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Cyanide and selected nutrients content of different preparations of leaves from three cassava species

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Cassava leaves are largely consumed as vegetable in African, but contain a toxic compound, cyanide. To ascertain their safety and contribution to human nutrition, after a number of pre-treatments preceding their boiling in water, cyanide, vitamin C, β -carotene, crude protein, iron, calcium, phosphorus, potassium and zinc contents were assessed in leaves from bitter, sweet and wild cassava species, boiled for 15 and 30 min after differently processed by: (1) pounding un-dried (UND), (2) drying before pounding (DBP) and (3) drying after pounding (DAP). Blanching headed drying was done in a tunnel solar dryer. Results showed that cassava species, processing procedures, and boiling time significantly ($p < 0.05$) reduced cyanide and the nutrients. However, except vitamin C, eliminated to almost nil, other nutrients were retained at considerable levels. Sensibly decreased by drying and/or boiling, cyanide levels ranged from 32 - 50 mg HCN/kg (dry matter basis) after boiling for 30 min. These levels, above the recommended level (10 mg HCN/kg) for foods, were safe with regard to cyanide toxicity based on the fact that the vegetable is served in small quantities as side food. consumed quantities of relishes as side foods. Nevertheless, it was advisable not to make them the everyday foods, especially to lower body weight such as children, and to extend time of cooking.

Key words: Cassava leaves, cassava species, processing procedures, cyanide and nutrients, Rwanda

INTRODUCTION

Cassava is a very important crop in the tropics and a staple food for over 800 million people (Nassar et al., 2007), growing over a range of climates and altitudes and on a wide variety of soils (FAO/IFAD, 2005). In Africa, Rwanda included, cassava is primarily used as food, consuming roots as starchy food (Nweke et al., 2002), but leaves are also largely consumed as green vegetables (Achidi et al., 2005).

Producing a valuable and safe food from cassava and cassava leaves involve certain challenges. In fact, cassava and cassava leaves contain cyanide, in the form of cyanogenic glucosides, primarily linamarin and small

lotaustralin (Uyoh et al., 2007). The cyanogenic glucosides are distributed throughout the cassava plant, with highest levels in leaves (Etonihu et al., 2011). Under high temperature, pressure, and use of enzyme (linamarase), or mineral acids, cyanogenic glucosides are decomposed into acetone cyanohydrins which, at pH above 5 or temperatures above 35°C, is broken down, spontaneously, into poisonous compound, hydrogen cyanide (HCN) (Siritunga and Sayre, 2004). Cyanohydrins are the most dangerous form of the cyanide because at the elevated pHs and temperatures present in the human body, it rapidly decomposes to release the poisonous

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hydrogen cyanide. The continued consumption of high dietary cyanogens has been linked with a number of chronic health disorders, and occasionally death, depending on the level of cyanogens, frequency of cyanogens exposure and, quality and quantity of protein intake status (Cliff et al., 2011; Nhassico et al., 2008; CCDN, 2007). For the human body detoxification, unbound cyanide is converted to less toxic thiocyanate (SCN) and is excreted in the urine. The synthesis of thiocyanate requires sulphur-containing amino acids, as a consequence of protein intake (CCDN, 2007).

Despite the presence of the poisonous component, numerous publications have provided evidence on potential contribution of cassava leaves in human nutrition by providing protein, minerals and vitamins, depending on preparation techniques (Akinwale et al., 2010; Mulokozi et al., 2007; Ayodeji, 2005). Faber and Van Jaarsveld (2007) revealed that improved handling, such as optimizing time of thermal treatment, drying process and preliminary preparations can preserve quality of treated food. In Rwanda, leaves from three species of cassava, bitter (*Manihot utilissima*), sweet (*Manihot dulcis*) and wild (*Manihot glaziovii*) are valued and highly utilized as green vegetables. They are usually cooked freshly harvested, but preservation by sun-drying to extend their shelf life is also reported by Umuhozariho et al., (2011). The direct exposure to sunlight is known to reduce the quality (colour and vitamin contents) of the final product (MMA, 2008). However, solar drying is reported among strategies to combating nutrients losses in processed food stuffs and extending the availability of the nutrient-rich foods, beyond the season in which they are in abundance (Oguche Gladys, 2011; Thompson and Amoroso, 2011).

