanic acid in beans was used. One hundred (100) ml of H_2O was added to 3 g of the sample in a 500 ml Kjedahl flask for steam distillation. The distillate was collected in 20 ml 0.02 N $AgNO_3$ acidified with 1 ml HNO_3. The apparatus was adjusted so that the tip of the condenser dipped below surface of the liquid in the receiver. After 150 ml had passed over, excess $AgNO_3$ was titrated with 0.02 N KSCN using Fe alum as indicator. The results were calculated and reported as mean values on dry matter basis.

Statistical analyses

Statgraphics software version 3.0 (STSC, Inc., Rockville, MD, USA) was used to analyze the data for analysis of variance (ANOVA). Least significant difference (LSD) was used to separate and compare the means, and significance was accepted at 5% level (p<0.05). All treatments and analytical determinations

Table 1. Biochemical composition and cyanogenic glycosides of cassava varieties as affected by processing and genotype

Processing method	Variety	Starch (%)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	CNp (mg HCN/kg)
Chips	Chila A	57.59±0.07	0.94±0.05	5.78±0.21	6.72±0.65	30.1±0.9
	Chila B	57.03±0.04	1.91±0.04	5.43±0.75	7.34±0.48	33.0±0.9
	Mweulu	63.60±0.04	1.58±0.08	6.48±0.01	8.06±0.78	39.7±0.6
	Bangweulu	63.61±0.03	1.68±0.06	4.80±0.30	6.48±0.64	64.3±0.2
	Tanganyika	62.77±0.08	2.46±0.03	6.53±0.02	8.99±0.97	36.4±0.1
	Kampolombo	58.06±0.03	2.53±0.07	6.41±0.04	8.94±0.74	50.0±0.8
Grated	Chila A	54.74±0.46	0.97±0.05	5.37±0.05	6.34±0.76	17.5±0.9
	Chila B	59.16±0.36	1.94±0.08	4.13±0.06	6.07±0.45	20.1±0.01
	Mweulu	76.34±0.84	1.58±0.05	6.40±0.10	7.98±0.68	18.9±0.9
	Bangweulu	60.90±0.45	1.58±0.03	4.34±0.41	5.92±0.96	16.2±0.01
	Tanganyika	65.77±0.37	2.25±0.08	6.77±0.91	9.02±0.75	12.1±0.5
	Kampolombo	55.17±0.85	2.12±0.06	4.88±0.93	7.00±0.54	12.1±0.6
Soaked	Chila A	53.86±1.29	1.09±0.64	6.07±0.51	7.16±0.96	16.1±0.01
	Chila B	60.56±0.06	1.96±0.07	5.36±0.45	7.32±0.15	18.7±0.8
	Mweulu	72.78±0.12	1.66±0.06	6.85±0.09	8.51±0.85	14.8±0.6
	Bangweulu	58.87±0.03	1.71±0.09	4.80±0.11	6.51±0.75	13.5±0.8
	Tanganyika	66.56±0.46	2.36±0.08	6.92±0.42	9.28±0.43	13.4±0.08
	Kampolombo	53.12±0.94	2.31±0.04	6.74±0.92	9.05±0.56	16.2±0.01

of data were performed in triplicates and mean values along with their standard deviation were reported.

Results and Discussion

Biochemical composition

Table 1 gives the biochemical composition of the various cassava varieties processed into flour using the three processing techniques (chipping, grating and fermentation). The starch content of the cassava varieties on dry weight basis (DW) varied among the varieties and processing technique. Grated Mweulu recorded the highest starch content of 76.34% while soaked Kampolombo had the least starch content of 53.12%. Soaked Mweulu and Tanganyika as well as grated Tanganyika also recorded relatively higher starch content of 72.78%, 66.56% and 65.77% respectively. Even though the starch content of all the varieties were below 80% for all the three processing methods, Mweulu, Bangweulu and Tanganyika in the form of chips and as grated had fairly high starch content (>60%). Similarly, in the soaked form Chila B, Mweulu and Tanganyika also had starch content >60%. Potentially these varieties could be used for many commercial products like starch, alcohol and glucose. Chijindu and Boateng (2008) found the starch content of fermented (fermentation by soaking in water for 48 hours) of Afisiafi and Yebesi varieties (cassava varieties commonly grown in Ghana) to be 76.82% and 78.75% respectively and that of the chips

also to be 82.62% (Afisiafi) and 84.66% (Yebesi). Analysis of the data revealed that varietal difference and processing technique significantly ($p < 0.05$) influenced the starch content of the cassava samples (Table 3).

