

Estimation of Nutrients and Anti-nutrients Components of Three Nigerian Leafy Vegetables.

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Abstract

Three Nigerian leafy vegetable species were analyzed for their proximate composition, mineral constituents, energy values and their anti-nutritional factors. The mean values for the proximate composition were 19.04, 13.62, 7.07, 1.84 35.68, 22.74 and 77.26% for crude protein, crude fibre, Ash, fat, carbohydrate, moisture and dry matter contents respectively, and the mean gross energy value was 235.47 Kcal/100g. Moderate variations were observed in the proximate composition of the vegetables analyzed as indicated by the moderately high coefficient of variation, except for moisture which was high 82.59%. The ten minerals analyzed (Na, K ,Ca, P, Fe, Mg, Cu, Zn, Mn and Co) were all present in all the three samples, with Cu been least in Ugwu (*Telfaria occidentalis*) (0.24±0.01), Ebolo (*Crassocephalum crepidiodes*) (0.20±0.01) and Mn least in *Manihot esculenta* (0.10+0.01) mg/100g. Phosphorus was the most abundant in Ugwu (14.60+0.04) and Ebolo (6.23±0.04)mg/100g, while K was the most abundant (7.73±0.05) mg/100g in *Manihot esculenta*. The Na/K ratio was less than one (<1) in all the samples but the Ca/P ratio was very high for *Manihot esculenta* 6.20. Marked variations were observed in the mineral composition of the samples as indicated by the high co-efficient of variations. The anti-nutrients analyzed Tannin, Saponin, Phytic acid, oxalate and HCN were significantly present with tannin been the most abundant in *Manihot esculenta* (4108. 67±0.90) mg/100g and HCN been the least abundant in Ugwu (3.10±0.02) mg/100g. There were distinct familial differences in these anti-nutrient constituents as indicate by the high co-efficient of variation of 160.78, 145.36, 139.80, 90.20 and 67.28% for Tannin, Saponin, oxalate, phytic acid, and HCN, respectively. The nutritive potentials of the samples are highlighted, dietary implications of the anti-nutrients are also mentioned, and the need to develop food/feed safe programmes involving these inherent factors discussed.

Key words: Proximate composition, minerals, anti-nutritional constituents.

1. Introduction

Efficient exploitation of available food resources is needed to feed the growing population and to develop agriculture especially in developing countries. Conventional food resources do not always meet the nutritional needs of humans. This justifies increasing research in the development of non conventional food resources especially high protein content food from plants.

Fruits and vegetables have various chemical components and their main contribution to human diet being vitamins, minerals, and sometimes carbohydrate which are of great importance to body metabolism. They are important sources of vitamin C, Fe and Ca (Baid, 1980). Several vegetables species abound in Nigeria which are utilized either as condiments or spices in human diets, or as supplementary feeds to live stock such as rabbits, cattle, swine and poultry. These vegetables are harvested at all stages of growth and fed either processed, semi-processed or fresh to man while they are usually offered fresh to livestock (Aletor and Adeogun, 1995).

Fluted pumpkin *(Telfairia Occidentalis)* is a tropical crop that belongs to the cucurbitaceae family (Irvine, 1969). It is reported to be indigenous to the west tropical rain forest area of Nigeria (Akoroda, 1990). The cultivation of fluted pumpkin (Esiaba, 1982, Okoli and Mgbeogu, 1983), its yield (Oloyo, 2001) and the chemical composition have been reported by Maduewesi, (1977) and Longe et al, (1983). Fluted pumpkin is mainly cultivated as a leafy vegetable (Okigbo, 1977) that are used for soup while its fruit and seeds are wasted.