The present study was undertaken to improve drying of cassava leaves by using tunnel solar dryer and evaluate levels of cyanide and nutrients in the dried and un-dried leaves, from the three cassava species and after preparation as human food.

MATERIALS AND METHODS

Collection of cassava leaves

In June, 2012, tender cassava leaves, the first matured up to leaf position five were harvested from three species of cassava, bitter (*Manihot utilissima*), sweet (*Manihot dulcis*) and wild (*Manihot glaziovii*). Varieties named "Seruruseke" (5280), ISAR 1961 and "Igicucu" were chosen for sweet, bitter and wild, respectively. In order to minimize the effects of age, environment and soil type on chemical composition, leaves samples of the same age were selected from the same field, Rwanda Agricultural Board (RAB)'s field at the Karama Research Station, in Bugesera District of Eastern Province of Rwanda.

Sample preparation

Samples were collected in the field and transported in closed polyethylene bags, which were stored in a cool box containing ice. Each sample was divided into two portions, first portion was analyzed in fresh condition and for the second portion analysis was

done after blanching and drying. Blanching was done by submersion in boiling water for 4-5 min, and then immediately cooled in tap water at ambient temperature as described by Kendall et al. (2010).

Three different preparation procedures were conducted, namely: (1) Un-dried (UND) (2) dried before pounding (DBP) and (3) dried after pounding (DAP) leaves. Pounding was done using wooden mortar and pestle, while drying was done using a tunnel solar dryer at Sokoine University of Agriculture. The products obtained by the three different preparation procedures (Figure 1) were chemically analyzed, un-boiled and boiled for 15 and 30 min. Moisture, cyanide, protein and minerals (Ca, Fe, K, P and Zn) were determined. The first four analyses were conducted at Sokoine University of Agriculture laboratories, while vitamins (Ascorbic acid and β -carotene) analyses were done at the Tanzania Food and Drug Authority (TFDA), in Dar-Es-Salaam. All chemical analyses were carried out in quadruple.

Drying procedures

After blanching, pounded and un-pounded leaves from bitter, sweet and wild cassava were dried using a tunnel solar dryer. Temperatures inside the dryer were recorded at 8 a.m., noon and 8 p.m. each day, averaging 38°C. The complete drying was when the samples were dried until they became brittle. The dried samples were immediately packed in plastic materials, sealed and transported to laboratories, in opaque cartons to avoid light effect before analysis.

Cooking procedures

The cooking consisted of boiling for 15 and 30 min, in distilled water (1:2) and (1:9) respectively for un-dried and dried samples as volume of sample by volume of water, in stainless steel and without cover. The dried samples were first soaked in water for about 5 min before starting the fire. Un-dried and cooked samples were kept frozen before analysis.

Cyanide (HCN) and nutrients determination

Cyanide (HCN) levels in the samples were determined by alkaline titrating method as described by AOAC (1995), official method 915.03B. Moisture content of samples was determined as outlined by AOAC (1995), official method 934.01. For minerals, sample ashes and solutions were obtained respectively by official methods 965.09 and 982.23 described by AOAC (1995). Total phosphorus (P) was obtained using ascorbic acid blue color procedure and by reading the absorbance at a wavelength of 884 nm on a UNICAM 5625 UV/visible spectrometer (Okalebo et al., 1993). Calcium (Ca) and potassium (K) were measured by flame photometry, reading their absorbance at 422.7 and 766.5 nm respectively on a Cole-Parmer instrument, model 2655-00 Digital flame Analyzer. Iron (Fe) and zinc (Zn) were determined by reading their absorbance at 248.3 and 213.9 nm, respectively on a UNICAM 919 Atomic Absorption Spectrometer (AAS) using Hollow Cathode lamps (Okalebo et al., 1993). Crude protein content was determined by using the micro-Kjeldahl method (AOAC, 1995), official method 920.87. Vitamin C (ascorbic acid) content was determined as outlined by ISO (1984), method 6557/2. B-carotene was measured using a high performance liquid chromatography (HPLC), equipped with a Photodiode Array (PDA) detector fitted with a 436 nm wavelength. For sample preparation, aliquots were extracted by solvent n-Hexane (Priadi et al., 2009; Tee Siong and Lam, 1992). Further extraction and clean-up was done using a dispersive Solid Phase Extraction (dSPE) technique as described in AOAC (2007), official method 2007.0.1.

