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Determination of the Proximate Composition, Total Carotenoid, Reducing Sugars and Residual Cyanide Levels of Flours of 6 New Yellow and White Cassava (*Manihot esculenta* Crantz) Varieties

C.O. Eleazu and K.C. Eleazu
Department of Biochemistry, National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria

Corresponding Author: C.O. Eleazu, Department of Biochemistry, National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria Tel: +2348034164686

ABSTRACT
The proximate composition, total carotenoid, reducing sugars and residual cyanide levels of flours of 6 new elite yellow and white cassava varieties: UMUCASS 38, UMUCASS 36, UMUCASS 37, TMS05/0473, TMS05/1636 and TMS98/0505 were determined using standard techniques. Results indicated that all the cassava varieties had low moisture contents with TMS98/0505 having the highest moisture contents (12.28±0.95%) than other varieties studied and TMS05/1636 having the least (8.40±0.00%) while the reverse was the case for their dry matter contents which was observed to be high with TMS05/1636 having the highest dry matter (91.60±0.00%) and TMS98/0505 having the least (87.73±0.95%). There were no observed significant differences ($p>0.05$) in the crude fibre and ash contents of all the cassava varieties investigated. UMUCASS 37 was observed to have significantly higher quantities of fats ($p<0.05$) among the cassava varieties studied (2.75±0.31%) while TMS05/1636 had the least (0.80±0.57%). The flours were observed to have low residual cyanide which was higher with the yellow varieties compared with the white varieties. The yellow varieties were also observed to have higher quantities of reducing sugar and carotenoid compared with the white varieties. Results indicate that the yellow varieties may have dual utility both for human consumption and for industrial purposes while the white variety may be confined to domestic use. In addition, their low moisture and high dry matter contents suggest longer storage lives and better cooking qualities for the cassava varieties.

Key words: Cyanide, cassava varieties, cyanide, carotenoid

INTRODUCTION
Cassava (*Manihot esculenta* Crantz) is a cheap and reliable source of food for more than 700 million people in the developing countries and is Africa's second most important staple food after maize in terms of calories consumed, with Nigeria being the largest producing country in the world (FAO, 2003; Eleazu *et al*., 2011a).

Vitamins are essential food receptors which are important for the proper utilization of the proximate principles of foods such as carbohydrates, proteins and fats in addition to the maintenance of good health in humans and experimental animals (Pamela and Champe, 1994; Robert *et al*., 1993).

Carotenoids are compounds found in plants that can enhance the human health immune response and reduce the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts, etc. (Astorg, 1997; Burri, 1997; Mayne, 1996; Olson, 1999; Olson and Krinsky, 1995) and these have been attributed to their antioxidant property, specifically, their ability to quench singlet oxygen and interact with free radicals (Palozza and Krinsky, 1992). This source of vitamin A from carotenoids (β-carotene) in vegetables and fruits is the main source for most people in developing countries. It makes up 70-90% of all their dietary vitamin A intake. Studies have indicated a significant reduction in maternal mortality and postpartum infection with either vitamin A or β-carotene supplementation.

Vitamin A deficiency is among the most common and serious of all nutritional deficiency diseases. Many studies have investigated the effect of vitamin A supplementation on various infections in children, the most dramatic benefit being obtained in measles with a marked reduction in its mortality (Donald and Martin, 2001).

In Africa, eye lesions often occur in measles and this is frequently due to a combination of xerophthalmia, measles itself (which directly affects the eye) and damage caused by traditional eye medical practitioners. The outcome has been reported to be improved with vitamin A supplementation in diseases. Infants born to women with HIV infection have a higher mortality, poorer growth and morbidity than those on vitamin A supplementation (Donald and Martin, 2001). Studies carried out show that about 50% of the preschool children in the developing countries are vitamin A deficient and this is a cause for concern for those living in those regions. It has been calculated that improvement of vitamin A status would reduce mortality rate in young children aged under 5 years by about 23% (Donald and Martin, 2001). This has thus necessitated the need for supply of foods that contain significant quantities of carotenoids especially for young children or the fortification of existing foods to meet the vitamin A requirement.

