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Chemical composition and potential of some underutilized tropical biomass. I: fluted pumpkin (*Telfairia occidentalis*)

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The seeds of *Telfairia occidentalis* have been subjected to standard chemical analysis to evaluate their properties. Proximate analysis indicated a low moisture content (6.30 ± 0.50%). The ash content was slightly higher than the range recommended for compounding of animal feed (3.44 ± 0.06%). The carbohydrate content was low (16.5 ± 0.12%). Starch, however, constituted the dominant carbohydrate (62.5 ± 0.48), while three sugars, glucose, fructose and sucrose were detected in the seed. The crude protein in the seed was high (16.0 ± 0.03%), a value which compared favourably with high protein seeds and nuts. In all, 16 amino acids were detected in the protein. Glutamic acid showed the highest concentration (16.4 g $100\,g^{-1}$), while lysine showed the lowest (2.6 g $100\,g^{-1}$). The brown oil extracted from the seed (yield 48.6 ± 0.94) had the following physicochemical properties; acid value, 3.05 ± 0.80 g, saponification value 166 ± 1.34 mg/KOH g^{-1}, free fatty acids, 0.3 g and peroxide value 3.02 ± 0.07 mg Eq kg^{-1}. The iodine value (80.1 ± 0.10)g $100\,g^{-1}$ indicated a preponderance of unsaturated fatty acid. Four fatty acids were detected whilst unsaturated acids constituted 61.3 g. Triglyceride was the dominant lipid species while hydrocarbons, waxes, sterols and sterol esters and higher alcohols, were detected in the unsaponifable matter. Results of nutritionally valuable mineral elements indicated that potassium occurred at the highest concentration. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The fluted pumpkin (*Telfairia occidentalis*) belongs to the family *Cucubitaceae*. It is a dicotyledonous seed vegetable indigenous to the west tropical rain forest area of Nigeria (Akoroda, 1990*a*).

The plant consists of a flexible succulent stem with trifoliate leaves. At maturity it gives rise to flowers which are white in colour with purple spots and fruits (usually 1 or 2 per plant), which have an average of 60 seeds per fruit. The seeds are usually covered with thick and hard kemels (Akoroda, 1990*b*).

Much research has been focused on the leaves of the plant. This is because they constitute the usual ingredients of soups which are important sources of protein, vitamins and minerals to the mainly starch-based diets in Africa south of the Sahara (Achinewhu, 1983; Faboya, 1983; Sanni, 1983; Akpapunam, 1984; Okoli and McEvans, 1986; Ossom, 1986; Gupta *et al.*, 1989; Badifu *et al.*, 1995). Apart from this, the plant provides an appreciable cash income to small-scale farmers in Nigeria (Lucas, 1988; Akoroda, 1990*b*). On the other hand, there is a dearth of information on the composition and utilization of the seeds.

To date, only the solvent extraction of the seeds, fixed oil and some scanty data on the composition have been reported (Longe *et al.*, 1983; Taylor *et al.*, 1983; Attah and Ibemesi, 1990). Unfortunately, recent reports have cast doubt on the edibility of vegetable leaf. In their evaluation of some popular Nigerian vegetables for their methylating potential due to nitrosamide formation, Atawodi *et al.*, (1996) reported a methylating activity of 1200 fg kg^{-1} which was the highest concentration ever detected in any vegetable in the world. This suggests it can be a potential carcinogen. Earlier Obidoa and Okoro, (1987) sounded a note of warning on the effect of consumption of the vegetable on the liver. In view of this, there is therefore a need to focus more attention on the detailed compositional analysis of the seed.

This paper reports the detailed proximate chemical composition of the seeds and the amino acid pattern of

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the protein. Also analysed are physicochemical parameters, triglycerides, other lipid classes and the fatty acid content. The work also involves comparative analysis with other known oils, in addition to the determination of nutritionally valuable minerals.

