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Nutritional and phytogeriatological studies of three varieties of *Gnetum africanum* ('afang')

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Abstract

Three varieties of *Gnetum africanum* viz. the Asutan, Oron and Ikom, commonly consumed in the Southern parts of Nigeria, were assayed for their proximate nutritional composition and fibre characteristics as they related to age of the leaves. Nutritionally, the Asutan variety was significantly ($p < 0.05$) superior to the other two. It had the highest levels of ash, protein, lipid and highest caloric value. It also had the lowest contents of antinutrients such as tannins, oxalates, glucosinolates and hydrocyanic acid, which were all below documented toxic levels. Bioassay using albino rats confirmed the high nutritional value of the Asutan variety. Phytogeriatological assay showed that, in the Asutan variety, protein content, crude fibre, fibre length and fibre width increased with age of the plant, peaking at 6–12 months. There was positive correlation between crude fibre, fibre length and age ($p < 0.05$) whereas crude protein, in vitro digestibility and fibre width were negatively correlated with age. The findings are discussed with special reference to the possible contribution of *G. africanum* to the nutrient requirements of man in health and disease. A suggestion is also made for the selective cultivation of the Asutan variety over the other two.

1. Introduction

The tata leaf, *Gnetum africanum* (welw.) is a lesser known leafy vegetable common to Southern Nigeria (latitudes 4°32′ and 5°53′ North; longitudes 7°25′ and 8°25′ East), where it grows wild in the tropical forest as a vine and is cultivated in backyard gardens of rural areas mainly for subsistence. Eyo, Mohme, and Abel (1983) had earlier reported some distribution in Cameroun, Gabon and Angola. Thus, it is a vegetable eaten by at least 15–20 million peoples across 4–5 countries. Indeed, the vegetable has gained wide popularity among gourmets across Nigeria in recent times and many restaurants now regularly serve the dish. The canned soup has furthermore been recently introduced into the market by Lisabi Mills Plc. With this development, spatterings of farmlands devoted solely to its cultivation have sprung up.

1.1. Botanical description and local nomenclature

Gnetum africanum belongs to the family Gnetaceae and order Gnetales (Dutta, 1981). Mostly vines, they produce large oval and entire net—veined leaves resembling those of dicotyledons. The leaves are opposite and decussate, while the plant is dioecious (Hutchinson & Datziel, 1963).

In Southern Nigeria, *G. africanum* has as many names as there are ethnic groups that use it. The Efiks and Ibibios call it 'afang', the Northern Cross Riverians call it 'nkani' and the Igbos, 'okasi'. However, the different varieties are named according to the various localities from which they originate. For instance, the Oron variety is found in the Oron local Government area of Akwa Ibom State and is otherwise called 'Afia afang', i.e. white afang, because it has the lightest green colour of all the known varieties. Other varieties include Ikom, Asutan, Ekpupa and a few others not harvested from the forest at all due to their unpopular, bitter taste, highly fibrous and sometimes poisonous nature.

A survey of literature regarding less commonly consumed leafy vegetables in this region reveals paucity in variety specification of botanical species examined (Ifon & Bassir, 1979; Odutola & Carl, 1983; Ekpa & Ebana, 1991). The authors are of the opinion that it is important to specify varieties whenever nutritional or other research is carried out on leafy vegetables in the tropics because the lushness of the tropical belt presents wide

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variation in botanical species, many of which have medicinal, rather than nutritional leanings.

1.2. Culinary presentation

The tata leaf has a very distinct flavour and may be eaten raw as a vegetable salad when shredded and mixed with palm oil and salt (Eyo et al., 1983). It is usually cooked as a soup in conjunction with other vegetables such as chopped waterleaf (*Talinum triangulare*), okro fruit (*Abelmoschus esculentus*), or ground melon seeds (*Citrullum vulgaris*), in addition to other ingredients such as sea meats and dietary oils. It is eaten with starchy root tuber mashes and thus forms a rich and complete meal. Literature on the nutritive value of this leafy vegetable is very sparse indeed. Some earlier work had been done by Eyo et al., 1983 and Udoessien, Ifon, and Oko (1985). However, these studies omitted to specify the variety used.

It was the aim of this study to investigate and establish the nutritive values of the different varieties of this vegetable and relate the findings to their known culinary characteristics, as well as their age. These parameters could then provide fundamental bases for selective cultivation and harvesting of the leaves for maximal nutritive exploitation.

2. Materials and methods

Sample collection and post-harvest treatment involved the following. Fresh *G. africanum* leaves were harvested in composite branchlets. Each composite had an average of 10 leaves. An average of about 10 random composites were collected per sample in April of 1992 and 1993. Leaves were finely shredded with a sharp knife. Oven-drying (Gallenkamp Blue M) followed (50°C/48 h), subsequent to which the dried samples were milled (National MX—491N) into powder and stored in air-tight, brown bottles.