One of the characteristics of cassava plant (*Manihot esculenta* Crantz) is the general presence of linamarin, a glycoside that is hydrolysable under certain circumstances and releases cyanide (HCN) in a process called cyanogenesis (Fig. 1). The use of cassava products for food or feed consumption is strongly influenced by these toxic cyanogenic glycosides and the international literature is concerned about the residual levels remaining in cassava food (Brito *et al.*, 2010; Baltha and Cereda, 2006). Whilst there are few reports of poisoning and death due to cyanide intake from cassava consumption (Akintonwa *et al.*, 1994), there are several disorders which have been associated with regular intake of cassava (either long term or short term) such as goiter and cretinism which are due to both iodine deficiency and intake of cyanide.

In order to develop strategies to deal with and hopefully prevent further outbreaks of these crippling disorders, it is important to have available good methods for determination of the

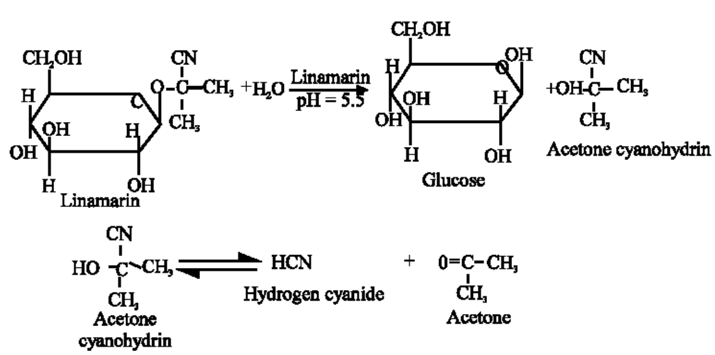

Fig. 1: Hydrolysis of linamarin (Cooke, 1978)

cyanogenic potential of cassava roots and the processed products. Many methods have been developed for determination of the total cyanide content of cassava (Bradbury *et al.*, 1994, 1995; Cooke, 1978). The limitation has been that most of them are either not easily reachable or affordable especially for those in developing countries where cassava is a staple diet. In addition, most of these methods can only be carried out properly by skilled personnel in a reasonably well equipped laboratory. To be able to monitor the levels of cyanogenic potential of processed cassava flour, a simple method is needed that can be used by an unskilled personnel.

The picrate method which forms a red colored complex with cyanide has been reported as the method of choice in determining the total cyanide content of plants of importance for human food (Egan *et al.*, 1998; Williams and Edwards, 1980).

In a bid to enhance the nutritive qualities of cassava through the supply of pro-vitamin A varieties with low cyanogenic potentials, six of the elite new yellow and white cassava varieties of National Root Crops Research Institute, Umudike were screened for their total carotenoid, residual cyanide, reducing sugar, moisture, dry matter, ash and crude fibre contents and the results are reported in this study.

MATERIALS AND METHODS

Preparation of plant materials: Six elite new yellow and white cassava varieties: UMUCASS 38 (Yellow), UMUCASS 36 (Yellow), UMUCASS 37 (Yellow), TMS05/0473 (Yellow), TMS05/1636 (Yellow), TMS98/0505 (White) were freshly harvested from the experimental farm of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria. The tubers were washed, peeled, sliced, oven dried at a temperature of 60°C for 24 h and ground to flour. The flour was used to analyze the proximate, cyanide and reducing sugar contents. For the analysis of total carotenoids, the fresh samples were used.

Proximate analysis: The moisture, dry matter, ash, crude fibre and lipid contents of the cassava varieties were determined using the AOAC (1990) methods.

Carotenoid analysis: The carotenoid content of the fresh cassava samples was determined using the method of Rodriguez-Amaya and Kimura (2004).

