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Antinutritional Factors Reduction from Cassava (*Manihot esculenta Crantz***) Roots by Grating or Chipping Processing Technique in Mtwara Tanzania**

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LMK, GMN and EET contributed to concept development and data interpretation, manuscript preparation and finalization. Authors LK, CC and DM contributed to manuscript preparation, samples collection. Author MEK contributed to data analysis and interpretation and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Regular intake of diets high in cyanogenic amount in iodine deficiency areas with limited sulfur supply diets have been associated with the development of hypothyroidism, goiter and cretinism in young and adult populations. This study aimed at the investigation of the suitable processing technique for the production of high-quality cassava flour (HQCF) with acceptable consumption level of cyanide residues from different cassava varieties available at Mmongo village in Mtwara region. Grating technique was used to produce wet and dried cassava grits, whereas chipping technique produced wet and dried cassava chips. The wet and dried cassava products were all produced in a single day and under the same environmental conditions. The results showed that the hydrogen cyanide (HCN) in fresh cassava roots was 62.18–139.28 mg HCN/kg. Upon processing, the cyanide was lower (*P*<0.05) in wet cassava grits 24.27–76.74 mg HCN/Kg but higher in wet cassava chips 44.81–92.57 mg HCN/kg. After sun drying, the HCN retention was higher in dried cassava chips (10.7–14.2%) and lower in dried cassava grits (7.3–9.1%). Grating, efficiently reduced HCN from bitter cassava roots to the level within the WHO/FAO recommended safe limit (10 mg HCN/kg) as chipping only suite for sweet cassava roots. Therefore, HQCF can be nutritionally strategic cassava product consumed regularly and during food shortage without causing potential health problems even in iodine deficiency areas.

Keywords: Cassava; cyanide; cassava chipping; cassava grating; chinyanya.

1. INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is very unique staple crop which is utilized in sub-Saharan Africa to fight hunger during drought and lean harvests, but it can be potentially antinutritional and lethal toxic owing to the presence of cyanogen [1,2]. Cyanogen in cassava is bound to sugar molecules in the form of cyanogenic glycosides that protect the plant against herbivores [3]. The cyanogenic glycoside is composed of lotaustralin (5%) and linamarin (95%) which upon hydrolysis yields cyanohydrins that eventually breaks down into HCN [4,5]. The anti-nutritional factor is due to their impact on aggravating goiter and cretinism in iodine deficiency areas as the cyanogen inhibits the uptake of iodine by the thyroid grand [6,7]. The deficiency can have serious effects in children because of their small body size impairing both body growth and intellectual development [8–10]. Moreover, cyanogen limits bio-availability of sulfur-containing amino acids in marginally protein-deficient diets just as sulfur is utilized in the detoxification of thiocyanate that formed when cyanide reacts with thiosulphate [2]. The cyanogen may also combine with hydroxyl cobalamine which excreted in urine and bile [11].

Tanzania assumed universal salt iodation as measure to prevent iodine deficiency among children and adults in early 1990s [12]. Goiter prevalence declined form 25% to 6.9% (95% CI: 6.8-7.0) in 2004 (12). Despite of the progresses evidenced by the decrease of goiter to 7.0% (2015) along with the national median urinary iodine concentration increasing from 160 µg/L (2010) to 180 µg/L (2015), Mtwara remains one of the areas with potential iodine deficiency. Over 75% of women of child bearing age (15-49 years) tested urinary iodine below150 µg/L [13,14]. Conversely, the possibility of low-income families to consume cyanogen from bitter cassava varieties is relatively high especially during food shortage since some of cassava contains up to 822mg HCN equivalent/kg [15] and inadequately removed. For instance, *Chinyanya* which is prepared by peeling cassava root, pounded and sun-dried for a single day, the cyanide level is reduced to an average 75 mg HCN equivalent/kg on a dry basis [15]. C*hinyanya* dominates staple diet at time of food shortage because of accessibility and cost affordability of cassava [15].