This work is in continuation of our efforts to exploit lesser known and underutilized tropical biomass and it is hoped that the data generated from the work will form a sound basis for its industrial utilization.

MATERIALS AND METHODS

Collection and sample pretreatments

Telrairia occidentalis

Samples were obtained in the markets in Ibadan, Benin, Warri and Calabar in Nigeria. They were identified at the genetic resources unit of the International Institute of Tropical Agriculture, Ibadan. The samples were stored in plastic bags at 4°C. Prior to analysis the seeds were ground with a laboratory mill.

Analytical methods

Crude protein, ash, crude fibre and moisture were determined using standard methods already described in the AOAC, (1980). Carbohydrate fractions were analysed for free sugars, water-soluble polysaccharides and starch. Sugars were determined by thin-layer chromatography (TLC). To extract free sugars, 1 g of the defatted seed powder was extracted 5 × with 80 ml ethanol on the steam bath. The supernatant was concentrated by reducing the volume to 10 ml. Protein in the extract was precipitated with 1 ml lead acetate (0.1 M) followed by the adjustment of pH to 7.0 with $NaHCO_3$ (0.5 M). The protein was centrifuged at 3000rpm for 10 min. Standard glucose, fructose and sucrose were spotted onto the TLC plates and run on the mobile phase n-propanol: water: ethylacetate: ammonia (24: 12: 4: 1). The columns were developed by spraying with acetone: $AgNO_3$ mixture. Starch was determined by the Clegg method (Clegg, 1956).

Oil extraction was carried out using Soxhlet extraction with petroleum ether 40–60°C for 72 h. For the determination of amino acids, seed protein was hydrolysed with 6N HCl for 3 h at 7 150°C in-vacuo. The amino acid solution was derivatized with an amino acid derivatizer/analyzer model 420A using phenylisothiocyanate PITC in the presence of diisopropylethyl amine DIEA. The derivative was extracted and analysed with an HPLC microseparation system model 130 A.

Analysis of oil

Iodine value, saponification value, peroxide value, free fatty acid and refractive index were determined by the methods already described in the AOAC (1980). Carotenoids were determined using a Perkin Elmer 202 spectrophotometer. The sample was dissolved in cyclohexane (2–5% w/v) and the spectra recorded in a range of 380–550 nm. For the sample, absorbance was read at 415 nm and carotenoids were calculated using the Scherle equation (Vasconcellos *et al.*, 1980).

$$\text{mg carotenoids kg}^{-1} = \text{Absorbence sample vol (ml)}/0.204 \text{ sample weight (g)}$$

Fatty acid composition was analysed by gas chromatography. To 0.1 g of oil, 5 ml methanol and 1 ml CH_2Cl_2 were added. The mixture was then cooled in ice. Acetyl chloride (0.6 ml) was added slowly to the mixture; 1 ml of the solution was withdrawn into the hydrolysis tube and heated for 1 h at 110°C. The contents were discharged into 10 ml NaCl (1%) solution in a separating funnel. The non-polar organics were extracted three times with 4 ml hexane into a 50 ml flask. The value was reduced to 0.5 ml using a rotary evaporator. This was transferred into a silica gel column and eluted successively with hexane (6 ml) and CH_2Cl_2 (4 ml). The hexane fraction was discarded while the CH_2Cl_2 fraction was injected 'on column' into a Chrompack CP 9001 gas chromatograph equipped with computer software, using column PB 5, 25 m long, 0.25 mm diameter. The determination involved temperature programming; 35°C for 3 min; temperature was increased 20°C per min up to 120°C; 5°C per min up to 230°C and 230°C for 5 min.

The unsaponifiable matter was determined by refluxing 1.0 g of the oil with 200 ml 2M KOH for 1 h. The solution was then transferred into a 500 ml separating funnel. The unsaponifiable matter was extracted with 3 × 50 ml diethyl ether and washed with 100 ml NaOH (3%). The weight was determined on the Mettler balance. The unsaponifiables were separated on a silica gel 20/20 column with hexane: diethyl ether (1:1) and the bands observed under UV. The bands were identified by comparing with known R_F values and standards.