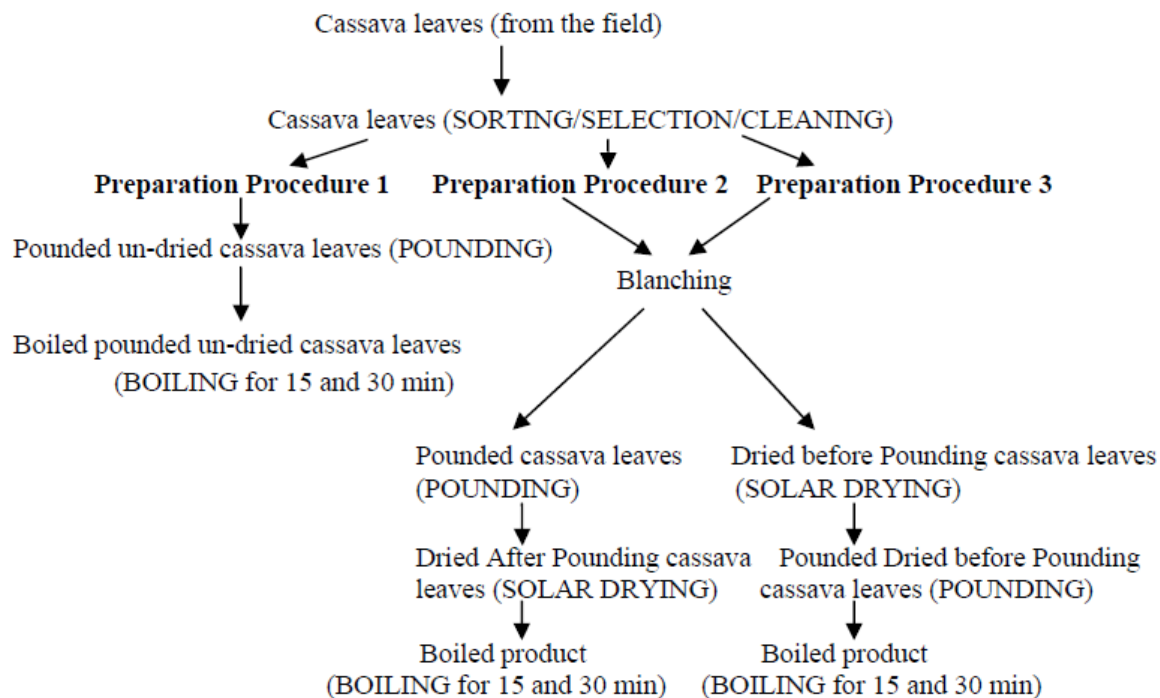


Figure 1. Flow diagram illustrating preparation procedures of cassava leaves.

Statistical data analysis

Data from the results of chemical analyses of samples were subjected to statistical analysis, using SAS 9.2 (SAS Institute, 2008). Kolmogorov-Smirnov test was first carried out to assess the normality of the data (Kutner et al., 2005). Multiple ways (species with three levels specifically bitter, sweet and wild, processing methods with three levels namely un-dried, dried before pounding and dried after pounding, boiling time with 2 levels, that is, 15 and 30 min) analysis of variance (ANOVA) was applied after assuming the normal distribution of the data. Where the treatments had statistical significant effect on the response variables of interest, Fisher's least significant differences (LSD) test was used to separate the means. The treatments were judged statistically significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Cyanide, ascorbic acid, β -carotene, protein, iron, calcium, phosphorus, potassium and zinc were chemically determined in cassava leaves from three species (bitter, sweet and wild). Overall effect of species and processing procedures on contents are shown in Table 1. From the results in the table, it was noticeable that for un-dried and dried samples, before boiling, wild species had the highest concentrations in all determinations (cyanide, ascorbic acid, β -carotene, protein, iron, calcium, phosphorus, potassium and zinc). Bitter species had also high protein content as the wild. Sweet species was less concentrated in cyanide, β -carotene, crude protein, calcium, phosphorus and potassium than wild and bitter. Bitter and sweet species had similar concentrations of ascorbic acid and

iron. Bitter species was less concentrated in zinc than wild and sweet.