The key constraint of traditional processing methods is their incompetence to remove cyanogen from bitter cassava roots to a safe level [15]. It is obvious that Mtwara's families who subsist on monotonous diet of cassava unfavorably are exposed to increased intake of cyanogen from bitter cassava causing cyanogen from bitter cassava accumulation of toxins and antinutrients in the body. And body detoxification of thiocyanate is likely to be poor due to consumption of marginal protein deficient diets [16]. Mlingi et al. [17] reported children and young adults to have elevated urinary and serum thiocyanate levels. Thus, based on the above evidences necessitated a proper HCN step reduction techniques that could mitigate its level in *chinyanya* and other related traditional cassava products [2,17]. To ascertain this, cassava processing groups which composed mostly women from low-income households were formed and equipped with skills on how to process high-quality cassava flour (HQCF) [18]. HQCF is unfermented cassava flour produced in a single day which is organoleptically safe and has extended shelf life that enables farmers to reach a wider market for better prices [19]. HQCF was produced by using either grating or chipping technique (Fig. 1). Both grater and chipper machines are well described in [20] and have higher performance and size reduction efficiency over 85%. Moreover, cassava
processing groups were guided during processing groups were preparation of HQCF and each step monitored by assessing the reduction potential towards achieving minimal residual cyanide content in cassava products which can be safely consumed by low-income families who are at high risk of dietary exposure. Several key parameters associated with HQCF quality and safety deprivation were also examined.

2. MATERIALS AND METHODS

2.1 Materials

The cassava roots at the age of 18 months were purchased from farmers surrounding the Mmongo cassava processing center at Mmongo village in Mtwara region. Where they were harvested during the morning and transported to the processing center, where were immediately processed into grits or chips on the same day and environmental conditions. The cassava varieties were from two local most bitter varieties (Salanga and Nanjenjeha) and one local most

sweet variety (Nankongoha). *Linamarase* enzyme was extracted from cassava peels and stored frozen until further use.

2.2 Production of Cassava Grits and Cassava Chips

In each experiment, 1000 Kg of the fresh cassava roots were washed before and after peeling to remove all the sands and other contaminants. The cassava grits were obtained when freshly harvested cassava roots were peeled, washed and grated by the use of mechanical grater machine (Intermech Engineering Co. LTD, Morogoro, Tanzania). The fine cassava wet mash was placed in clean jute bags and water squeezed out using a presser machine (Intermech Engineering Co. LTD, Morogoro, Tanzania). After the dewatering process, the pressed cake was disintegrated into wet granules/grits using the same mechanical grater machine and immediately was subjected to direct sun drying on an open space. The

cassava chips were produced immediately after peeling and washing steps. The cleaned cassava roots were placed in the chipper machine (Intermech Engineering Co. LTD, Morogoro, Tanzania) chipped into chips that were subjected to sun drying on the open space. Both cassava grits and cassava chips were processed at the same time and under the same environmental conditions. The detailed flow diagram for the production of both grits and chips is described in Fig. 1.

2.3 Proximate Composition

The determination of the physical and chemical composition of dried grits and chips samples viz: moisture content, ash content, protein content, fat content, sugar content, titratable acidity (TA) and starch content were determined by the methods described by Helrich K [21]. The pH of the high-quality cassava flour was measured using the pH meter as described by Mlingi L. V. [15].

Fig. 1. Flow diagram for production of high quality cassava flour (HQFC) production

2.4 Determination of Hydrogen Cyanide

Sample extraction was done according to Essers AAJ [22] as follows: Fresh cassava root was cut into 1 $cm³$ and randomized. A sample of 50 g was homogenized in 250 mL refrigerated extraction medium in a blender. For dried cassava chips or dried cassava grits 4g were ground and swirled in 25 mL of refrigerated extraction medium in 50 mL closed tube and the homogenates centrifuged at 10000 x g for 15 minutes and the was used as an extract. The extract was stored refrigerated until needed for analysis. Total cyanide analysis: About 0.1mL of extract was added to 0.4 mL buffer pH7 in a test tube, followed by addition of 0.1 mL *linamarase* solution, incubated for 15 minutes at 30° C, then 0.6 mL of 0.2M NaOH was added and left for 5 minutes, then followed by addition of 2.8 mL buffer pH 6. Colorimetric procedure: Chloramine T 0.1 mL reagent was added to the 4 mL buffered extracts in the stopped test tube and mixed, after five minutes 0.6 mL of color reagent was added and mixed. The absorbance was measured by using spectrophotometer at 605 nm after 10 minutes.

$$
HCN = \frac{x (v + s \times \frac{m}{100})}{s (1 - \frac{m}{100}) \times d} \times 0.026
$$

Whereby HCN is hydrogen cyanide; χ is quantity of cyanogen in tube (*nmol*) from calibration curve; V is volume of extraction media (mL); S is Sample weight (g); m is moisture content (%); d is volume of extract assayed (mL).