Lipid classes were separated on 0.75 mm plates (20*20 cm) coated with silica gel (Merck). Plates were developed vertically in a 80/20/1 volume mixture of petroleum ether: diethyether: acetic acid. They were developed according to the method of Sanders and identified by reference to known R_F values and standards (Sanders, 1980).

The various bands were located under iodine vapour, thereafter evaporated and the bands scraped into small chromatographic columns and eluted exhaustively with diethyl ether. The diethyl ether was removed completely and lipid content was expressed as weight percent. Mineral elements were determined by accurately weighing 2 g of the sample into a silica crucible and heating on the bunsen flame for 10 min. This was then transferred into the muffle furnace at 975°C for 6 h. Two drops of concentrated $HN0_3$ were added as ashing aid and returned into the furnace for further 4 h. The residual

white ash was dissolved in 10 ml 0.1 M $HN0_3$ and filled up to 100 ml with distilled-deionised water. Sodium and potassium were determined with flame photometer (Carning model 45), other metals by atomic absorption spectrometer (Pye Unicam SP9).

RESULTS AND DISCUSSION

The summary of the proximate composition of *Telfairia occidentalis* seed is shown in Table 1. The moisture content of the seed is quite low (6.30 ± 0.5 g) and falls within the range of moisture contents of similar seeds (Ukhun and Ifebigh, 1988; Aletor and Aladetimi, 1989). This might be advantageous in terms of the shelf life of the seeds. The ash content of the seed averaged 3.44 ± 0.06%. Ash content determination is significant in food for various reasons. Among others, it is an index of the quality of feeding materials used by animal feed compounders for poultry and cattle feeding. It has been established by Pomeranz and Clifton, (1981) that ash content of nuts, seeds and tubers should fall in the range of 1.5–2.5% in order to be suitable for animal feed. The ash content recorded in this work falls slightly outside the standard range which makes it rather unsuitable for compounding of feed. The carbohydrate content is also low (16.5 ± 0.12%). As shown in Table 2, starch constituted the major carbohydrate (62.5 ± 0.48%), while three sugars were detected in the seed (glucose 1.57 ± 0.02%, fructose 3.82 ± 0.06% and sucrose 4.46 ± 0.03%). The high starch content of the seed makes it a good source of industrial starch.

Crude protein in the seed was 16.0 ± 0.03%. This value compared favourably with high protein seeds and nuts like lima beans (19.8%) and chickpeas (19%); however, it is lower than others, soybeans (35%), cowpea (22.7%) (Oshodi, 1993). In all 16 amino acids were detected in the protein concentrate as presented in Table 3. Glutamic acid showed the highest concentration (16 g 100^{-1}), followed by arginine (11.9 g $100\,g^{-1}$) and lysine showed the lowest value (2.6 g $100\,g^{-1}$). The fairly high concentration and the wide spectrum of the amino acids detected in the seeds make it suitable for fortification of foods. Soxhlet extraction of the seed oil with petroleum ether (40°C–60°C) gave a brown oil with a yield of 48.6%. The oil had excellent keeping quality as the colour was intact several months after extraction. Thereafter, on analysis by standard methods, some physico-chemical properties were obtained (Table 4).

The peroxide value was low (3.02 ± 0.01 g kg^{-1}). The concentration of free fatty acid was 0.3%, while the acid value was 3.05 ± 0.8 mg KOH g^{-1}. The free fatty acids as well as the acid value were lower than the limits recommended for virgin and non-virgin edible oils by the Codex Standards (≤0.08% for free fatty acids and < 4.0 mg KOH g^{-1} for the acid value).