Cassava *(Manihot esculenta*, Crantz) leaves a byproduct of cassava root harvest (depending on the varieties) is rich in protein (14-40% dry matter), vitamin B_1, B_2, C and carotenes (Eggum, 1970, Adewusi and Bradbury, 1993). Available literature clearly suggest, that apart from lower methionine, lysine and perhaps isoleucine content, the amino acid profile of cassava leaf protein compares favourably with those of milk, cheese, soyabean, fish and egg (Ayodeji,2005). In spite of these qualities, the nutritional potentials of cassava leaf meal and cassava protein concentrates remain currently under-researched. The major drawback to the widespread use of cassava leaves as food in Nigerian is "cyanide scare" as its content of cyanogenic glucosides could, depending on the variety, be 6 times higher than in the roots (Ayodeji, 2005). Apart from cyanide, tannin and possibly phytin (Reddy et al, 1982) may limit the nutritional value of cassava leaves.

The upsurge in the consumption of vegetables in many Nigerian homes due, perhaps, to the high cost of meat, indicates a need for a comprehensive list of nutritional and anti-nutritional characteristics. While most studies (especially on anti-nutritional factors) relate to their presence in European and North American species, it is conceivable that the presence of these compounds and the resultant effects on human health, are of more importance in the diets of the less well developed countries (LDCs). In these countries, the diets may be less varied and any physiological effects caused by these compounds may exacerbate existing problems of malnutrition and/ or under nutrition.

This paper presents the results of the proximate, mineral, and anti-nutritional composition of three Nigerian leafy vegetable in order to highlight their nutritional significance.

2. Materials and methods

The three vegetables analyzed were *Telfairia occidentalis*, *Crassocephalum crepidiodes* and *Manihot esculenta.* All the samples were collected in fresh conditions from the local farmers in Offa Local Government area of Kwara State Nigeria. They were air dried, blended, and stored in polythene bags inside the refrigeration prior to analysis.

2.1 Proximate Composition And Gross Energy

The proximate constituents of the air-dried samples (moisture, crude fibre, Ash, and fat) were carried out in triplicate using the methods described by AOAC, (1990). Nitrogen was estimated by the micro-Kjeldhal method and the percentage nitrogen was converted crude protein by multiplying with 6.25. The carbohydrate was determined by simple difference, while calorific values were obtained by the summation of multiplied values of protein, fat, and carbohydrate by their respective Atwater factors of 4, 9, and 4 Kcal/g (Udosen,, 1995and Eneche, 1999).

2.2 Mineral Analysis

The mineral sodium and potassium were determine by flame photometry, while other minerals were determined after wet digestion with a mixture of nitric ,sulphuric ,perchloric acids using atomic absorption spectrophotometer (AAS, SP9 model), and phosphous was determined by the vanodo-molybdate method (AOAC, 1990).

2.3 Determination of anti-nutrients

2.3.1 Phytic acid.

The extractions and precipitation of the phytin in the samples were done by the method of wheeler and Ferrel, (1971) while iron in the precipitate was determined as described by Makower (1970). Using a 4:6 Fe/P ratio to calculate phytin phosphours by 3.55 as suggested by young and Greaves, (1940). Where extracts were deeply coloured, they were decolorized by activated charcoal.

2.3.2 Tannin (Polyphenols)

The vegetable samples, finely milled (250mg in 10ml of 70% aqueous acetone) were extracted for 2hrs at 30^{o}C using Gallenkamp orbital shaker (Survey, UK). Pigments and fats were first removed from the vegetable by extracting with diethyl ether containing 1% acetic acid. Thereafter, the total polyphenols (as tannic equivalent) were determined in 0.05, 0.2, or 0.5ml aliquot using Folin Cocalteu (sigma) and standard tannic acid (0.5mg/ml) as described by Makkar and Godchild (1996).

2.3.3 Hydrocyanic acid (HCN)

The cyanogenic potential of the samples were determined (after an initial extraction for 2-3 min of 5-8g material in 0.1M H_3PO_4 by a 2M H_2SO_4) (100^{o}C for 50mins) hydrolysis followed by reaction with chloramines-

T pyridine barbituric acid (Konig Reaction) as developed by Bradbury et al (1991). KCN dried over concentrated H_2SO_4 was used to calibrate the standard curve from a stock solution contain 75mgKCN /100ml.

