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CHEMICAL COMPOSITION OF BITTER COLA (*Garcinia kola*) SEED AND HULLS

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The chemical composition of *Garcinia kola* seed and hull was determined using standard methods. Results show that crude protein, lipid extract, ash, and crude fibre ratios are: 39.52 and 99.92 g/kg, 43.25 and 42.91 g/kg, 11.42 and 18.62 g/kg, 114.02 and 153.44 g/kg respectively. Carbon : nitrogen ratios for the seed and hull are 57.88 and 29.01, respectively. *Garcinia kola* hull had significantly higher ($p<0.05$) protein and fibre but has comparable values for lipid and ash. Potassium and phosphorus were the most abundant mineral elements in *Garcinia kola* seed (334.82 and 242.61 mg/kg, respectively) while phosphorus predominates in the hull (288.61 mg/kg). The seed had significantly higher values for sodium, potassium, copper and cobalt while chromium, molybdenum, manganese, nickel, selenium, lead and mercury were not detected. The dominant saturated, monosaturated, and polyunsaturated fatty acid, in the seed and hull are palmitic (31.55 and 276.01 mg/kg), oleic (38.36 and 52.77 mg/kg) and linoleic acids (36.16 and 235.83 mg/kg) respectively. The hull has significantly higher ($p<0.05$) α-linolenic acid content. Glutamic acid is the dominant non-essential amino acid in seed and hull (6.80 and 8.10 g/kg, respectively) while lysine and valine (2.40 and 7.10 g/kg, respectively) are the dominant essential amino acids. The proportion of essential amino acid in the total amino acid is 44.52% in the hull and 35.81% in the seed. *Garcinia kola* seeds and hulls may find use in food and feed formulations by virtue of their chemical composition.

INTRODUCTION

Bitter cola (*Garcinia kola*) seeds are smooth elliptically shaped, with yellow pulp and brown seed coat. *Garcinia kola* has economic value across West African countries where the seeds are commonly chewed and used for traditional ceremonies. The seeds are also used in folk medicine, many herbal formulations and have potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds [Akintonwa & Essien, 1990; Adegoke *et al.,* 1998; Tona *et al*., 1999; Farombi *et al*., 2000, 2002; Pietta, 2000; Okunji *et al.,* 2002; Farombi, 2003]. The potential utilization of *Garcinia kola* in brewing operations as hop substitutes in lager beer brewing has also been reported [Aniche & Uwakwe, 1990; Dosunmu & Johnson, 1995; Ogu & Agu, 1995; Eleyinmi & Oloyo, 2001; Eleyinmi *et al.,* 2004]. These applications require only the pulp while the hull is discarded. The presence of beneficial bioactive compounds have been reported in the seed coats of almonds, peanuts (*Arachis hypogea*), lotus seeds (*Nelumbo nucifera*) and African yam bean (*Sphenostylis stenocarpa*) [Adeyeye, 1998; Frison & Sporns, 2002; Yu *et al.,* 2005], thereby opening up the possibility of the presence of potentially beneficial compounds in the seed coat of other plant materials with bioactive components in their pulp. If beneficial compounds are found in the seed coat, it would add value to the hull, hitherto regarded as waste, and open up an array of studies into their potential utilization in various food formulations and mammalian systems. Although there has been considerable interest in the bioactive compounds of *Garcinia kola* seed mainly from the medicinal perspectives, there are limited reports on the chemical composition of this seed and its hull with a view to investigating its potentials from a nutritional perspective.

The objective of this study was therefore to determine the chemical composition of *Garcinia kola* seeds and its hulls and examine their potential for use in food and feed formulations.

MATERIALS AND METHODS

Materials and reagents. The major raw material used in this work was freshly harvested *Garcinia kola* seeds obtained from a local farm in Akure, Ondo State, Nigeria. The samples were collected randomly (complete randomized design) and taken to the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria for identification and packed in clean sterile sample bags.

Sample preparation. *Garcinia kola* seeds were loaded into a conventional laboratory oven set at 50°C for 10–12 h and manually dehulled. *Garcinia kola* seed and hull were collected separately, dried further (50°C, 24–36 h), milled, packaged in sterile 1-mm thick high-density polyethylene sachet, labeled and stored in a refrigerator (3±1°C) until used.