The presence of cyanide in all the studied leaves confirmed the earlier reports that all cassava cultivars contain cyanogenic glucoside, in a wide variation according to varieties (CIAT, 2007). The levels of cyanide in fresh leaves of the studied species were 1905, 1480 and 2179 mg HCN/kg respectively, for bitter, sweet and wild. The values were in the ranges reported by earlier researchers, from 189 to 2466 mg HCN/kg fresh weight basis by Fukuba et al. (1982) and 800 to 3200 mg HCN/kg dry matter by Ravindran (1995). The high values in the studied leaves were not surprising as cyanide is known to be distributed throughout the cassava plant, but with highest levels in leaves (Etonihu et al., 2011). However, depending on the varieties, moderate and low cyanide content cassava leaves have been discovered by Burns et al. (2012). As continued consumption of high dietary cyanide has been linked with a number of chronic health disorders (Nhassico et al., 2008; CCDN, 2007), it is evident that, cassava leaves in the present study need to be properly processed to reduce their cyanide before consumption by humans.

Nutrients varied significantly ($p < 0.05$) according to cassava species. Sarkiyayi and Agar (2010) revealed significant differences in protein and mineral contents when investigating sweet and bitter roots and it may be the same phenomenon in the leaves. In un-dried samples, the average values of crude protein (35-36%), iron (230-278 mg/kg), calcium (7373-8822 mg/kg), phosphorus (4413-4907 mg/kg), potassium (15110-17119 mg/kg) and

Table 1. Mean levels of cyanide and selected nutrients of cassava leaves according to species and processing procedure.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	1905.0±25.0 ^b	8.41±0.03 ^b	51.2±0.5 ^b	36.6±0.5 ^a	230.4±1.6 ^b	7441.0±60.3 ^b	4607.7±48.8 ^b	16309.9±102.9 ^b	64.2±0.7 ^c
	Sweet	1480.5±18.9 ^c	8.39±0.02 ^b	40.6±0.3 ^c	35.2±0.8 ^b	230.4±1.3 ^b	7373.5±63.3 ^c	4413.3±45.6 ^c	15110.4±116.8 ^c	67.0±0.6 ^b
	Wild	2179.7±29.1 ^a	13.27±0.09 ^a	80.4±0.3 ^a	36.6±0.2 ^a	278.3±2.1 ^a	8222.5±61.8 ^a	4907.4±50.2 ^a	17119.9±109.5 ^a	76.2±0.7 ^a
DBP	Bitter	562.8±24.2 ^b	0.00075±0.00 ^b	43.7±0.8 ^b	36.8±0.7 ^a	222.5±0.6 ^b	6956.1±65.0 ^b	4590.4±43.5 ^b	15520.6±139.4 ^b	61.9±0.7 ^c
	Sweet	467.8±27.6 ^c	0.00075±0.00 ^b	39.8±0.2 ^c	35.1±0.9 ^b	221.8±1.9 ^b	6587.0±60.3 ^c	4212.0±49.7 ^c	14358.1±150.2 ^c	65.5±1.2 ^b
	Wild	873.3±27.3 ^a	0.00090±0.00 ^a	65.9±0.2 ^a	36.1±0.1 ^a	243.0±0.3 ^a	8070.2±62.1 ^a	4877.4±48.6 ^a	16157.4±133.0 ^a	73.2±0.8 ^a
DAP	Bitter	413.6±21.7 ^b	0.00070±0.00 ^b	39.1±0.1 ^b	36.6±0.1 ^a	215.2±0.1 ^b	6360.2±68.7 ^b	4454.6±48.6 ^b	14693.3±156.2 ^b	59.7±1.3 ^c
	Sweet	352.4±23.6 ^c	0.00070±0.00 ^b	29.1±0.0 ^c	35.2±0.1 ^b	215.3±0.2 ^b	6124.7±69.9 ^c	3957.2±52.3 ^c	12922.2±151.0 ^c	62.1±0.2 ^b
	Wild	684.9±21.6 ^a	0.00080±0.00 ^a	63.5±0.2 ^a	36.3±0.1 ^a	228.9±0.5 ^a	7898.8±63.6 ^a	4607.2±48.7 ^a	15765.7±118.7 ^a	69.5±1.6 ^a

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within columns superscript by similar letter are not significantly different from each other ($P < 0.05$) by Fisher's least significant difference (LSD). PP = Processing procedures, CS = cassava species, HCN = hydrogen cyanide, AA = ascorbic acid, β -C = β -carotene, CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding

zinc (64-76 mg/kg) agreed with values in earlier reports (Dada and Owuru, 2010; Ravindran, 1995). Values of β -carotene (406-804 mg/kg) were in agreement with values mentioned by Priadi et al. (2009). Considering these nutrient levels, it can be said that cassava leaves are good source of β -carotene, protein and minerals and similar observation has been written by Achidi et al. (2005) and Ayodeji (2005). Mulokozi et al. (2007) and Akinwale et al. (2010) noticed also the potential contribution of cassava leaves, especially in vitamin A and suggested to properly prepare them for more profit from their present nutrients.