2.3.4 Oxalate

Oxalate content was determined by the titrimetric method of Moir (1953) as modified by Ranjhan and Krishna (1980). Where extracts were intensely coloured, they were decolorized with activated charcoal (Balogun and Fetuga, 1988).

2.3.5 Saponin

The method used was that of Obadoni and Ochuko, (2001). The samples were grounded and 20g of each were put into a conical flask and $100cm^3$ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for four hours with continuous stirring at about 55^oC. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^oC. The concentrate was transferred into a 250ml seperatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60ml of n-butanol was added. The combined n-butanol extract were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated as mg/100g.

3. Statistical Analysis

The experiments were performed in triplicate and the means $\pm$ standard deviation of three values were reported. Mean values for all the parameters within the samples were assigned coefficients of variation (Steel and Torrie, 1960) and Skewness.

4. Results

The proximate composition (%) and the gross energy values (Kcal/100g) of the three leafy vegetables are presented in Table 1. The crude protein (CP) ranges from 14.41$\pm$0.1 % for *Manihot esculenta* to 25.20$\pm$0.1% for *Telfairia occidentalis* and a co-efficient of variation of 29.20%. The mean crude fibre (CF) content was 13.62%, with a range of 7.87$\pm$ 0.02% in *M. esculenta* to 18.00$\pm$ 0.4 in *Crassocephalum crepidiodes* with a CV of 38.18%. The fat content was average 1.84% and ranges 1.50$\pm$01% in C. *Crepidiodes* to 2.50$\pm$0.2% in *T. Occidentalis*, with a CV of 30. .98% and ash averaged 7.07%.

Table II presents the mineral composition (mg/100g) of the three leafy vegetables which varies thus K (4.40$\pm$0.02 to 7.73$\pm$0.03), Ca (1.24$\pm$0.03 to 2.63$\pm$0.01), Mg (0.80$\pm$0.01 to 2.40$\pm$0.03), P (0.20$\pm$0.01 to 14.60$\pm$0.04), Na (3.20$\pm$0.02 to 5.37$\pm$0.02); Fe (1.50$\pm$0.01 to 3.26$\pm$0.03), Mn (0.10$\pm$0.01 to 3.72$\pm$0.02), Cu (0.20$\pm$0.01 to 0.50 $\pm$ 0.01), Zn (2.93$\pm$0.03 to 5.25$\pm$0.03) and Co (1.20$\pm$0.01 to 2.20$\pm$0.02). The samples differed markedly in P, Mn, Mg, Cu, Fe, Ca, Zn and Co content as indicated by the high CV of 103.14, 87.26, 52.94, 51.61, 41.44, 38.67, 34.31, and 31.29% respectively. All the minerals were positively skewed except Cu that was negatively skewed. The Na/K ratio varies from 0.68 in *T. Occidentalis* to 0.73 in *C. Crepidiodes* and Ca/P varies from 0.18 in *T.occidentalis* to 6.2 in *M. esculenta*.

The anti-nutritional composition (mg/100g) was presented in table III. The mean values were 1438.36, 5.93, 282.52, 364.03, and 721.33mg/100g for Tannin, HCN, Saponin, phytic acid, and oxalates respectively. There was marked difference in their composition as indicated by their high co-efficient of variation 160.78%, 148.36, 139.80, 90.20 and 67.28% respectively for tannin, saponin, oxalate, phytic acid and HCN. They were all positively skewed.

5. Discussion

The data on the proximate, energy and mineral contents of the three leafy vegetables clearly indicate their potential as food or feed resources. For example, their mean content of crude protein 19.04%, crude fibre 13.62% and ash 7.07% compare favourably with and in certain cases, surpass those reported for most legumes (except groundnut and soyabean) grown in West Africa (FAO, 1973; Ologhobo, 1980; Aletor and Aladejimi, 1989). Also, their protein contents were generally higher than those reported for some cassava leaf varieties