Cyanide analysis: The residual cyanide levels of the flours of the cassava varieties were determined using the alkaline picrate method (Onwuka, 2005) with modifications. Five gram of each sample was dissolved in 50 mL distilled water and allowed to stay overnight. The sample was filtered and the filtrate was used for the cyanide determination. To 1 mL of the aqueous extract was added, 4 mL of alkaline picrate (obtained by dissolving 1 g of picrate and 5 g of Na_2CO_3 in 200 mL of distilled water) and the whole setup was incubated in a water bath at a temperature of 50°C for 5 min. The formation of a dark red color was read spectrophotometrically at 490 nm against a reagent blank which contained 1 mL of distilled water and 4 mL of alkaline picrate solution. The cyanide content of the flours was extrapolated from a standard curve that was prepared by diluting potassium cyanide (KCN) standard (in water, acidified with HCL) to varying concentrations of 0.01 to 0.05 $\mu g\ mL^{-1}$ in 0.01 increments as shown in Fig. 2 using the equation of the standard graph: $Y = 3.23x+0.217 *10$ ($R^2 = 0.775$) Where Y = Unknown concentration of the sample; 3.23 = slope of the graph, x = absorbance of the sample; 0.217 = intercept and 10 = dilution factor.

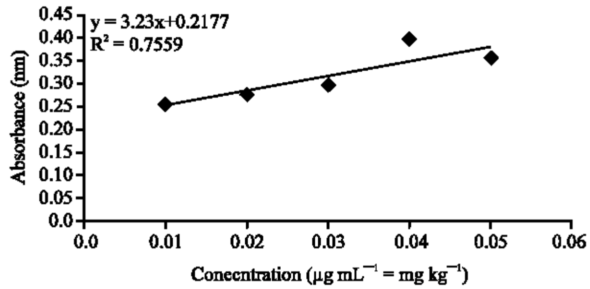

Fig. 2: Cyanide standard curve

Reducing sugar analysis: The dinitrosalicyclic acid reagent (DNS) method (Miller, 1972) was employed in the determination of the reducing sugar composition of the 6 cassava varieties.

Statistical analysis: Data was subjected to analysis of variance using the Statistical Package for Social Sciences (SPSS), version 15.0. Results are presented as Mean±standard deviations. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $p<0.05$ using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

The percentage moisture contents of the cassava varieties (dry wt basis) as observed in Table 1 ranged from 8.40 to 13.23 with TMS98/0505 whose moisture content did not significantly differ from that of UMUCASS 36, UMUCASS 37 and TMS05/1636 having the highest moisture content (12.28±0.95) among other varieties studied while TMS05/1636, though not significantly different from that of UMUCASS 36, UMUCASS 37 and TMS98/0505 had the least (8.4±0.0). All the cassava varieties had low moisture contents and this is a good attribute for storage. In addition, the lower moisture contents of TMS05/1636 compared with the other varieties analyzed shows that it may have longer storage lives if packaged well and stored.

Crude fibre represents that portion of food not used up by the body but mainly made up of cellulose together with a little lignin and is known to increase bulk stool (Eleazu *et al.*, 2011b). Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60-80% of the cellulose and 4-6% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity. Values obtained in all the cassava varieties analyzed, as shown in Table 1 indicate that there were no significant differences in the fibre contents of all the cassava varieties investigated ($p>0.05$).

Fats are vital to the structure and biological functions of cells and are used as alternative energy source. UMUCASS 37 had significantly higher fat contents (2.75±0.31%) ($p<0.05$) than other cassava varieties investigated while that of TMS05/1636 which did not significantly differ ($p>0.05$) from the fat contents of UMUCASS 38, UMUCASS 36, TMS05/0473 and TMS98/0505 respectively as shown in Table 1 was the least (0.8±0.52%).

Ash is a reflection of the inorganic mineral elements present in the samples. Some of the samples investigated contained significant quantities of ash which did not significantly from each other ($p>0.05$) (Table 1).