The cassava leaves were differently processed before cooking: (1) "Un-dried (UND)", (2) "Dried before pounding (DBP)" and (3) "Dried after pounding (DAP)". DBP and DAP leaves were blanched before solar drying, principally for inactivating potentially deleterious enzymes. The leaves were dried to brittle and on average, water content of the leaves varied from 83.5% for fresh to 4.7% for dried. As it is mentioned by James and Kuipers

(2003), green vegetables contains less sugar, and thus, they can be dried to brittle and water content, 4-8%, depending on the type of vegetable. Un-dried samples were more concentrated in cyanide, ascorbic acid, β -carotene, iron, calcium and potassium than dried, but crude protein seemed not to be sensibly affected by drying. Dried before pounding and dried after pounding samples were the first in protein and second in iron and potassium. Dried un-pounded (DBP) samples retained more cyanide and nutrients than the dried pounded leaves (DAP). Drying reduced deeply the ascorbic acid content of the samples. The differences between un-dried and dried leaves were due to the combination effects of blanching and drying, because before drying leaves were blanched. It has been revealed that blanching and drying reduce the poisonous compound, cyanide, but unluckily accompanied by nutrients losses (Oguche Gladys, 2011; Anhwange et al., 2011; Eze, 2010; Abah Idah et al., 2010; Udofia et al., 2010). From the same table (Table 1), it was

observable that pounding promoted cyanide and nutrients removal. The decrease, probably due to leaching or solubility in evaporated or drained water, was facilitated by small sized particles of dried after pounding products.

After the solar drying, the residual cyanide was still high (684-873 mg HCN/kg dry matter). An additional treatment was necessary for safety of the foodstuffs. Cooking by boiling in water is well known to reduce sensibly cyanide (Gernah et al., 2012; Ubi et al., 2008). Therefore, the UND, DBP and DAP were boiled, in distilled water, using stainless materials, for 15 and 30 min. In addition to cyanide reduction, findings in this study showed a significant ($p < 0.05$) decrease in protein, vitamins and minerals with cooking time and it has been the same observation in earlier study of Gernah et al. (2012). The concern was to assess a state of the cooked cassava leaves in regards to cyanogens and nutrients. Means of cyanide, ascorbic acid, β -carotene, protein, iron, calcium, phosphorus, potassium and zinc of the vegetables,

Table 2. Mean levels of cyanide and selected nutrients of boiled cassava leaves for 15 minutes.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	571.2±5.6 ^b	5.78±0.33 ^b	34.1±1.9 ^b	36.8±0.7 ^b	144.7±0.4 ^b	4230.4±11.9 ^c	3644.0±30.6 ^c	8917.0±87.9 ^b	45.8±0.8 ^c
	Sweet	501.9±1.4 ^c	5.39±0.05 ^c	33.1±2.6 ^c	35.5±0.4 ^c	142.5±1.4 ^c	4622.7±34.1 ^b	3872.9±33.9 ^b	8905.2±149.4 ^c	58.8±0.9 ^b
	Wild	696.5±3.3 ^a	11.49±0.04 ^a	49.1±3.1 ^a	36.9±0.8 ^a	210.4±1.8 ^a	6729.3±19.8 ^a	4533.0±31.6 ^a	8984.3±119.6 ^a	68.7±0.8 ^a
DBP	Bitter	532.8±4.2 ^b	-	35.9±0.2 ^b	36.8±0.7 ^b	92.5±0.6 ^c	4186.2±25.0 ^c	3199.4±23.5 ^b	8520.6±139.4 ^b	45.7±2.3 ^c
	Sweet	467.8±4.6 ^c	-	33.0±4.8 ^c	36.7±0.2 ^b	97.8±1.9 ^b	4487.0 ±20.3 ^b	3212.0±20.7 ^b	8858.1±150.2 ^a	58.1±0.2 ^b
	Wild	543.3±4.3 ^a	-	39.8±0.2 ^a	37.2±0.9 ^a	113.0±0.3 ^a	6370.2±32.1 ^a	4346.3±33.4 ^a	8857.4±133.0 ^a	63.5±1.6 ^a
DAP	Bitter	303.6±3.7 ^b	-	34.2±0.1 ^b	35.3±0.2 ^b	92.2±0.1 ^c	4160.2±28.7 ^b	3177.4±28.6 ^b	8493.3±156.2 ^c	45.1±0.1 ^c
	Sweet	292.4±3.6 ^c	-	29.1±0.1 ^c	35.2±0.1 ^b	95.3±0.3 ^b	4424.7±16.9 ^b	3157.2±42.3 ^b	8822.2±151.0 ^b	58.3±0.2 ^b
	Wild	314.9±3.6 ^a	-	35.5±0.2 ^a	36.7±0.1 ^a	112.9±0.5 ^a	6298.8±36.6 ^b	4307.2±38.7 ^a	8855.7±118.7 ^a	86.3±0.1 ^a