(Eggum, 1970, Ravindran et al, 1987; Ayodeji, 2005) and comparable to that reported for several leguminous browse plants (Aletor and Omodara, 1994) and several tropical leafy vegetables (Alefor and Adeogun, 1995). They were richer in crude protein and ash but lower in fat than Mango seed and Malina fruit with mean values of crude protein 12.79%, Ash 5.5%, and fat 28.75% (Etong et al, 2013). They were lower in protein but comparable in crude fibre to defatted cake from two Euphorbiaceae (oil seed plant) from Cameroon, total protein 51.45% and crude fibre 13.30% (Clerge at al 2006) and were also better than *Irvingia gabonensis* nut 7.4% crude protein, 2.5% ash and 0.9% crude fibre (Ejifor et al, 1987). There was no marked difference in terms of their proximate composition except for moisture content as indicated by the CV 82.59%. Carbohydrate, crude fibre and dry matter were negatively skewed. However, their high crude fibre content average 13.62% are of nutritional concern (especially in non-ruminants) since high dietary fibre can cause intestinal irritation, lower digestibility, and overall decreased nutrient utilization (Johnson, 1987).

The high mineral element contents, particularly P, K, Na, Zn and Fe in the vegetables when compared with other plants, such as legumes and tubers confirm their importance as rich sources of dietary minerals, P (14.60$\pm$ 0.4) and 6.23$\pm$ 0.04 and K 7.73$\pm$ 0.05mg/100g were the most abundant, while Cu 0.24$\pm$0.01 and 0.20$\pm$0.01 and Mn 0.10$\pm$0.01mg/100g were the least abundant in *T.occidentalis C.crepidiodes* and *M. esculenta* respectively.

The mineral levels were lower than that reported by Aletor and Adeogun, (1995) Ca (2.49), P (1.41), K (3.7), Na (3.80 and Mg (1.2) g/100g for some tropical leafy vegetables, melina fruit and mango seed mean values Ca (20.52), Mg (49.56), Na (162.01), K (195.26), Zn (23.84), and Cu (44.82)mg/100g (Etong et al, 2013), *Citrus sinensis* seeds ripe and unripe Na (0.54-0.57), P (0.87-0.97), K (1.13-1.28), Ca (0.30-0.39)g/100g, *Carica papaya* seeds Ca (0.59), Na (0.69), K (8.15), P (0.89),Mg(0.41), Zn(25.89)g/100g (Abulude,2000) and fish (Adeyeye, 1996). The ratios of sodium to potassium (Na/K) and calcium to phosphorus are shown in Table II. These ratios are of great importance in the control of high blood pressure in the body (Aremu et al, 2006). Since the Na/K values for the vegetables are less than ones (< 1), meeting the recommended value of < 1, the samples might be good sources for lowering blood pressure, which is in accordance with the flour of some Nigerian underutilized legumes which are good sources for lowering blood pressure (Aremu et al, 2006).

The concept of Ca/P ratio, made it clear that modern diets which are rich in animal proteins and phosphorus tend to promote the loss of calcium in urine. If Ca/P ratio is low, high amount of calcium may be loss in the urine, resulting in a decrease in the calcium levels of bones (Nieman et al, 1992). A Ca/P ratio of $\geq$ 0.5 is termed a good source while < 0.5 is termed poor sources of food. Therefore, since the Ca/P ratio for *T. Occidentalis* and *C. Crepidiodes* are 0.18 and 0.26 respectively, they are termed poor sources but *M. esculenta* 6.2 is termed good sources of minerals for bone formation.

Result in Table III suggest low level of HCN (3.10 $\pm$ 0.02 – 10.50 $\pm$ 0.01) mg/100g in all the samples, moderate level of saponin and tannin in *T. occidentalis* and *C. crepidiodes* but high level of tannin (4108.67$\pm$0.9), oxalate (1878.50$\pm$0.80), Saponin (756.67$\pm$0.60) and phytic acid (741.60$\pm$0.50)mg/100g in *M.esculenta.* They were richer in phytic acid and oxalate than that reported by Aletor and Adeogun (1995) for some fresh tropical leafy vegetables, oxalate 20, and phytic acid 110mg/100g. *Citrus sinensis* and *Carica papaya* seeds phytic acid 23.2 and 32.55 mg/100g respectively (Abulude, 2000), also higher than that of foliage of some leguminous trees (Oduguwa et al, 1999). The HCN level was lower, phytic acid in *T. occidentalis* and *C. crepidiodes* comparable but in *M. esculenta* higher than that reported for some cassava leaf varieties (Ayodeji, 2005). The presence of phytic acid in all the vegetables agrees with an earlier report (Lolas and Markakis, 1975) of a widespread occurrence of phytic acid in plants.

