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ESTIMATION OF CALCIUM, ZINC, HYDROCYANATE, OXALATE AND PHYTATE IN *Dioscorea bulbifera* TUBER

A. E. ABARA, E. O. UDOSEN and O. U. EKA

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ABSTRACT

Calcium, zinc, oxalate, hydrocyanate and phytate were estimated in the wet and dry matter of the peeled, unpeeled and peels of *Dioscorea bulbifera* tuber. Calcium ranged from 36.50 to 56.50mg% wet matter (WM) and 209.00 to 323.00mg% dry matter (DM) while zinc ranged from 0.56 to 1.5mg% WM and 1.40 to 3.80mg% DM of the peeled, unpeeled and peels respectively. The values of 1.19, 2.77 and 2.98mg% WM and 3.03, 7.77 and 8.10mg% DM for oxalate; 5.41, 7.71 and 8.22mg% WM and 2.06, 2.59 and 2.73mg% DM for hydrocyanate; 118.07, 129.25 and 147.95mg% WM and 275.00, 286.53 and 338.07mg% DM for phytate were found in the peeled, unpeeled and peels respectively. The calculated phytate:zinc, calcium:phytate and (calcium)(phytate)/(zinc) molar ratios for the peeled (edible) portion were 20.77, 5.10 and 18.96 WM and 19.40, 12.53 and 101.34 DM respectively. The discussion focused on toxic levels of hydrocyanate, oxalate and phytate, and the significance of the molar ratios in predicting the bioavailability of dietary zinc.

Key Words: Calcium, zinc, oxalate, hydrocyanate, phytate.

INTRODUCTION:

Yams are widely used staples in the tropics and in Nigeria, it is a very popular food crop. There are several yam species, some of which by reason of quality are better patronised than others. One species, *Dioscorea bulbifera* which is commonly found in the South of Nigeria, is consumed mainly boiled, fried or roasted along with meats, vegetables or with palm oil spiced with pepper. Nigeria alone accounts for 78% of total world production *Dioscorea bulbifera* (Tindall, 1983).

The nutritional value of a food depends not only on the level of nutrients in it but also on the presence of toxic factors and anti-nutrients in the food. The common toxic factors and anti-nutrients in food especially those of plant origin include hydrocyanate, phytate, oxalate and tannins. These substances are present in food in variable amounts and act as antagonists by combining with other nutrients in the food to make them nutritionally unavailable. Phytates, and oxalates form complexes with cations of metals such as calcium, zinc and iron while tannins form complexes with proteins (Berk, 1976). Hydrocyanate is a potent cytochrome oxidase inhibitor and interferes adversely with the aerobic respiratory system. Besides its toxicity, hydrocyanate has also been implicated in chronic degenerative neuropathy (Oshuntokun *et al*, 1969). There are so far no reports on the oxalate and phytate contents of *Dioscorea bulbifera* in the available literature.

The mineral content of *Dioscorea bulbifera* has been reported by a number of workers. FAO (1968, 1972), Eka (1978), and Egbe and Treche (1984) have reported the calcium content of *Dioscorea bulbifera* to range from 6.5 to 45.5mg% wet matter of edible portion while Barquar and Oke (1977) reported a value of 0.45% on a dry weight basis. Zinc content of *Dioscorea bulbifera* has been reported by Egbe and Treche (1984) to be 0.51mg% of the edible wet matter while Barquar and Oke (1977) found 18.52ppm in the edible dry matter.

These reports show that the level

DR. A. E. ABARA (Author for Correspondence). Department of Chemical Sciences, The Polytechnic, Calabar, Nigeria.
DR. E. O. UDOSEN, Department of Biochemistry, University of Calabar, Calabar, Nigeria.
PROF. O. U. EKA, Department of Biochemistry, University of Calabar, Calabar, Nigeria.

of calcium and zinc in *Dioscorea bulbifera* is low.