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within sub-columns superscript by similar letter are not significantly different from each other ($P < 0.05$) by Fisher's least significant difference (LSD). PP = processing procedures, CS = cassava species, HCN = hydrogen cyanide AA = ascorbic acid, β C = β -carotene, CP=Crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding.

boiled for 15 and 30 min are given respectively in Tables 2 and 3.

After boiling for 15 min (Table 2), depending on species and processing procedure, the residual cyanide levels ranged from 209 to 696 mg HCN/kg and remained high so that an extension of cooking time was highly indispensable. After boiling for 30 min (Table 3), the remaining levels of cyanide, across species and processing procedures, varied from 32 to 50 mg HCN/kg. From Tables 2 and 3, processing procedure that excluded blanching (UND) was more effective in removing cyanide by heating than those procedures that included blanching (DBP and DAP). This was attributed to the action of endogenous linamarase on cyanogenic glucosides, following the intimate contact in the finely-divided tissues, during pounding, between linamarin and the hydrolyzing enzyme, linamarase, which promotes rapid breakdown of cyanogens glucosides into a free form, hydrogen cyanide (HCN) (White et al., 2003), while blanching inactivated enzymes in the dried samples and limited easier hydrolysis of cyanogenic gluco-

sides into hydrogen cyanide. The hydrogen cyanide is known to be easily removed by heat during boiling. Similar to drying, after boiling, it was visible that pounding and then drying (DAP) improved cyanide reduction. The reason may be the same for drying, small sized particles of boiled products.

Cassava leaves as a safe human food

After cooking for 30 min, moisture content of the called "relishes" was on average 87%. Therefore, one kilogram (dry weight basis) is equivalent to about 4 kg of the cooked vegetable as it is eaten (relishes). Under normal circumstances, this volume is shared by many persons in one meal considering an adult person can eat up to 100-200 g of the vegetable relish. Furthermore, different studies reported that an acute oral lethal dose of hydrogen cyanide (HCN) is proportional to body weight (WHO, 2004). But a large variation of the doses showed a lack of precision. For example, levels ranging from 30-210 mg of HCN for a 60 kg adult

have been recorded by Montgomery (1980). Committee of experts in codex standards concluded that a cyanide level of up to 10 mg HCN/kg of cassava flour is not associated with acute toxicity (FAO/WHO, 1993) and the level became recommended by FAO/WHO (1991) as safe for human foods. Therefore, the cassava leaves in this study, un-dried and solar dried, after being boiled for 30 min, can be said to be safe for human consumption in regards to cyanide toxicity, based on the acute oral lethal doses, but also by considering that the quantities of green vegetables are usually small by serving, as a side relish for the starchy based food. Besides, relish from the leaves was found as source of protein, and the nutrient is known to be helpful in cyanide human body detoxification (Nhassico et al., 2008; CCDN, 2007).

Potential contribution of cassava leaves to human nutrition

For the nutrients, the nine vegetable relishes

Table 3. Mean levels of Cyanide and selected nutrients of boiled cassava leaves for 30 minutes.