2.5 Statistics and Data Analysis

Three replications of the study were performed for measurements of all parameters and the data analyzed by one-way ANOVA, using the IBM SPSS statistics 22. Comparison of mean values ± SD (standard deviation) of n=3 between treatments was made by post hoc using Tukey's multiple range tests for chemical and cyanide reduction parameters. Statistical significance was identified at the 95% confidence level (*P*<0.05).

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The chemical composition of dried cassava grits and chips (Table 1) showed the moisture content for dried chips was 6.68–7.89% and dried grits was 8.54–11.54%. The moisture loss was related to shape of cassava products, grits were small rounded whereas chips composed of thin layer that easily loses the moisture. Grits made from Salanga were the only with moisture content above 10%. The overall observation noted dried chips contained moisture content 2.4% lower than that their counterpart dried grits and was significant difference (*P*<0.05). The variation in moisture content between chips and grits concur to the findings reported by other authors [3].

The value of crude fiber for dried chips 2.08– 2.47% and dried grits 1.23–2.47% were slightly higher to established standard for East Africa for HQCF [23]. Crude fat for dried chips was 0.32– 3.5% and 0.24–0.37% for dried grits. Protein for dried chips was 1.31–1.42 mg/100 g and 1.03– 1.34 mg/100g for dried grits. Ash content for dried chips was 1.35–1.69% and dried grits 1.14–1.5%. Sugar content for chips was 1.47– 2.59% and dried grits was 1.19–3.08%. A low content of Crude fiber, protein, ash and sugar observed in all samples were in agreement with other findings for dried cassava flours [24,25]. The pH for dried chips was 5.80–6.19 and dried grits was 5.67–6.99. Starch for dried chips 66.48–68.73% and dried grits was 53.77– 69.14%. The dried chips and grits composed largely amount of starch (*P*>0.05) but relatively lower in grits due to the dewatering process. The starch values are in agreement with that established for HQCF in east Africa standard except for Nanjenjeha which has 6.23% lower to the minimum amount [23]. The variation could be due to variety, harvesting seasonal and maturity level of cassava [26]. Cassava starch may be beneficial to diabetics due to its low glycemic index that allow the release of glucose into the bloodstream at a steady and sustained rate, keeping the body's metabolic processes and energy levels balanced [27]. The pH between chips and grits showed no significant difference (P>0.05) except for Salanga cassava variety. These pH values were within the range (5.5–7.0) for HQCF which is denotes unfermented flour [23]. The titratable acidity (TA) for chips was 0.24–0.34% and dried grits 0.09–0.22%, these values are in agreement with those specified level for HQCF [23] except for Salanga (0.3) and Nakongoha (0.31) which were slightly above. The TA value was inversely proportional to respective pH but slightly high in chips (*P*<0.005) except for Nanjenjeha cassava variety.

Table 1. Chemical composition of dried chips and dried grits produced from local cassava varieties in Mmongo village

*Value are mean ± SD (standard deviation) of n=3. Value within the same raw with different superscript (a-c) is statistically different at P<0.05. ** Bitter cassava variety, * Sweet cassava variety*

Table 2. Cyanogen concentration in fresh cassava roots, cassava products and their retentions

*Value are mean ± SD (standard deviation) of n=3. Values within the same column with different superscript (a-f) are statistically different at P<0.05. ** Bitter cassava variety; * Sweet cassava variety*