The presence of oxalates and phytates in *Dioscorea bulbifera* may further reduce the level of calcium and zinc because of the tendency of these substances to combine with divalent cations. Turnlund *et al* (1984) have reported the effect of phytate:zinc and (calcium)(phytate)/(zinc) molar ratios on bioavailability of dietary zinc in humans while Wise (1983) have reported on the effect of calcium: phytate molar ratios on phytate precipitation.

The main objective of this study was to estimate the levels of calcium and zinc in the tuber of *Dioscorea bulbifera* and determine the effect of phytate and calcium levels on the bioavailability of dietary zinc in *Dioscorea bulbifera*. The significance of hydrocyanate and oxalate levels in *Dioscorea bulbifera* was also examined.

MATERIALS AND METHODS

Dioscorea bulbifera tubers were harvested from an experimental farm at The Polytechnic, Calabar, on a typical loamy soil to which no manure was added. Samples were prepared from the tubers as described below and used for analysis.

1. PREPARATION OF SAMPLES:

(a) **UNPEELED SAMPLE:** A set of randomly selected tubers was crushed in a mortar with a pestle and the crushed material divided into two portions. One portion was used directly for analysis while the other portion was dried in a draught oven (Gallenkamp model Ov-160) at 60°C to constant weight after which it was crushed to powder for use in mineral and antinutrient determinations on dry matter basis.

(b) **PEELED SAMPLE:** Another set of randomly selected tubers was peeled with a knife and the peels collected. The peeled material was divided into two portions one of which was crushed in a mortar with pestle and used directly for analysis while the other was dried as in the unpeeled above, crushed to powder and subsequently used for analysis.

(c) **PEELS ONLY:** The peels obtained in the sample (b) above were also divided into two portions. One portion was crushed as in the unpeeled sample (a) above and used directly for analysis while the other portion was dried and crushed to powder as in latter sample (a) and used for analysis.

2. DETERMINATION OF CALCIUM AND ZINC;

The wet digestion method of A.O.A.C. (1975) was applied to 1g portions of samples of *Dioscorea bulbifera*. The diluted digests were treated with 5% lanthanum oxide solution and the calcium and zinc contents were determined by atomic absorption spectrophotometry (Pye Unicam, SP2900).

3. DETERMINATION OF OXALATE:

Oxalate was determined using 4g of each sample by the method of Dye (1956). The sample was digested for 4 hours at 50°C by addition of dilute HCl. An aliquot of the digest was evaporated to a brownish suspension which was filtered and the filtrate treated with conc. ammonia. Oxalate was precipitated from the solution by treatment with dilute $CaCl_2$ solution at 90°C. The precipitate was solubilised with hot dilute H_2SO_4 and titrated against a dilute $KMnO_4$ solution. The oxalate content was calculated taking 1 ml of 0.05 $KMnO_4$ as equivalent to 2.2mg of oxalate.

4. DETERMINATION OF PHYRATE:

A modification of the method of McCance and Widdowson (1935) was used to estimate phytate. Phytate was extracted from the sample with dilute HCl and precipitated from the solution as ferric phytate by addition of $FeCl_3$ solution. The precipitate was subsequently solubilised by adding a dilute NaOH solution with heating to give sodium phytate solution which was treated with a mixture of conc. H_2SO_4 and 65% perchloric acid to liberate phytate phosphorous. The inorganic phosphorous was determined by the A.O.A.C. (1975) method. Phytate content was calculated from inorganic phosphorous taking the molecular weight of phytic acid to be 660.

5. DETERMINATION OF HYDROCYANATE:

The hydrocyanate content of the samples of *Dioscorea bulbifera* was determined according to the A.O.A.C. (1975) method. A 10g sample was soaked for 4 hours in distilled deionised water. The suspension was subsequently steam-distilled into a dilute NaOH solution. The distillate was then treated with dilute KI and titrated against $AgNO_3$ to a faint and permanent turbidity. The

hydrocyanate was calculated taking 1 ml of 0.02 $AgNO_3$ as equivalent to 1.08mg HCN.