Moisture content, ash, crude fat, crude protein, carbohydrate and mineral element composition were determined using the standard methods of the AOAC, (1975; nos. 7.007–7.100). This involved for ash (AOAC no. 7.009), incinerating a known weight of the sample in a muffle furnace (Gallenkamp 2799A) at 600°C/24 h and weighing the difference. Crude fat determination (AOAC no. 7.061) involved exhaustive Soxhlet extraction using petroleum ether (bp. 40–60°C); crude protein (AOAC no. 7.025–7.032) involved the use of routine Kjeldahl nitrogen assay ($N \times 6.25$) while mineral element composition (AOAC no. 3.013–3.016) involved the use of an atomic absorption spectrophotometer (UNICAM 919) subsequent to acid and alkaline digestion of the samples. Crude fibre estimates (AOAC No. 7.066–7.070) were obtained from the loss in weight on ignition of dried residue following digestion of fat-free samples with 1.25% each of sulfuric acid and sodium hydroxide solutions under specified conditions. Carbohydrate content was by difference while caloric values were obtained by the summation of multiplied mean values for protein, lipid and carbohydrate by their respective Atwater factors (4, 9, 4). Quality control procedures stipulated in each of the methods applied were strictly adhered to.

Estimates for toxicants involved defatted samples and were as follows: for tannins, the vanillin/HCl method of Price and Beutler (1977) was used, while oxalate was by the permanganate titration method of Dye (1956). Hydrocyanic acid was by the picrate screening method of Harborne (1973), while glucosinolate levels were by the enzyme-liberated, acid titration method of Croft (1979).

Leaf protein extract was obtained by heating the sample in a waterbath (Gallenkamp 1049H) (60°C/30 min) using a bicarbonate buffer and sieving while hot. The extract was left to settle (40°C/48 h) and the thick supernatant was protein precipitated with acetone. This was severally repeated. The mixture was left overnight, sieved and the residue oven-dried (50°C/24 h), weighed and milled to a fine powder.

Feeding trials were conducted using 24 weanling albino rats of Wistar strain and uniform weight range (30–50 g). The animals were starved 24 h before weighing, then randomly distributed regardless of sex and litter origin into individual metabolic cages (Northkent Cages Ltd., UK) that had been divided into four groups. Each group was maintained on the four diet regimens including control (Table 1). The raw, powdered leaves were used in compounding the diet and moistened food was provided in 17 cm dia. porcelain bowls, to minimize spillage. Animals were conditioned for 4 days followed by an experimental period of 14 days. Water was supplied *ad libitum* in plastic water bottles with stainless steel nozzles. All cages were placed away from direct sunlight but near a window, for adequate ventilation. Temperature averaged 27 ± 2°C, while lighting regimen was 14 to 10 h of light and dark, respectively.

Parameters measured included feed intake, weight gain, protein efficiency ratio (PER), net protein retention (NPR), and percent true digestibility (TD%).

Fibre metrology featured as part of the geriatological assay. This involved microscopic fibre length/width determination of isolated leaf fibres. Fat-free samples of leaves were digested using hydrogen peroxide and acetic acid (1:1 v/v, 60°C/12 h). Washing of the neatly-separated and bleached fibres followed and measurements of single fibres were taken (50 random samplings each), using a microscope with calibrated occulars ($\times 10$) and stage graticules.

The use of crude fibre estimates is often discouraged in the industialized world (Hillemeier, 1995), due to the emergence of better defined, albeit arbitrary, fibre groups such as soluble and insoluble fibres, as well as because of improved methods of chemical analysis. The crude fibre index is nonetheless, still reflective of the physiologically important, dietary fibre content, regardless of such ambiguities as underestimations. Crude fibre therefore, still finds usefulness in under-developed nations, where access to modern and sophisticated equipment is limited.

In vitro digestibility was measured for both the raw and cooked vegetables. Samples (10 g) were boiled for 15 min (simulating actual culinary practice) in 100 ml distilled water, prior to analysis, which was by the enzymic method of Saunders, Cannon, Booth, Bichoff, and Kohler (1973).

Statistical analysis involved the calculation of standard deviations for triplicate determinations. Student's *t*-test (95% level) and ANOVA were used to measure significant differences. Linear correlation statistics were also used (Book, 1977).