Dry matter content relates to good cooking quality. Higher dry matter contents suggests better cooking qualities and extended storage lives. The high dry matter contents of the all the cassava

Table 1: Proximate composition of 6 cassava varieties (%)

Par	UMUCASS38	UMUCASS36	UMUCASS37	TMS05/0473	TMS05/1636	TMS98/0505
Moisture content	9.85±0.07[a]	9.60±1.27[b]	9.08±0.04[b]	8.63±0.30[a]	8.40±0.00[b]	12.28±0.95[b]
Crude fibre	2.02±0.02[a]	2.32±0.40[a]	1.84±0.33[a]	1.65±0.11[a]	2.0±00.47[a]	1.78±0.49[a]
Fat	1.38±0.78[b]	0.98±0.35[b]	2.75±0.31[a]	1.15±0.03[b]	0.80±0.57[b]	0.95±0.40[b]
Ash	2.35±0.35[a]	2.15±0.17[a]	1.48±0.31[a]	1.54±0.47[a]	1.44±0.52[a]	1.92±0.73[a]
Dry matter	90.15±0.07[a]	90.30±1.13[a]	90.93±0.04[a]	91.38±0.39[a]	91.60±0.00[a]	87.73±0.95[a]

Values in each row with the same superscript are not significantly different from each other ($p>0.05$); N: 6 cassava varieties replicated three times

varieties analyzed (Table 1), which did not differ significantly from each other($p>0.05$) suggest better cooking qualities of the flours that would be produced from these cassava varieties in addition to their longer storage lives. This is a significant finding in this present study.

Due to the concern about the residual levels of cyanide remaining in the cassava plants after being processed, the cassava roots were classified according to their potential toxicity to humans and animals as: non toxic (less than 50 mg HCN kg^{-1} in fresh roots), moderately toxic (50-100 mg HCN kg^{-1} of fresh roots) and dangerously toxic (above 100 mg HCN kg kg^{-1} of fresh roots) (Delange *et al*., 1982). The lethal dose of cyanide in humans has been reported by several authors as ranging between 50 to 300 mg kg^{-1} body weight (Bolhuis, 1954; Akiyama *et al*., 2006).

The residual cyanide levels in the flours of all the cassava varieties investigated ranged from 2.43 to 3.4 mg kg^{-1} with UMUCASS 37 having the highest cyanide level among the cassava varieties investigated (3.08±0.32 mg kg^{-1}) while TMS98/0505 had the least (2.56±0.13 mg kg^{-1}). The low cyanide levels of the flours of all the cassava varieties investigated as shown in figure 1 is attributed to their method of processing which employed both soaking in water (for 24 h) and oven drying as both methods of processing have been reported to reduce the cyanide contents of cassava (Bradbury, 2004; Eleazu *et al*., 2011b), with the former (soaking in water) tending to bring about fermentation due to the introduction of moisture. The implication is that the usage of the flours made from these varieties of cassava for human consumption may not confer any toxic effect to the user. This is because the body has a natural metabolic pathway to detoxify cyanide that employs rhodanese. Thus any residual cyanide in the cassava varieties may serve as a substrate for rhodanese *in vivo* in the liver. However, consumption of these cassava varieties as staple food may need to be matched with a proteinaceous diet from exogenous sources. This is because cassava is known to contain very low quantities of protein and the body's detoxification mechanism of cyanide through the catalytic action of rhodanese (conversion of cyanide to thiocyanate) uses up part of the pool of the sulfur containing essential amino acids: methionine, cysteine and cystine that can only be obtained from the food consumed. Thus any shortfall of these sulphur containing amino acids would limit protein synthesis and could cause stunted growth in children. More so, consumption of doses of cyanide for a long period of time may overwhelm the detoxification mechanism of rhodanese, there by leading to devastating effects such as: Cerebral brain damage, konzo, parkinsonism, etc. However, some studies reported that bitter yellow cassava roots could be promising sources of proteins (Chavez *et al*., 1999), although this was not determined in this work. Even if this were to be the case for these cassava varieties, it still may not preclude the need for dietary supplementation of their proteins from other sources, as plant proteins can deficient in some essential amino acids. This is approached from a biochemical point of view. This study thus underscores the need for the assay of the total protein and possibly amino