RESULTS:

The results with standard deviations as shown in Tables 1 and 2 are means of three replicates. The calcium, zinc, hydrocyanate, oxalate and phytate contents of the wet and dry matter of unpeeled and peels of *Dioscorea bulbifera* are as shown in Table 1 while the computed molar ratios for phytate: zinc, calcium: phytate and (calcium) (phytate)/(zinc) are as shown in Table 2.

The calcium content of *Dioscorea bulbifera* ranged from 49.20mg% in the unpeeled through 36.50mg% in the peeled sample to 56.50mg% in the peels on wet matter basis while values of 281.20mg%, 209.00mg% and 332.00mg% were found in the unpeeled, peeled and the peels respectively on dry weight basis.

The zinc contents of *Dioscorea bulbifera* samples on wet weight basis ranged from 11.28mg% in the unpeeled sample through 0.56mg% in the peeled edible portion to 7.30mg% in the peels while on dry weight basis the value for zinc ranged from 3.20mg% in the unpeeled sampled through 1.40mg in the peeled sample to 18.30mg% in the peels.

The hydrocyanate contents of the wet matter of the peels, the peeled and the unpeeled of *Dioscorea bulbifera* were 8.25mg%, 5.40mg% and 7.71mg% respectively; while values obtained on dry weight basis were 2.73mg%, 2.06mg% and 2.50mg% in the peels, peeled and unpeeled sample, respectively.

Soluble oxalate content was 2.77mg% in the unpeeled sample, 1.19mg% in the peeled edible portion and 2.92mg% in the peels of *Dioscorea bulbifera* on wet weight basis. The soluble oxalate contents of the samples on dry weight bases were 7.77mg%,3.0mg% and 8.10mg% in the unpeeled, peeled and the peels, respectively.

The phytate contents of the unpeeled, peeled and the peels of *Dioscorea bulbifera* on wet weight basis were 129.25mg%, 118.07mg% and 147.95mg% respectively; while on dry weight basis the values for phytate were 286.53mg%, 275.00mg% and 338.07mg% in the unpeeled, peeled and peels, respectively.

The results showed that the peels of *Dioscorea bulbifera* contained significantly higher levels of the substances analysed than either the

TABLE 1. CALCIUM, ZINC, HYDROCYANATE, OXALATE AND PHYTATE CONTENT OF *Dioscorea bulbifera*

	UNPEELED		PEELED		PEELS ONLY	
	WET MATTER	DRY MATTER	WET MATTER	DRY MATTER	WET MATTER	DRY MATTER
Calcium Mg/100g	49.30 ± 0.42	281.20 ± 0.05	36.50 ± 0.71	209.00 ± 4.24	56.50 ± 0.04	323.00 ± 0.34
Zinc "	1.28 ± 0.03	3.30 ± 0.14	0.56 ± 0.01	1.40 ± 0.16	7.30 ± 0.08	18.30 ± 0.41
Hydrocyanate "	7.71 ± 1.89	2.59 ± 0.65	5.41 ± 0.62	2.06 ± 0.29	8.22 ± 2.02	2.73 ± 0.67
Soluble Oxalate "	2.77 ± 0.05	7.7 ± 0.12	1.19 ± 0.11	3.03 ± 0.28	2.92 ± 0.08	8.10 ± 0.20
Phytate "	129.25 ± 8.04	286.53 ± 18.83	118.07 ± 7.35	175.00 ± 17.11	145.95 ± 9.20	338.07 ± 21.04

Mean ± SD of three replicates

TABLE 2. METAL:PHYTATE RATIOS IN *Dioscorea bulbifera*

	PHYTATE:ZINC	CALCIUM:PHYTATE	(CALCIUM)(PHYTATE)/(ZINC)
WET MATTER	20.77 ± 6.00	5.10 ± 1.50	18.96 ± 1.08
DRY MATTER	19.40 ± 13.00	12.53 ± 4.04	101.34 ± 1.37

Mean ± SD of three replicates

unpeeled sample or the peeled edible portion ($P<0.01$). The results also showed that in all the substances analysed apart from hydrocyanate, the dry matter of *Dioscorea bulbifera* contained higher levels than the wet matter, which is to be expected.