3. Results and discussion

The results of the study are summarized in Tables 2–7. The nutritive profiles of the three *G. africanum* varieties were found to be comparable to those of other leafy vegetables (Oke, 1965, 1969; Udoessien et al., 1985; Ekpa & Ebana, 1991). Moisture contents (12–14% fresh weight) and ash contents (2–3% dry weight) were low compared with those of other common leafy

Table 1
Composition of diets (% of ingredients)

Ingredients	Diet 1 (protein free)	Diet 2 (casein)	Diet 3 (whole leaf)	Diet 4 (leaf extract)
Corn starch	65.0	53.4	28.0	50.6
Sucrose	20.0	20.0	20.0	20.0
Caesin	–	11.6	–	–
Non-nutritive cellulose	5.0	5.0	–	5.0
Salt mixture	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0
Palm oil	5.0	5.0	5.0	5.0
Gnetum africanum (whole leaf)	–	–	42.0	–
Gnetum africanum (extract)	–	–	–	16.4

Table 2
Proximate composition (% dry matter) of three varieties of *G. africanum*[a]

Varieties	Moisture (% wet wt)	Ash	Crude fibre	Ether extract	Crude protein	Carbohydrate (total)	Caloric value (kcals)
Asutan	13.0	3.0	28.6	6.7	17.9	43.8	307.1
	±0.1	±0.5	±6.2	±2.2	±1.8	±5.2	
Oron	14.0	2.0	34.2	9.6	15.5	38.7	303.2
	±0.0	±0.6	±3.2	±1.5	±6.2	±4.8	
Ikom	12.0	3.0	37.8	2.4	12.8	44.0	248.8
	± ±0.8	±0.5	±3.3	±0.8	±2.3	±6.2	
Asutan leaf							
Protein	–	–	–	–	60.9	–	–
extract	–	–	–	–	±5.6	–	–

[a] Mean ± SD of triplicate determinations ($p < 0.05$).
–Not determined.

Table 3
Mineral element composition (ppm) of three varieties of *G. africanum*[a]

Varieties	Ca	Mg	Fe	Zn
Asutan	130	89.0	76.1	1.3
Oron	165	39.0	78.3	1.0
Ikom	249	39.0	121	0.8

[a] Automated analysis.
N.B. Cadmium and lead not detected.

Table 4
In vitro digestibility of raw and cooked samples (% total N)

Varieties	Digestibility	
	Raw	Cooked
Asutan	39.4±5.6	71.1±4.2
Oron	27.4±3.8	75.5±1.4
Ikom	36.3±4.2	67.2±2.8

Mean ± SD of duplicate determinations ($p < 0.1$).

Table 5
Some antinutrient levels (mg/100 g dry matter) in three varieties of *G. africanum*[a]

Varieties	Oxalate		Tannins	Glucosinate
	Total	Soluble	(mg/g catechin)	(%)
Asutan	18.3	15.0	8.1	1.1
	±8.2	±2.4	±1.2	±0.1
Oron	16.1	12.7	4.0	1.3
	±8.2	±2.1	±1.6	±0.8
Ikom	21.7	17.3	2.4	1.1
	±1.6	±2.6	±0.9	±0.0

[a] Mean ± SD of triplicate determinations ($p < 0.1$).
N.B. HCN not detected.

vegetables such as *Corchorus aliterus*, *Amaranthus hybridus*, *Telfaria occidentalis*, and *Talinum triangulare* which range from 23 to 25% and 11 to 17% for moisture and ash, respectively (Ifon & Bassir, 1979). However, their crude fat and protein contents were moderate, when compared with other reports (Bassir & Fafunso, 1975; Ifon & Bassir).

Problems associated with plant proteins are usually three-fold, namely low concentration (usually 1–2 g/100 g), low digestibility and low biological value (Devlin, 1982). From the observations made in this investigation, protein quality, as regards the first two parameters, i.e. low concentrations and low digestibility, was quite high in *G. africanum* leaves (concentration here was 12–18 g/100 g, while in vitro digestibility was 67–75%), especially the Asutan variety.

The protein extract contained 61% protein. In vitro digestibility of raw and cooked leaves showed digestibility to improve by as much as about 50% with cooking, especially in the Oron variety. Given the fact that the whole leaf diet used in the feeding study was raw and not cooked, a better biological value than recorded for the raw leaf diet should be expected for the cooked leaves. True digestibility of the raw, whole leaf diet, was 73% as compared with 90% in the protein extract diet and 98% in the reference (caesin) diet (Table 1). Again, better digestibility is expected if cooked leaves are used.

The high quality of *G. africanum* leaf proteins suggests the possibility of using the vegetable in the preparation of leaf protein concentrates for livestock feed preparations. Eyo et al. (1983) had earlier shown a good amino acid profile.

Table 6
Bioassay parameters[a]

Parameter	Diet I	Diet II	Diet III	Diet IV
Feed intake (g)	60.3 ± 0.0	82.0 ± 0.00	57.5 ± 0.0	80.0 ± 0.0
Weight gain/loss (g)	−6.3 ± 1.2	8.4 ± 0.1	−4.3 ± 2.0	−6.4 ± 0.4
PER	—	2.1 ± 0.2	−1.7 ± 0.4	3.9 ± 0.1
NPR	—	2.3 ± 0.2	1.1 ± 0.2	2.0 ± 0.1
TD (%)	—	98.1	72.5	89.6

[a] Figures significant at ($p < 0.1$).