Table 1. Proximate chemical composition of *Telfairia occidentalis* seeds

Parameters	Composition %[a]
Moisture	6.30 ± 0.50
Oil yield	48.6 ± 0.94
Crude protein	16.0 ± 0.30
Fibre	9.25 ± 0.16
Ash	3.4 ± 0.06
Total carbohydrate	16.5 ± 0.12

[a]Mean ± sd of three replicate analyses.

Table 2. Percentage carbohydrate in the seed

Parameters	Composition %[a]
Sugars	9.85 ± 0.14
Glucose	1.57 ± 0.02
Fructose	3.82 ± 0.06
Sucrose	4.46 ± 0.03
Soluble polysaccharides	7.22 ± 0.13
Starch	62.5 ± 0.48
Other polysaccharides	20.4 ± 0.26

[a]Mean ± sd of three replicate analyses.

Table 3. Amino acid composition of *Telfairia occidentalis* seed (g $100\,g^{-1}$ amino acid)

Parameters	Composition
Aspartic acid ASP	6.1
Glutamic acid GLU	16.4
Serine SER	4.9
Glycine GLY	6.2
Histidine HIS	2.7
Arginine ARG	11.9
Threonine THR	3.3
Alanine ALA	6.3
Proline PRO	4.9
Tyrosine TYR	3.4
Valine VAL	7.3
Methionine MET	8.8
Cysteine CYS	8.8
Leucine LEU	8.7
Phenylalanine PHE	5.6
Lysine LYS	2.6

Table 4. Physicochemical characteristics of *Telfairia occidentalis* oil

Parameters	Composition
Colour	Brown
Specific gravity	0.978 g cm^{-3}
Refractive index	1.4832
Acid value %	3.05 ± 0.80
Saponification value	166 ± 1.34 mg KOH g^{-1}
Iodine value	80.1 ± 0.10 g $100\,g^{-1}$
Peroxide value	3.02 ± 0.07 m Eg kg^{-1}
Unsaponifiable matter %	0.03
Free fatty acid %	0.3
Free hydrocarbons %	0.2
Carotenoids mg kg^{-1} oil	181

Table 5. Comparative analysis of *Telfairia occidentalis* oil with other known and underutilized oils

Parameters	Soy bean[a]	Cotton seed[a]	Corn[a]	Safflower[a]	Bliphia sapida[b]	Curcubita foetidissima[b]	Telfairia occidentalis[c]
Oil (%)	21.0	22.9	4.5	30.5	21.0	36.0	48.6
Free fatty acid (%)	0.5	0.7	1.5	0.4	7.2	0.5	0.3
Acid value (mg KOH g^{-1})	1.0	1.4	3.0	0.8	14.1	1.1	3.05
Iodine value (mg Iodine g^{-1})	126	105	128	145	64.3	130	80.1
Peroxide value (mEq kg^{-1})	N/A	N/A	N/A	N/A	4.34	N/A	3.02
Saponification value (mg KOH g^{-1})	193	195	191	191	176	192	166
Carotenoids (mg kg^{-1})	40	167	N/A	N/A	N/A	110	181
Refractive index	1.4730	1.4700	1.4720	1.4750	1.4751	1.0720	1.4832
Specific gravity (g cm^{-3})	0.919	0.917	0.918	0.927	0.942	0.972	0.978

[a]Known oils, (Vasconcellos *et al.*, 1980).
[b]Underutilized oils (Vasconcellos *et al.*, 1980; Esuoso and Odetokun, 1995).
[c]Present study.
N/A = not available.