Parameter		Cyanide (mg/kg)	Vitamin (mg/100g)		Crude Protein (%)	Mineral (mg/kg)				
PP	CS	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn
UND	Bitter	35.4±2.0 ^b	0.24±0.01 ^b	33.0±1.0 ^b	33.7±0.6 ^b	199.4±1.4 ^b	3939.8±27.8 ^c	3564.9±16.5 ^c	8849.3±102.6 ^b	45.4±0.4 ^c
	Sweet	32.8±1.5 ^c	0.20±0.03 ^c	32.6±0.4 ^c	33.8±0.4 ^b	182.7±0.8 ^c	4391.4±24.7 ^b	3795.5±28.2 ^b	8834.1±159.1 ^b	57.9±0.1 ^b
	Wild	40.7±1.5 ^a	0.30±0.01 ^a	43.2±1.9 ^a	34.3±0.2 ^a	219.6±2.5 ^a	5939.3±27.5 ^a	4321.7±23.2 ^a	8960.3±173.5 ^a	68.4±1.2 ^a
	Bitter	47.7±1.1 ^b	-	33.5±0.6 ^b	35.8±0.1 ^a	144.5±2.8 ^b	3509.7±26.3 ^c	3120.9±35.5 ^c	8216.6±124.3 ^c	45.1±0.2 ^c
DBP	Sweet	45.2±0.8 ^c	-	32.6±0.8 ^c	35.0±1.2 ^b	143.9±1.4 ^c	3654.3±28.3 ^b	3200.4±23.6 ^b	8521.8±169.7 ^b	54.4±0.7 ^b
	Wild	50.4±0.4 ^a	-	37.7±0.3 ^a	35.9±0.6 ^a	205.9±3.5 ^a	5436.0±25.6 ^a	4166.9±4.2 ^a	8861.1±180.9 ^a	63.0±1.2 ^a
DAP	Bitter	38.0±2.9 ^b	-	33.2±0.2 ^b	35.3±0.9 ^a	87.1±3.6 ^c	3221.6±33.3 ^c	2982.9±25.0 ^b	7840.7±181.6 ^b	44.5±0.7 ^c
	Sweet	36.4±1.8 ^c	-	29.0±1.8 ^c	35.4±0.5 ^a	95.0±0.6 ^b	3400.2±22.6 ^b	2737.8±25.1 ^c	7008.9±171.6 ^c	51.9±0.4 ^b
	Wild	41.8±2.1 ^a	-	34.3±0.1 ^a	35.0±0.4 ^b	103.9±1.1 ^a	4888.4±18.9 ^a	3898.3±16.9 ^a	8746.5±168.0 ^a	61.7±0.6 ^a

Values are means and SE of nine independent determinations, dry matter basis in quadruple. Means within sub-columns superscript by similar letter are not significantly different from each other (P < 0.05) by Fisher's Least Significant Difference (LSD). PP = Processing procedures, CS = cassava species, HCN = hydrogen cyanide AA = ascorbic acid, βC = β-carotene, CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, PM = processing method, SP = species, UND = un-dried, DBP = dried before pounding and DAP = dried after pounding.

retained appreciable levels of the studied nutrients, except vitamin C, for which levels were too small to be considered as traces in relishes from dried and cooked cassava leaves. The severe reduction of ascorbic acid may be related to the fact that it is thermo-labile at mild heating and very sensitive to blanching, drying and cooking (Faber and Van Jaarsveld, 2007). To understand the contribution of the relishes to human nutrition, their content levels of β-carotene, protein, iron, calcium, phosphorus, potassium and zinc were compared with Recommended Dietary Allowances (RDAs). For β-carotene, because the body converts all dietary sources of vitamin A into retinol, it is explained as retinol activity equivalent (RAE) and believing that 1 μg of retinol is equal to 6 μg of β-carotene (Food and Nutrition Board, 2001), the β-carotene mean values were calculated into RAE before being compared to RDA. Results of the comparison are shown by Table 4.

From the Table 4, the amounts in grams (dry matter basis) of relish from un-dried (UND) leaves

(128, 50, 190, 205, 202, 263, and 14 g) to meet respectively protein, iron, calcium, phosphorus, potassium, zinc and β-carotene RDAs, were less than the amounts of relishes from DBP (128, 60, 208, 228, 210, 272 and 15 g), and DAP (132, 105, 228, 249, 228, 287 and 16 g), needed to meet the respective nutrients (protein, iron, calcium, phosphorus, potassium, zinc and β-carotene) RDAs.