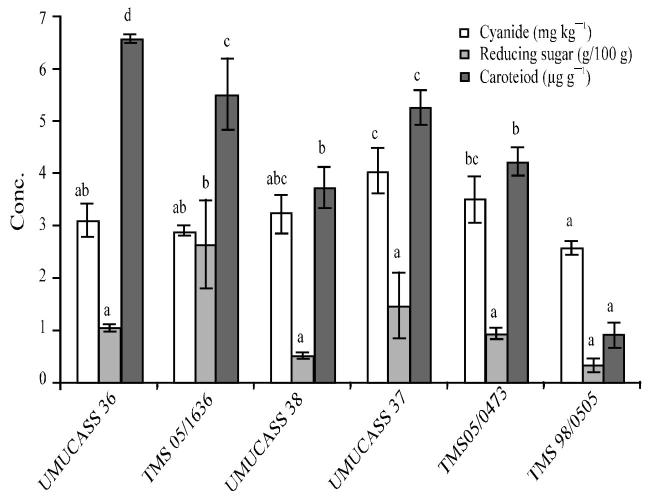

Fig. 3: Residual cyanide, total carotenoid and reducing sugar contents of 6 cassava varieties, Values with the same superscripts for each parameter are not significantly different from each other ($p<0.05$)

acid profiles of these cassava varieties. In addition, all the yellow cassava varieties had significantly higher cyanide contents ($p<0.05$) than the white variety (TMS98/0505) (Fig. 3) and this is the most significant finding in this present study. Several authors have reported that yellow cassava varieties have bitter tastes (Iglesias *et al*., 1997). This bitter taste can be attributed to the cyanide contents of the yellow cassava varieties. Although, there are no facts in literature to substantiate this as many scientists have died trying to elucidate this, the report given by the Indian goldsmith who reported the taste of cyanide as being acrid gives us a clue. In addition, hydrogen cyanide is an acid and acids are known to have a sour taste. Thus its only plausible to attribute the bitter tastes that were reported by the authors in the yellow cassava varieties to the cyanide contents of these cassava varieties.

Carotenoids compounds as found in plants, enhance the human health immune response and reduce the risk of degenerative diseases such as cancer, cardiovascular diseases etc and these have been attributed to their antioxidant and free radical scavenging activities. All the yellow cassava varieties investigated as observed in Fig. 3, had significantly higher quantities of carotenoids than the white variety and this may confer antioxidant potentials on these yellow cassava varieties. In addition, the predominant carotenoid in these yellow cassava varieties is β-carotene (makes up about 90% of total carotenoid in cassava) and being that six micrograms of β-carotene equals to one microgram of vitamin A, there's the need for the dietary supplementation of these cassava varieties as their consumption may not meet the Recommended Daily Allowance (RDA) for vitamin A in men (750-100 µg daily), women (750 µg daily) and children (400-600 µg daily). However, their consumption will reduce amount of dietary supplement needed to meet the RDA of vitamin A which is a good attribute for them.

Cassava flour presents a good substrate for alcohol production due to its high content of fermentable sugars and stable shelf-life (Ocloo and Ayernor, 2010).

The reducing sugar contents of TMS05/1636 (2.62±0.00 g/100 g) which was significantly higher than other cassava varieties investigated ($p<0.05$) shows promising potentials of its utilization in ethanol production while this may not be applicable to TMS98/0505 whose reducing sugar content (0.31±0.01 g/100 g) was observed to be the lowest amongst the cassava varieties investigated. In addition, the yellow cassava varieties were observed to have higher quantities of reducing sugars than the white varieties and this is another significant finding in this present study (Fig. 3). The higher amounts of reducing sugars observed in the yellow varieties are attributed to varietal differences and possibly to the phenolic compounds that may be present in them. This is subject to further investigation and confirmation.

CONCLUSION

Results showed that all the cassava varieties investigated had low quantities of residual cyanide. In addition, all the yellow varieties had higher cyanide, reducing sugar and total carotenoids which confers upon them, a dual utility both for human consumption and for industrial purpose while the white variety may be confined to domestic use. Most of the cassava varieties were observed to contain significant quantities of minerals and utilization of the yellow varieties for vitamin A purposes may require dietary supplementation with vitamin A from exogenous sources.

Finally, the low moisture and high dry matter contents of all the cassava varieties suggest longer storage lives and better cooking qualities for them.

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