Several studies including Rackis (1994) Reddy et al, (1982), Forbes and Erdman, (1983), and Aletor, (1990) have implicated dietary phytic and oxalic acids in the impairment of the efficient utilization especially of divalent minerals such as calcium and magnesium and the subsequent development of rickets when certain legumes and cereals are fed. Dietary phytin is of particular importance in monogastric animals (including man) that lack phytase which breaks down phytin to release phosphorus for metabolism. Phosphorus utilization has become an importance current issue on the question of environmental pollution. This arises from the poor digestibility of phosphorus, especially, in food of vegetable origin (Huisman, 1991) where a high proportion of the phosphorus may be present as the poorly digested phytin-phosphorus in monogastric animals.

The concentration of phytic acid in forages ranged between 28.55-31.22mg/g (Oduguwa et al, 1991). According to Aletor and Adeogun (1995) and Ayodeji (2005) suggested that processing methods reduce the concentrated of anti-nutritional factor. Therefore supplementation of divalent minerals and addition of the enzyme, phytase to foods high in phytin as advocated by Simons and Versteegh, (1990) as a way of enhancing P digestibility and utilization, will go a long way to improving the food values of these samples.

6. Conclusion

From the above analytical result, this study indicates the high potential of the three leafy vegetables as an unconventional protein, crude fibre and mineral elements resources for both humans and animals. However, the principal problem that could undermine this potential is the high presence of anti nutrients typified by oxalates, phytic acid, tannin, saponin and HCN, which their effect could be reduced by processing methods, supplementation of diverted mineral and addition enzyme phytase to enhance their nutritive value. These vegetables are therefore tipped as a veritable replacement to the conventional protein and mineral sources that are presently of very high cost and in high competition with human consumption.

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Table 1. Proximate composition (%) and gross energy of three Nigerian leafy vegetables (means, n=3)[a]

Vegetables	*Family*	*CP*	*CF*	*Ash*	*Fat*	*CHO*	*Moisture*	*DM*	*Gross Energy (Kcal/100g)*
Ugwu *(T. Occidentalis)*	Cucurbita ceae	25.20 ± 0.1	15.00 ± 0.30	10.00 ± 0. 2	2.50 ± 0.2	39.80 ± 0.4	7.50 ± 0.2	92. 50	282.50
Ebolo *(C. Crepidiodes)*	Compositae	17.50 ± 0.3	18.00 ± 0.4	5.00 ± 0.1	1.50 ± 0.1	41. 00 ± 0.50	17.00 ± 0.5	83.00	247.50
Cassava *(M. esculenta)*	Euphorbia cea	14.41 ± 0.1	7.87 ± 0.02	6.22 ± 0.02	1.53 ± 0.01	26.25 ± 0.2	43.72 ± 0.2	56.28	176.41
Mean ($\bar{X}$)		19.04	13.62	7.07	1.84	35.68	22.74	77.26	235.47
SD		5.56	5.20	2.61	0.57	8.19	18.78	18.78	54.06
% CV		29.20	38.18	36.92	30.98	22.95	82.59	24.31	22.96
SK		0.83	-0.80	0.98	1.63	-1.51	0.92	-0.92	-0.67
Range		14.41-25.20	7.87-18.00	5.00-10.00	1.50-2.50	26.25 - 41.00	7.50-43.72	56.28-92.50	176.41-282.50

[a] Values are means ± standard deviations of triplicate determination

CP = Crude protein; CF = Crude fibre; CHO = Carbohydrate ; DM = Dry matter ; SD = Standard deviation ;

SK = Skewness ; CV = Co-efficient of variation

Table 2: Mineral composition (mg/100g) of three Nigerian leafy vegetables (Means, n =3)[a].