Analytical methods

Proximate and mineral analysis. The milled seed and hulls were analysed for crude protein (Method No 978.04),

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fat (Method No 930.09), crude fibre (Method No 930.10) and total ash (Method No 930.05) as described by AOAC [1990]. The nitrogen free extractives was obtained by difference. The moisture content was determined by drying the wet sample to a constant weight in an air circulating oven at 70–80°C. The mineral contents, namely: Na, K, Ca, Mg, Cu, Mn, Co, Cr, Hg, Pb, Mo and Se contents were determined as described by Whiteside & Milner [1984] using a Pye Unicam SP9 Atomic Absorption Spectrophotometer connected to an SP9 computer (Pye Unicam Ltd, York Street, Britain). Total phosphorus was determined by the spectrophotometric molybdovanadate method (No 970.39) as described by AOAC [1990]. Regression equations were used to calculate the amount of metals in each sample (using their absorbance and dilutions).

Elemental analysis. The total carbon, nitrogen and sulfur were determined using an Elemental Analyser (Model EA/108, Carlo – Erba Instruments, Italy) in the Chemistry Department of the University of Alberta, Canada. The instrument was calibrated with known standard compounds using the linear regression method.

Fatty acid (FA) analysis. Oil was extracted from *Garcinia kola* seed and hull using the Goldfisch apparatus, saponified and then esterified using 10% (v/v) boron trifluoride-methanol (Method No 969.33) as described by AOAC [1990] to fatty acid methyl esters (FAMEs). The FAMEs were analysed on a cool on-column injection gas liquid chromatography (GLC) (Varian 3400, Varian Inc, Walnut Creek, CA) equipped with a flame-ionisation detector and a SP2560 fused silica capillary column (100m × 0.25mm id; Supelco Inc). The carrier gas was helium with a flow rate of 1.5 mL/min. The injector temperature was programmed from 50 to 230°C at 150°C/min and maintained for 9.4 min. Column temperature was programmed from 45 to 175°C at 13°C/min (maintained for 27 min), then to 215°C at 4°C/min (maintained for 45 min). Detector temperature was set at 270°C. Peak area integration for fatty acids was made using a Shimadzu Ezchrom Data System (Shimadzu Scientific Instruments Inc., Columbia, MD). The fatty acids were identified by comparing their relative retention times with reference standards (Nu-Chek-Prep, Inc, Elysian, Mn 56028) of known composition. The degree of unsaturation (DU) was determined as described by Porzucek & Raznikiewicz [1990] using the following equation with computed values obtained from GLC after comparison with reference standards:

$$DU = \frac{1\times(\%wt\ MUFA)+2\times(\%wt\ DUFA)+3\times(\%wt\ PUFA)}{100}$$

where: MUFA represents monounsaturated fatty acid; DUFA – diunsaturated fatty acid and PUFA – polyunsaturated fatty acid.

Amino acid profile. Separation and quantification of amino acids was accomplished on hydyrolysed samples with a High Performance Liquid Chromatography and a Fluorichrom detector (excitation 340 nm emission 450 nm) as described by Sedgwick *et al*. [1991]. Separation was achieved using Supelcosil LC-18 reverse phase column (4.6 × 150 mm; Supelco) equipped with a guard column (4.6 × 50 mm) packed with Supelco LC-18 reverse phase packing. Amino acid standard solution (Sigma, St Louis, Missouri 63103 USA) was used for calibration and quantification.

Statistical analysis. Means of triplicate readings and standard errors were determined and subjected to Student's t-test (α=0.05) using standard procedures.