With the exception of Tanganyika, soaking increased the reducing sugars of the different cassava varieties. This is because during the soaking process, some degree of fermentation takes place where microorganisms hydrolyze the starch to produce reducing sugars. Chijindu and Boateng (2008) also reported high reducing sugar content in both Afisiafi (4.69%) and Yebesi (3.83%) cassava varieties soaked for 48 hours compared to the Afisiafi chips (3.41%) and Yebesi chips (2.78%). Tanganyika chips had the highest reducing sugars of 2.46% while Chila A chips and grated recorded the least reducing sugar content. Total sugars were highest for soaked Tanganyika sample (9.28%) and least for grated Bangweulu sample (5.92%). Non-reducing sugars formed the major component of the total sugars of the cassava varieties for all the processing methods. Total sugars and non-reducing sugars were significantly affected ($p < 0.05$) by both processing technique and varietal differences. Varietal differences significantly ($p < 0.05$) affected the reducing sugars of the samples while processing technique did not significantly ($p > 0.05$) influence the reducing sugars of the samples (Table 3).

Table 2. Colour of cassava flour as affected by processing technique and genotype

Processing method	Variety	L*- value	a*- value	b*- value
Chips	*Chila A*	70.12±0.03	-1.40±0.02	+9.53±0.01
	Chila B	68.81±0.02	-1.39±0.04	+9.92±0.04
	Mweulu	68.36±0.00	-1.65±0.01	+8.23±0.02
	Bangweulu	70.58±0.01	-1.75±0.03	+7.99±0.01
	Tanganyika	69.58±0.03	-1.17±0.01	+7.12±0.04
	Kampolombo	68.66±0.02	-1.71±0.01	+9.92±0.01
Grating	*Chila A*	70.37±0.52	-1.21±0.01	+6.42±0.05
	Chila B	69.85±0.54	-1.09±0.04	+8.01±0.10
	Mweulu	71.21±0.19	-1.34±0.02	+8.37±0.07
	Bangweulu	75.72±0.46	-1.18±0.01	+9.22±0.07
	Tanganyika	71.75±0.01	-1.10±0.01	+7.65±0.00
	Kampolombo	77.54±1.07	-1.03±0.01	+8.76±0.11
Soaking	*Chila A*	71.04±0.01	-0.93±0.00	+8.46±0.03
	Chila B	68.12±0.18	-0.92±0.05	+7.51±0.05
	Mweulu	71.51±0.08	-0.88±0.03	+8.28±0.04
	Bangweulu	68.48±0.62	-0.83±0.03	+6.69±0.05
	Tanganyika	72.49±0.86	-0.93±0.01	+7.25±0.10
	Kampolombo	78.40±0.32	-0.74±0.00	+8.13±0.01

Colour of cassava varieties

Colour is one of the most important attributes of food, both for its aesthetic value and for quality judgment (Vamos-Vigyazo, 1981). It affects our overall judgment on the worth of food from both an aesthetic and safety point of view (Clydesdale, 1984). It plays an important role in taste thresholds, flavour identification, food preferences, pleasantness, acceptability and ultimately food choice. However its role is elusive and difficult to quantify (Clydesdale, 1984). Colour of cassava roots both as fresh and processed is an important criterion for quality, especially for use in the food industries. Ideally, cassava roots or cassava flour should be clear, have a good white colour, and be free from any off-colour for better acceptability. The results from the colour determination given in Table 2 indicate that grating and soaking caused a significant (p<0.05) increase in the L*-values of the cassava flours compared to the cassava chip flours. The L*-value increased from 68.36 for Mweulu chips to 71.21 (grated Mweulu) and 71.51 (soaked Mweulu). However, fermented Chila B and Bangweulu flour samples had a lighter colour (L*-values) less than their respective flour made from the chips. Soaked Kampolombo had the highest L*-value of 78.40 suggesting that the soaked Kampolombo cassava sample was whiter than the rest of the samples. These findings are novel and quite innovative as they explain as shown in Table 4, the relative appearance properties of different cassava flours. Furthermore, the results confirm the perceptions of farmer's and statements that flour produced from some cassava cultivars is much whiter and thus more preferred (Chiwona-Karltun *et al.*, 1998).