3.2 Hydrogen Cyanides Retention in Dried Cassava Grits or Chips

Grating and chipping are the newest techniques currently used by village cassava processing groups (VCPGs) and small and medium enterprises (SMEs) to process high-quality cassava flours (HQCF) [18,24].The findings on cyanide content (Table 2) in fresh bitter cassava were within the range 100–400 mg HCN equivalent/Kg on fresh weight basis as reported by others [10,28]. Similarly, the cyanide content in fresh sweet cassava roots was less 100 mg HCN equivalent/Kg as reported by [28]. The process of grating followed by sun drying was the most effective technique for reducing hydrogen cyanides to the level acceptable to consumers. Results in Table 2 showed that cassava roots with the initial 62.8–139.28 mg HCN equivalent/Kg was reduced to 5.67–11.21 mg HCN equivalent/Kg of dried grits and the HCN retention was only 7.3–9.9%. While the chipping technique reduced the same HCN content to 8.83–17.30 mg HCN equivalent/Kg of dried chips and the HCN retention was 10.7–14.2%, in respective order. Dried cassava chips produced from bitter variety had HCN content above the FAO/WHO recommended safe limit (10 mg HCN/kg cassava products) compared to dried cassava chips (8.83 mg HCN equivalent/kg) of sweet cassava variety. The cyanide content in dried cassava grits from all cassava varieties was within the FAO/WHO recommended safe limit (10 mg HCN equivalent/kg) [15]. Meanwhile, the findings noted the wet grits before subjected to sun-drying had 39.03–67.07% HCN retention while wet chips had 61.49–81.48% in for better and sweet cassava in respective order. The difference observed in cyanide residual between cassava grits and cassava chips is that wet grits production involved de-watering step through which some of HCN precursors such as cyanohydrins which are water soluble may have been washed away in the liquor [15]. Besides, the grating is relatively harsh in disrupting cassava tissues as compared to chipping method enabling direct contact between the *linamarin* and its hydrolyzing enzyme *linamarase*, that facilitate the breakdown of *linamarin* to hydrogen cyanide, which being volatile escaped into the air [4,15]. The breakdown of glucosides during sun-drying upon tissue disintegration depends on enzymatic hydrolysis. This requires a lengthily contact time between the enzyme and its substrates before the inactivation of the enzyme at low moisture content [15]. This is evidenced by dried chips from bitter cassava roots which had slightly higher HCN content (*P*<0.05) than their counterpart dried grits and concur to the results on moisture (Table 1).

3.3 Cyanogen Reduction between Processing Technique (Grating or Chipping) and Sun-drying

A great variation of cyanogen loss occurs between processing steps when cassava tissues are disrupted. Tissues disruption allows enzymes and their substrate (linamarin and lotaustralin) to come into contact and broken to cyanohydrin. The pH 5.8-7.0 (Table 1) is favorable range for cyanohydrin degradation to HCN which dissipate into the air. The results in Figs. 2 and 3 noted a remarkable cyanide loss between peeling and wet grits production that yielded average loss of 50% for bitter and 61% for the sweet variety. The effect was assumed contributed by the cyanohydrin lost in water during de-watering
following adequate pressing. Therewithal following adequate pressing. Therewithal cassava tissues were severely disrupted accelerating enzymatic degradation of cyanogen leading to the evolved HCN escarping to the air. Conversely sun-drying had an average loss of 41.2% for bitter and 52.7% for the sweet variety which were assumed to be mainly enzymatic contribution. Chipping technique had least cyanide reduction 35.9% for bitter varieties and 28.0% for sweet varieties. In chipping, disruption of cassava tissues is mildest causing delay contact between enzyme and its substrate that consequently lead to a little escape of HCN and water loss is minimal. Meanwhile sun-drying attained average loss of 52.7% for bitter variety and 57.0% for sweet variety. Interesting observation is that cyanogen that could not be removed between peeling and wet mash (wet grits/chips) can still be removed during sundrying although grating followed by adequate pressing (de-watering) effectively contributed greatly to the reduction of cyanogen form bitter cassava roots. Chipping could be the insufficient technique in the removal of HCN in bitter roots. This is in agreement with other findings on cyanide glycosides removal in bitter roots [15,29]. A long-term intake of high dietary cyanogen ranging from 14 to 50 mg/day due to insufficiently processed roots of bitter cassava was reported to affect families in Mozambique and Nigeria [30].

Fig. 2. HNC removal efficiency between processing steps during production of dried grits *Key: FCR = Flesh cassava roots, ** Bitter cassava variety, * Sweet cassava variety*

Fig. 3. HNC removal efficiency between processing steps during production of dried chips *Key: FCR = Flesh cassava roots, ** Bitter cassava variety, * Sweet cassava variety*

4. CONCLUSIONS

In conclusion, cassava processing groups are strongly advised to use grater machines for the reduction of cyanogen from bitter cassava varieties. Chipping technique should be applied to cassava varieties with mild cyanogen content (< 61 mgHCN/kg fresh weight basis) as it still

attaining the recommendable 10 mg HCN equivalent/ Kg safe limit. Both grits and chips had appealing white color which is acceptable to most of consumers and cyanogen was adequately removed from bitter cassava within a single day to allow consumption without causing toxicity and anti-nutritional to regular consumers and at the time of food shortage. Moreover, the

perception that cassava is a food for poor households needs to be changed through enhancing market information interventions that will stimulate productions, consumptions and eventual transformation to commercial crop. From these findings, it is recommended that future research to consider monitoring HCN loss
due to extractability, degradation and due to extractability, degradation and volatilization.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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