The results showed that relish from un-dried leaves (UND) provides more nutrients than relish from dried leaves. This was attributed to blanching and drying, indispensable treatments for quality and storability (Oguche Gladys, 2011; Anhwange et al., 2011; Eze, 2010). Moreover, comparing the dried samples, drying before pounding (DBP) procedure provides more nutrients than pounding before drying treatment (DAP), but the latter contains less cyanide and then is safer for human consumption. B-carotene and iron are adequately contributed by the cassava leaves, considering the slighter required quantities of the greens to meet their RDAs (Table 4).

Conclusion and recommendations

Cassava leaves of bitter, sweet and wild species, when un-dried or solar dried, have potential to contribute to vitamin A, protein and mineral requirements. Vitamin C is very low in cooked (un-dried and dried) cassava leaves that a complement vitamin C rich-food is necessary to accompany cassava leafy meal. Leaves from wild species are the richest in nutrients, followed by bitter while sweet is the least. Fresh (un-dried) leaves give the more safe and rich foods, but for preservation purposes, solar drying is efficient, and pounding cassava leaves before they are dried is a more recommended drying method for cyanide reduction. Cyanide levels are significantly different in leaves from different cassava species, but after sufficient cooking, the difference is small that vegetable relishes from all the cassava species can be judged to be safe for human consumption. Blanching, drying and prolonged cooking treatments (30 min) reduce sensibly cyanide levels

Table 4. Average levels of nutrients in processed and cooked (30 min) cassava leaves and required amount of the relishes to meet the RDAs.

PP	CP (%)	Fe (mg/kg)	Ca (mg/kg)	P(mg/kg)	K (mg/kg)	Zn (mg/kg)	B-C (µgRAE/g)
UND	35.3±0.4	200.7±3.9	4756.5±59.9	3893.0±37.2	8881.2±180.4	56.6±1.1	61.5
DBP	35.5±1.3	164.1±2.5	4199.4±24.8	3495.5±28.8	8532.4±162.5	54.8±0.8	58.1
DAP	33.3±1.0	95.3±1.4	3836.7±32.6	3205.3±33.5	7864.7±165.4	52.5±1.3	54.4
RDA(mg)	45,000	10	800	800	1800	15	900 (µg RAE)
Required amount of the vegetable relishes (g) to meet the RDAs (dry matter basis)							
UND	128.5	50.2	190.5	205.4	202.6	263.6	14.6
DBP	128.3	60.7	208.0	228.7	210.5	272.3	15.4
DAP	132.3	105.2	228.6	249.6	228.6	287.7	16.5

Values are means and SE of six independent determinations, dry matter basis in quadruple. CP = crude protein, Fe = iron, Ca = calcium, P = phosphorus, K = potassium, Zn = zinc, PP = processing procedures, UND = un-dried, DBP = dried before pounding, DAP = dried after pounding, RDAs = Recommended Dietary Allowances by day and RAE = retinol activity equivalent.

(32-50 mg HCN/kg dry matter basis), but not at recommended level for human foods (10 mg HCN/kg dry matter basis). However, considering the small quantities by serving of green vegetables as side food, the protein level in cassava leaves, important nutrient in cyanide human body detoxification, and the acute oral lethal doses of hydrogen cyanide by bodyweight, the cassava leaves food can be said to be safe for human consumption. However, it is not advisable to consume cassava leaves as an everyday vegetable relish or in large quantities. In addition, cassava leaves meals may be limited for lower body weights such as children. The frequency may be reduced by promoting other greens such as amaranths, spinach and cabbage, even in arid areas, where cassava leaves are highly utilized as human food because other leafy vegetables cannot grow well in the present conditions. Cassava varieties with low levels of cyanide in leaves should be released for leaves consumption purposes to alleviate nutrient deficiencies, especially β -carotene and iron.

In general, amounts of nutrients retained after

cooking un-dried and dried cassava leaves (after 30 min) are significant that cassava leaves as food are judged to contribute nutritionally to human health, especially vitamin A and iron.

Protein content is also of interest, and as cassava leaves are affordable by even poor people whose access to protein rich-foods such as milk, meat and fish is hard, the leaves can be helpful. But time of cooking may be extended to at least 30 min to improve reduction of cyanide level in the cassava leaves relishes.

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