DISCUSSION:

The value of 5.41mg hydrocyanate per 100g of edible wet matter obtained in this study was lower than the value of 58.5mg per kilogram reported by Chakraborty and Eka (1978). The lethal dose of hydrocyanate is believed to be about 60mg per head per day in adult man (Oyenuga and Amazigo, 1957) and the result of this study suggests that hydrocyanate poisoning is unlikely to arise from the consumption of *Dioscorea bulbifera* since it would require the ingestion of very large amounts of yam to meet this level of hydrocyanate. Additionally, the human body is able to detoxify hydrocyanate through the enzyme, rhodanase, which converts the hydrocyanate in the presence of thiosulphates to thiocyanates which further reduces the risk of cyanide intoxication.

The nutritional significance of oxalate depends only on its content of soluble oxalate which inhibits calcium absorption and other divalent ions (Aremu, 1989; Chakraborty and Eka, 1978; Davidson et al, 1975). The soluble oxalate content of the edible portion of *Dioscorea bulbifera* found in this study was 1.19 and 3.03mg per 100g wet and dry matter respectively. The threshold of oxalate toxicity has been estimated to be between 2 and 5g (Munro and Basir, 1969) and the chances of oxalate toxicity arising from ingestion of *Dioscorea bulbifera* are slim in view of the low level of soluble oxalate in this tuber.

The phytate levels in the wet and dry matter of peeled *Dioscorea bulbifera* samples were found to be 118.07 and 275.00mg per 100g respectively. Phytate is deleterious because it precipitates metal cations of calcium, zinc and iron and thus render them unavailable for absorption although the threshold of dietary toxicity in humans and animals is unknown (Ferguson et al, 1980; Taylor and Coleman, 1979; Thompson and Weber, 1981). Turnlund *et al* (1984) have shown that phytate:zinc molar ratios of 15:1 may lead to reduced zinc bioavailability in humans. The phytate:zinc molar ratios of about 21:1 and 19:1 for the edible wet and dry matter respectively obtained in this study were far above the critical value of 15:1 reported, and indicated that the phytate level in *Dioscorea bulbifera* would lead to reduced bioavailability of zinc from the tuber. Wise (1983) reported that the solubility of phytate and the proportion of zinc bound to the complex in the intestine depended upon the dietary calcium levels.

According to this model, phytate precipitation is incomplete until dietary calcium:phytate molar ratios attain a value of 6:1. The calcium: phytate molar ratios of 5:1 and 13:1 for the edible wet and dry matter respectively obtained in this study suggest complete phytate precipitation in the dry but not in the wet matter. The values of about 19 and 101 per 100g obtained in this study in respect of (calcium) (phytate)/(zinc) molar ratios for the wet and dry edible portions respectively also indicate that zinc bioavailability from *Dioscorea bulbifera* may be adversely affected by the phytate in the tuber since molar ratios greater than 0.5mol per kilogram are reported to reduce the bioavailability of dietary zinc.

It may, therefore, be concluded that *Dioscorea bulbifera* on the basis of its phytate and calcium content is a poor source of dietary zinc. This point should be noted in area where consumption of *Dioscorea bulbifera* is high with a view to supplementing zinc intake from other sources.

Yam peels are known to be richer in certain nutrients such as the vitamins than the main flesh (Oyenuga, 1968). Consequently, cooking of small whole yam tubers (unpeeled) is favoured in the hope of retaining as much nutrients as possible in the yam food. However, in view of the high levels of hydrocyanate, oxalate, and phytate in the peels of *Dioscorea bulbifera*, the seeming wholesome practice of cooking small whole yams and of feeding yam peels to animals as feed may be inappropriate. Also, the low levels of zinc in the wet and dry matter of peeled *Dioscorea bulbifera* coupled with the unfavourable interaction of zinc with phytate as indicated by the phytate: zinc molar ratios found in this study, makes *Dioscorea bulbifera* a very poor source of dietary zinc.