Cyanogenic glycosides potential (HCNp)

The cyanogenic potential of all the six cassava varieties processed using the three processing techniques are shown in Table 1. Results showed that soaking the cassava tubers for 48 hours as well as grating cassava significantly (p<0.05) reduced the HCNp compared to just chipping the cassava. All the cassava chips were above 30 mg HCN/kg DW with Bangweulu chips recording the highest cyanogenic glucoside content of 64.3 mg HCN/kg DW. This suggests that cyanogenic levels of both the local and improved varieties were comparable with the exception of Bangweulu which had high levels of 64.3 mg HCN/kg DW. Grated Tanganyika and Kampolombo recorded the lowest HCNp of 12.1 and 12.1 mg HCN/kg DW respectively. Soaked Bangweulu and Tanganyika flour samples also recorded fairly low levels both of 13.5 and 13.5 mg HCN/kg respectively. Statistical analysis on the data showed that the cyanogenic levels of the different cassava varieties were significantly different (P<0.05). Processing techniques also significantly influenced the cyanogenic levels of the processed cassava (Table 3).

Table 3. ANOVA summary showing F-ratios of starch, reducing, non-reducing and total sugars and cyanogenic glycoside potential of cassava varieties during processing

Variables	Starch	Reducing sugars	Non-reducing sugars	Total sugars	CNp
Processing technique (PT)	67.91*	1.39	807.78*	909.14*	682.86*
Variety (V)	2097.72*	9.54*	2514.21*	2364.93*	207.53*
Interaction (PT X V)	242.77*	1.50	191.80*	116.64*	32.68*

*Significant at $p < 0.05$

Table 4. ANOVA summary showing F-ratios of appearance (colour) properties of cassava varieties as affected by processing technique and varietal variation

Variables	L-value	a-value	b-value
Processing technique (PT)	104.54*	1854.88	478.87*
Variety (V)	108.66*	53.92*	221.99*
Interaction (PT X V)	17.0763*	142.91*	265.40*

* Significant at $p < 0.05$

During grating, cassava tissues are disrupted which leads to the release of the enzyme, linamarase (Diop, 1998). When this enzyme is released, it hydrolyzes the cyanogenic glycosides into acetone cyanohydrins and glucose. Acetone cyanohydrins dissociate spontaneously to yield hydrogen cyanide which is removed either by volatilization or solubilization (Mkpong *et al.*, 1990). Soaking is also reported to allow for greater extraction of the soluble cyanogens into the soaking water. Tewe and Lutaladio (2004) reported that the latter process removes about 20% of the free cyanide in fresh root after 4 hours, although bound cyanide is only negligibly reduced and also a very significant reduction in total cyanogens is achieved if the soaking water is routinely changed over a period of 3–5 days. Chipping however, involves minimal disruption of the tissues and this might have accounted for the high cyanogenic residuals of the cassava chips. Results from this work however showed that even though grating cassava and soaking cassava in water for 48 hours as expected drastically reduced the HCNp, the levels were still above the acceptable limits of < 10 mg cyanide equivalents/kg DM for flour (FAO/ WHO, 1991). Thus more extensive processing would be required to reduce the potential for toxicity as it is traditionally done.

Conclusion

Processing of the cassava roots had variable effects on the biochemical composition, appearance and cyanogenic glycoside potential with all the different cassava varieties studied. The starch content of the flours from all the studied cassava varieties was below 80% irrespective of the processing method used. Mweulu, Bangweulu and Tanganyika chips and grated had fairly high starch content (>60%) as well soaked Chila B, Mweulu and Tanganyika and thus could be used for many commercial products like starch, alcohol and glucose. The results showed that the different processing techniques had only minimal but significant effects on the starch and sugar content of the different cassava varieties. The chips from the two local and four improved cassava varieties were comparable and had very high cyanogenic glucoside potentials with values ranging between 30.1 mgHCN/ kg in Chila A to 64.3 mgHCN/kg in Bangweulu. Grating and soaking of the roots however caused significant reductions in the levels of the cyanogenic glucosides in all the varieties. The high HCNp levels in the chips of Bangweulu were reduced drastically to 16.2 and 13.5 mgHCN/kg by the grating and soaking treatments respectively. Similar reductions in HCNp levels were noted for all the other varieties. Likewise, grating and soaking caused significant increase in the L*-values of the cassava flours compared to the cassava chip flours. Soaked Kampolombo had the highest L*-value of 78.40 suggesting that the soaked Kampolombo cassava sample was whiter than the rest of the samples. Primary processing of cassava roots from South-eastern Africa by grating and soaking are two methods that should be employed to enhance the safety and appearance properties of their derived flours.

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