This makes the oil suitable for use as a food oil. The oil is thicker than most drying oils with a specific gravity of 0.978 g cm^{-3}. The saponification value was 166 ± 1.34. The slightly high iodine value (80.1 ± 0.10 g $100g^{-1}$) indicated the preponderance of unsaturated fatty acids. Also a high level of carotenoids was recorded in the oil (181 mg kg^{-1} oil). Xanthophylls apparently are the predominant pigment present in the oil, as shown by absorption around 415 nm. This is in contrast to α and β carotenes which predominate in soy bean and red palm oil (Allen *et al.*, 1982). Comparison of these parameters with known and underutilized oils is presented in Table 5. This shows that *Telfairia occidentalis* oil closely resembles oils that are processed for food use. The oil content was the highest out of all the known and underutilized seeds presented. This shows the commercial potential of the oil, which is enhanced by the low free fatty acid and acid values, and high carotenoid contents.

The lipid classes and fatty acid composition are presented in Table 6. Unsaturated fatty acid was 67.3% while saturated fatty acid was 33.6%. Oleic acid showed the highest concentration (39.1%) while linoleic acid was 28.3%. On the other hand, palmitic acid was the predominant saturated acid. The concentration obtained was 17.8%. Triglyceride was the dominant lipid specie in the oil (85.8%). Mono and diglycerides were 2.3% and 3.3%, respectively, while the percentage of polar lipids was 4.4%. The unsaponifable matter of the oil consists mainly of hydrocarbons (35–60%) followed by steroids (12–20%). Higher alcohols had the lowest concentration (3–10%) (Table 7).

The result of determination of nutritionally valuable minerals is presented in Table 8. The predominant metal in the seed was potassium 3.03 ± 0.06 g $100\,g^{-1}$, followed by sodium, 1.59 ± 0.03%. These metals would provide the average body intake of minerals if used as press cake for animal feed.

In view of the high yield of the fixed oil, a parallel research work is in progress on the low temperature conversion of the seeds to fatty acids/diesel oil and activated carbon using the converter designed by Bayer and Kutubuddin (1981, 1982*a*,*b*). The work will also involve comparative analysis in terms of the yield and economics of the processes for food and non-food uses, with the aim of finding the most cost-effective route for the maximal utilization of the seeds and seed oils. These results will be published in the near future.

Table 6. Lipid classes and total fatty acid composition of *Telfairia occidentalis* oil

	Lipid classes[a]							
	HC/ST	TG	FFA	DG	ST	MG	GLPL	P_{HL}
% wt oil	1.6	84.8	0.3	3.3	2.4	2.3	1.6	2.8
Fatty acid composition in mol%[b]	16:0	18:0	18:1	18:2				
Oil	17.8	14.8	39.1	28.3				

[a]HC/ST Hydrocarbons/Steroids; TG Triglycerides; DG Diglycerides; ST Steroids; MG Monoglycerides; PL Polar lipids; GL Gycolipids; P_{HL} Phospholipids.
[b]16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid.

Table 7. Constituents of unsaponifiable matter of *Telfairia occidentalis* oil

Constituents	Composition %
Hydrocarbons	35.60
Waxes	6.80
Sterol esters	7.32
Higher alcohols	3.10
Unidentified	5.32
Steroids	20.10

Table 8. Nutritionally valuable minerals of elements in the seeds of *Telfairia occidentalis* g $100\,g^{-1}$

Element	Composition%[a]
Sodium	1.59 ± 0.03
Potassium	3.30 ± 0.06
Calcium	0.09
Magnesium	0.10 ± 0.01
Iron	0.14 ± 0.02
Zinc	0.36 ± 0.01
Copper	0.008
Manganese	0.006
Phosphorus	0.09 ± 0.01

[a]Mean ± sd of three replicate analyses.

CONCLUSIONS

The high protein content of the seed coupled with a fairly high concentration distribution of the amino acids and the absence of toxic metals in the ash make it suitable for fortification of foods. However, a high ash and crude fibre value with a low carbohydrate content make it rather unsuitable for compounding of animal feed.

The high oil yield of the seeds and its close resemblance to commercial food oils in terms of physico-chemical properties, make it a potential food oil source of the future.

Low temperature conversion of the seeds to fatty acids/diesel oil and activated carbon might provide an alternative route for the utilization of the seeds.

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