RESULTS AND DISCUSSION

Proximate and elemental composition

The proximate and elemental composition of dry milled *Garcinia kola* seeds and hulls are presented in Table 1. The crude protein, lipid extract and ash obtained for the seed in this work, 39.52, 43.25 and 11.42 g/kg, respectively on dry weight basis (dwb) differs from previous reports by Dosunmu & Johnson [1995] (78, 87 and 24 g/kg dwbs, respectively), and Arogba [2000] (70, 99 and 31 g/kg dwbs, respectively). The difference may be due to difference in the source of the materials used. Dosunmu & Johnson [1995] obtained their raw material from Calabar (South East, Nigeria), Arogba [2000] from Idah (Middle belt, Nigeria) while those used in this work were obtained from Akure (South West, Nigeria). These regions have different climatic and soil conditions which could influence the composition of the seeds. The hulls had significantly higher crude protein and crude fibre content and comparable values for lipid extract and crude ash with the seed. The crude protein obtained for *Garcinia kola* seed (4%) and hull (~10%) is comparable with 6.1–8.1% reported for *Chrysophyllum albidum* [Edem *et al.,* 1984] and higher than 0.44–0.94% reported for *Cola rostrata* [Dosunmu & Eka, 1989]. The crude protein values obtained in this work are lower than other vegetable protein sources commonly consumed in tropical regions, namely: melon (28.44% dwb), peanut flour (24.3% dwb) and canola (25% dwb). The high

TABLE 1. Proximate, elemental composition (g/kg, dwb) and energy content of *Garcinia kola* seeds and hulls.

Components	*Garcinia kola*	
	Seed	Hull
Proximate composition		
Moisture content	97.31	95.43
Crude protein	39.52[a]	99.92[b]
Lipid extract	43.25	42.91
Ash	11.42	18.62
Crude fibre	114.02[a]	153.44[b]
NFE	694.48	589.68
Elemental composition		
Carbon	434.11	452.62
Nitrogen	7.52[a]	15.61[b]
Hydrogen	64.12	51.72
Sulphur	1.42[a]	2.82[b]
Oxygen (by difference)	492.83	477.23
C:N ratio	57.88	29.01

Values are means of three replicate readings. Means followed by different superscript in the same row are significantly different (p<0.05).

crude protein obtained for hulls, when compared with the seeds suggests that it may find use in food and/or feed formulation (for ruminants) since it is presently discarded as waste. However, bioavailability of crude protein of *Garcinia kola* seed and hull has to be determined *via in-vivo* studies before use in food/feed formulation. The hulls have about twice as much of nitrogen and sulphur compared with the seed. Aini and Vimala [2002] reports that an initial C:N ratio of 30 offers less volatization of nitrogen and consistent compost maturity, hence *Garcinia kola* hull, with a C:N ratio of 29.01, may find use in composting.

Mineral analysis

The mineral composition of *Garcinia kola* seeds and hulls is shown in Table 2. Results show that potassium (335 mg/kg) and phosphorus (243 mg/kg) were the most abundant in the seed, while phosphorus (289 mg/kg) and calcium (41.5 mg/kg) were the most abundant in the hull. These values differ from earlier reports by Dosunmu & Johnson [1995] who reported 499 and 720 mg/100 g for potassium and phosphorus respectively in the seed; 990 and 200 mg/100 g for potassium and calcium respectively in the hull. These differences may be due to variation in the soil and climatic conditions. The K/Na ratio obtained for *Garcinia kola* seeds is more than twice that of *Garcinia kola* hulls while the reverse holds for Ca/Mg ratio. Na and K are required to maintain osmotic balance of body fluid, the pH of the body, regulation of muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth [NRC, 1989]. The [K/(Ca + Mg)] ratio was the highest in the seed (5.39). Marten & Andersen [1975] reported that the milliequivalent of [K/(Ca + Mg)] must be less than 2 to avoid hypomagnesia. The absence of toxic metals like lead and mercury suggests that the seed and its hull are safe from these metals which are poisonous.

TABLE 2. Mineral composition (mg/kg) and mineral ratio of nutritionally important elements in *Garcinia kola* seeds and hull.