REFERENCES

A.O.A.C., 1975. Official Methods of Analysis, 12 edition Association of Official Analytical Chemists, Washington, D. C.

Aremu, C. Y., 1989. Quantitative estimation of dietary contribution of phytate, oxalate and hydrocyanate by six popular Nigerian foodstuffs. Nig. J. Nutr. Sci ,10: 79-84.

Barguar, S.R. and Oke, O.L., 1977. Mineral constituents of Nigerian yams. Nutr. Reports Intern., 15:265 - 272.

Berk, Z., 1976. Braverman's Introduction to the Biochemistry of Foods. Elservier Scientific Publishing Company. Amsterdam, Oxford, New York.

Chakraborty, R. and Eka, O. U. 1978. Studies on hydrocyanic, oxalic and phytic acid content of foodstuffs. West Afri. J. Biol. Appl. Chem., 21:50-55.

Davidson, S., Passmore, R., Brock, J. F. and Truswell, A S., 1975. Human Nutrition and Dietetics. 6th edition, ELBS Churchill Livingstone, Edinburgh.

Dye, W. B., 1956. Studies on *Halgeton glomerulus*, Weed, 4:55-60.

Egbe, T. A. and Treche, S., 1984. Variability in Chemical Composition of Yams Grown in Cameroon. In: Tropical Root Crops: Production and Uses in Africa (Terry, E.E.; Doku, E. V.; Doku, E. V.; Arene, O. B. and Mahungu, N. M. eds).

Eka, O.U. 1978. Chemical evaluation of nutritive value of soya paps and porridges, the Nigerian weaning foods. Fd. Chem., 3:199 – 206.

F. A. O., 1986. Food composition Table for use in Africa. Food and Agricultural Organisation, Rome, Italy.

Ferguson, T.U., Haynes, P.H. and Spence, J. A., 1980. Distribution of dry matter and mineral nutrients in tubers of two cultivars of *Dioscorea alata* L. Tropic Agric. (Trinidad), 57:61 – 67.

McCance, R. A. and Widdowson, E. M., 1935. Phytin in human nutrition. Biochem. J. 29: 2694 - 2699.

Munro, O. and Bassir, O., 1969. Oxalate in Nigerian vegetables. W. Afr. J. Biol. Appl. Chem., 12:14-18.

Oshuntokun, B. O., Monekosso, G. L. and Wilson J., 1969. Cassava diet and chronic degenerative neuropathy: An epidemiological study. Nig. J. Science, 3: 3-15.

Oyenuga, V. A., 1968. Nigeria foods and feeding stuffs: Their Chemistry and Nutritive Value. 3rd edition, Ibadan University Press, Ibadan.

Oyenuga, V. A. and Amazigo, E. O., 1957. A note on the hydrocyanic acid content of cassava (Manihot utilissimaPohl). W. Afr. J. Biol. Appl. Chem. 1:39 – 43.

Taylor, T. G. and Coleman, J. W., 1979. A comparative study of the absorption of calcium and the availability of phytate phosphorous in Golden hamsters and the laboratory rat. Brit. J. Nutr., 42: 331 –334.

Thompson, S. A. and Weber, C. N., 1981. Effects of dietary fibre sources on tissue mineral levels in chicks. Poultry Sci., 60:840-845.

Turnlund, J. R., King, J. C., Keyes, W. R. and Michael, M. C., 1984. A stable isotope study of zinc absorption in youngmen: Effects of phytate and α-cellulose. Amer. J. Clin. Nutr., 40:1071 – 1077.

Wise, A., 1983. Dietary factors determining the biological activities of phytate. Rev. Clin. Nutr., 53: 791 – 806.