Fibre lengths and widths of leaves peaked at 6–12 months and later decreased in mature (36–48 months) leaves. The application of fibre metrics to nutritional assay is novel. From the study, the method could find usefulness in age and digestibility assessments of leaves. These could be extrapolated to physiological interpretations for functionality, as regards fibre. Fibre lengths showed positive correlation with crude fibre and age, while fibre width showed negative correlation with age, crude protein and digestibility. Crude fibre contents are high as are fibre lengths of the older leaves. It is suggested that the two to three month old leaves, which have lowest fibre lengths and hence higher digestibility (Tables 3 and 7) should be selectively harvested, for consumption.

It is a known fact that soups made from *G. africanum* leaves produce mild to severe purgative effects. From the present study, the older leaves should presumably produce a higher laxative effect than the younger ones, due to the longer fibres and lower digestibility observed; dietary fibre promotes gastric motility, emptying, water retention and soft, bulky stool (Eastwood, 1974; Hillemeier, 1995; Kwiterovich, 1995). The older leaves could therefore provide good dietetic inclusions in the regimens of hypertensives and atherosclerotic patients as they would aid in lowering plasma cholesterol levels by reducing bile acid resorption. Dietary fibre usually produces this effect (Kimm, 1995; Saldanha, 1995).

Caloric densities of most vegetables are low (30–50 kcals/100 g) (Devlin, 1982). The observations made here, however, show an appreciable range of 248–307 kcals/100 g. Mineral constituents were also appreciable, with the Ikom variety having the highest level of calcium (249 ppm) and iron (121 ppm) but also highest levels of the antinutrient, oxalate (21.7 mg/100 g), which antagonises both calcium and iron absorption. Fibre also mitigates calcium and iron bio-availability (Branch, Southgate, & James, 1975; Dwyer, 1995; Eastwood, 1974).

Antinutrient levels in each of the varieties were found to be below toxic levels. Oke, (1969) reported toxic levels of HCN to be 35 mg/100 g dry weight (compare

Table 7
Phytogeriatologic parameters[a]

Varieties	Age (months)	Crude fibre (%)	Protein (%)	In vitro digestibility (%)	Fibre length (mm)[b]	Fibre width (mm)[b])
Oron	2–3	17 ± 0.7	18 ± 0.6	60 ± 0.7	1.2 ± 0.0	0.0112 ± 0.0004
	6–12	29 ± 0.2	25 ± 0.4	48 ± 0.6	1.4 ± 0.1	0.0115 ± 0.0007
	36–48	49 ± 0.3	12 ± 0.0	36 ± 0.6	1.3 ± 0.1	0.0110 ± 0.0005
Asutan	2–3	14 ± 0.6	16 ± 0.0	67 ± 0.7	1.0 ± 0.1	0.0109 ± 0.0003
	6–12	24 ± 0.7	22 ± 0.1	53 ± 0.4	1.3 ± 0.1	0.0117 ± 0.0010
	36–48	42 ± 0.1	14 ± 0.6	44 ± 0.6	1.2 ± 0.0	0.0109 ± 0.0080

[a] Mean ± SD of triplicate determination ($p < 0.1$).
[b] Mean ± SD of 50 random samplings ($p < 0.05$).

with undetected amounts in this study), while Munro and Bassir (1969) reported toxic oxalate levels to be 2–5 g/100 g (compare 16–21 mg/100 g observed in this study). Tannin levels ranged from 2 to 4 mg/g catechin equivalent. It is pertinent to note here that the Oron variety is locally noted for its very astringent taste and thus usually requires larger amounts of 'buffer' vegetables such as waterleaf (*Talinum triangulare*), okro fruit (*Abelmoschus esculentus*), or melon seeds (*Citrullum vulgaris*), during culinary preparations. This may now be speculatively attributed to its relatively significant tannin level. Tannins are known astringents. It is also noteworthy that tannins detract from protein availability and this may adversely affect the bioavailability of the protein. The in vitro digestibility of the cooked Oron variety lends credence to this. The levels of glucosinolate, which is an inhibitor of iodine uptake, were low in all three varieties. The antinutrient has, however, in recent times been identified as a chemoprotective, dietary anticarcinogen (Bresnick, Birt, Wolterman, Wheeler, & Martin, 1990).

Based on the findings in the present study, it may be concluded that the general nutrient profile of *G. africanum* is good. That of the Asutan variety is notably superior to the other two. Selective cultivation of this variety for protein augmentation programmes in Nigeria and other West African countries where protein-calorie malnutrition is a problem Omololu (1976), is recommended.

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