Components	*Garcinia kola*	
	Seed	Hull
Na	86.4[b]	7.1[a]
K	335[b]	10.6[a]
Ca	34.1	41.5
Mg	28.1[b]	10.1[a]
P	243[b]	289[a]
Cu	38.4[b]	4.1[a]
Co	102[b]	10.9[a]
Cr	ND	ND
Mo	ND	ND
Mn	ND	ND
Ni	ND	ND
Se	ND	ND
Pb	ND	ND
Hg	ND	ND
[K]/[Na]*	3.87	1.50
[Ca]/[Mg]	1.21	4.11
[K]/([Ca+Mg])	5.39	0.21

Values are means of three replicate readings. Means followed by different superscript in the same row are significantly different ($p<0.05$). ND: Not Detected. * Mineral ratios based on the mean values of the respective metals.

Fatty acids

The fatty acid composition and lipid index of oils from *Garcinia kola* seeds and hulls are presented in Tables 3 and 4. The ratios of fatty acids to each other (Table 4) are important in the evaluation of the economic and nutritional value of *Garcinia kola* seed and hull oils. Results show the presence of saturated and unsaturated fatty acids. The dominant fatty acids in the seed are oleic (38 mg/kg), linoleic (36 mg/kg) and palmitic acid (32 mg/kg) while palmitic (276 mg/kg) and linoleic (235 mg/kg) acid predominate in the hull. The dominant saturated fatty acid in the seed, palmitic acid (32 mg/kg), is significantly lower ($p<0.05$) than in the hull (276 mg/kg). This value represents 23.68% of total fatty acid and 66% of saturated fatty acid in the seed and 32.09% of total fatty acid and 72.7% of saturated fatty acid in the hull. The dominant monounsaturated fatty acid is oleic acid with 28.79% of total fatty acid and 95.8% of monounsaturated fatty acid (seeds); 9.02% of total fatty acid and 74.6% of monounsaturated fatty acid (hulls) respectively. Although the percent composition of oleic acid is higher in the seed, the actual quantity present is higher in the hull (39 and 58 mg/kg, respectively). The dominant polyunsaturated fatty acid in the seed and hull is linoleic acid (28.35% and 33.02% of total fatty acids; 96.8% and 83.9% of polyunsaturated fatty acids, respectively). Linoleic acid content is significantly higher in the hulls (236 mg/kg) than in the seeds (38 mg/kg). The essential fatty acid is significantly higher in the hulls (281 mg/kg, 33% of total fatty acids) than in the seeds (38 mg/kg, 3.98% of total fatty acids).

It is noteworthy that 95.81 and 79.79% respectively of the total unsaturated fatty acids of *Garcinia kola* seed and hull oil can be attributed to oleic and linoleic acids, both of which are important from the nutritional and stability point of view. The high total unsaturated fatty acid, degree of unsaturation (0.89) and unsaturation/saturation index (3.48) in *Garcinia kola* seed oil suggests the predominance of unsaturated fatty acids which are nutritionally important. While low α-linolenic acid level in *Garcinia kola* seed oil may suggest that it has better stability properties than *Garcinia kola* hull oil, high polyunsaturated fatty acid levels are desirable because of their potential health benefits [Bonvehi & Coll, 1993; Cunnane *et al.,* 1993; Zwarts *et al.,* 1999]. The high levels of oleic acid and low levels of linoleic acid in *Garcinia kola* seed oil (29.5 and 1.22%, respectively) may make it more stable to oxidation than *Garcinia kola* hull oil which had comparable values for the two fatty acids (6.74 and 5.28%, respectively). *Garcinia kola* seed oil may thus be more suitable for cooking and frying, than *Garcinia kola* hull oil. With the current emphasis on lowering consumption of saturated fats, minimizing or eliminating trans fat, and increasing polyunsaturated and monounsaturated fats intake, the use of *Garcinia kola* seed oil (with a high oleic to stearic acid ratio) and *Garcinia kola* hull oil (with a high linoleic to oleic acid ratio) in food formulations may be acceptable.

TABLE 3. Fatty acid composition (mg/kg) of *Garcinia kola* seeds and hulls.