Minerals	*Ugwu (T. occidentalis)*	*Ebolo (C. crepidiodes)*	*Cassava (M. Esculenta)*	$\overline{X}$	*% CV*	*SD*	*SK*	*Range*
K	5.60 ± 0.02	4.40 ± 0.02	7.73 ± 0.05	5.91	28.60	1.69	0.55	4.40±0.02 - 7.73±0.05
Ca	2.60 ± 0.01	1.60 ± 0.03	1.24 ± 0.03	1.81	38.67	0.70	0.90	1.24±0.03 – 2.60±0.01
Mg	1.40 ± 0.02	0.80 ± 0.01	2.40 ± 0.03	1.53	52.94	0.81	0.48	0.08± 0.01 – 2.40±0.03
P	14.60 ± 0.04	6.23 ± 0.04	0.20 ± 0.01	7.01	103.14	7.23	0.32	0.20±0.01 – 14.60±0.04
Na	3.80 ± 0.02	3.20 ± 0.02	5.37 ± 0.02	4.12	27.18	1.12	0.86	3.20±0.02 – 5.37±0.02
Fe	1.90 ± 0.01	1.50 ± 0.01	3.26 ± 0.03	2.22	41.44	0.92	1.04	1.50±0.01 – 3.26±0.03
Mn	3.72 ± 0.02	2.55 ± 0.04	0.10 ± 0.01	2.12	87.26	1.85	-0.70	0.10±0.01 – 3.72±0.02
Cu	0.24 ± 0.01	0.20 ± 0.01	0.50 ± 0.01	0..31	51.61	0.16	1.31	0.20±0.01 – 0.50±0.01
Zn	5.25 ± 0.03	2.93 ± 0.03	3.10 ± 0.02	3.76	34.31	1.29	1.53	2.93±0.03 – 5.25±0.03
Co	2.20 ± 0.02	1.20 ± 0.01	1.50 ± 0.03	1.63	31.29	0.51	0.76	1.20±0.01 – 2.20±0.02
Na/K	0.68	0.73	0.69					
Ca/P	0.18	0.26	6.2					

[a] Values are means ± standard deviations of triplicate determination.

$\overline{X}$ = Mean; CV = Co-efficient of variation; SD = Standard deviation; SK = Skewness

Table 3: The Anti-nutritional composition (mg/100g) of three Nigerian leafy vegetables (Means, n=3)[a]

Anti-Nutrient	*Ugwu (T. occidentalis)*	*Ebolo (C. crepidiodes)*	*Cassava (M. esculenta)*	$\overline{X}$	*% CV*	*SD*	*Range*	*SK*
Tannin	101.20 ± 0.05	105.20 ± 0.02	4108.67 ± 0.90	1438.38	160.78	2312.56	101.20 ± 0.05 – 4108.67 ± 0.9	1.73
HCN	3.10 ± 0.02	10.50 ± 0.01	4.20 ± 0.01	5.93	67.28	3.99	3.10 ± 0.02 – 10.50 ± 0.01	1.29
Saponin	40.30 ± 0.04	50.60 ± 0.04	756.67 ± 0.60	282.52	145.36	410.66	40.30± 0.04 – 756 ± 0.6	1.69
Phytic Acid	145.20 ± 0.06	205.30 ± 0.05	741.60 ± 0.50	364.03	90.20	328.36	145.20 ± 0.6 – 741.60 ± 0.5	1.45
Oxalate	255.30 ± 0.08	30.20 ± 0.03	1878.50 ± 0.80	721.33	139.80	1008.44	30.20 ± 0.03 – 1878 .50± 0.80	1.39

[a] values are means ± standard deviation of triplicate determination.

$\overline{X}$ =Mean; CV = Co-efficient of variation; SD = Standard deviation; SK = Skewness