Fatty acid	Molecular name	*Garcinia kola*			
		Seed		Hull	
		Quantity of FA (mg/kg)	Composition (%)	Quantity of FA (mg/kg)	Composition (%)
Saturated					
Myristic	$C_{14:0}$	3.69[a]	2.77	10.33[a]	1.20
Pentadecanoic	$C_{15:0}$	0.66[a]	0.50	4.66[b]	0.54
Palmitic	$C_{16:0}$	31.55[a]	23.68	276.01[b]	32.09
Margaric	$C_{17:0}$	0.84[a]	0.63	5.89[b]	0.68
Stearic	$C_{18:0}$	11.04	8.28	35.58[b]	4.14
Arachidic	$C_{20:0}$	ND	0.00	19.74[b]	2.30
Behenic	$C_{22:0}$	ND	0.00	27.24[b]	3.17
Σ Saturates		47.78[a]	35.86	379.45[b]	44.12
Monounsaturated					
Myristoleic	$C_{14:1}$	ND	0.00	8.38[b]	0.97
Palmitoleic (trans)	$C_{16:1}$	1.71[a]	1.28	8.89[b]	1.03
Palmitoleic (cis)	$C_{16:1}$	ND	0.00	2.42[b]	0.28
Oleic (cis)	$C_{18:1\ n9}$	38.36[a]	28.79	52.77[b]	6.14
Vaccenic (cis)	$C_{18:1\ n7}$	0.94[a]	0.71	5.17[b]	0.60
Σ Monoenes		41.01[a]	30.78	77.63[b]	9.02
Polyunsaturated					
Linoleic (*cis*)	$C_{18:2}$	36.16[a]	27.13	235.83[b]	27.42
α-Linolenic	$C_{18:3\ n3}$	1.63[a]	1.22	45.39[a]	5.28
Eicosadienoic	$C_{20:2\ n6}$	ND	0.00	2.75[b]	0.32
Σ Polyenes		37.79[a]	28.35	283.97[b]	33.02
Σ Unsaturates		78.80[a]	59.13	361.60[b]	41.04
Σ Essential FA ($C_{18:2}$ + $C_{18:3}$)		37.79[a]	3.98	281.22[b]	32.7
Unidentifiable peaks (%)			4.99[a]		14.84[b]

Values are means of three replicate readings. Means of quantity of fatty acid followed by different superscript in the same row are significantly different ($p<0.05$). ND: Not detected.

TABLE 4. Lipid index of *Garcinia kola* seeds and hulls oil extract.

Fatty Acid Ratio	*USI Ratios	
	Seeds	Hulls
**$C_{14:1}/C_{14:0}$	-	0.81[b]
$C_{16:1}/C_{16:0}$	0.05	0.03
$C_{18:1\ n9}/C_{18:0}$	3.48[b]	1.48[a]
$C_{18:1\ n7}/C_{18:0}$	0.09	0.15
$C_{18:2}/C_{18:1\ n9}$	0.94[a]	4.47[b]
$C_{18:2}/C_{18:1\ n7}$	38.48[a]	45.60[b]
$C_{18:3}/C_{18:2}$	0.05[a]	0.19[b]
**$C_{20:2\ n6}/C_{20:0}$	-	0.14
**$C_{20:2\ n6}/C_{18:3}$	-	0.06

Values followed by different superscript in the same row are significantly different ($P < 0.05$)

* USI (unsaturation/saturation index) = $\Sigma\ n_i f_i / \Sigma$ saturates; where n_i is the number of double bonds and f_i is the relative content for each fatty acid;

** $C_{14:1}$ and $C_{20:2\ n6}$ were not detected in the seed oil.

Degree of unsaturation (DU) is 0.89 and 0.80 for the seed and hull, respectively.

$$DU = \frac{1\times(\%\ MUFA)+2\times(\%\ DUFA)+3\times(\%\ PUFA)}{100}$$

where MUFA represents monounsaturated fatty acid; DUFA – diunsaturated fatty acid and PUFA – polyunsaturated fatty acid.

Amino acids

The amino acid content of bitter cola seed sand hulls is shown in Table 5. Results show that the dominant essential amino acids are lysine, leucine and valine in the seed and valine, leucine and lysine in the hull. Glutamic acid (6.8 g/kg) and arginine (5.5 g/kg) are the predominant non-essential amino acid in *Garcinia kola* seed while glutamic acid (8.1 g/kg), aspartic acid (7.5 g/kg) and glycine (5.3 g/kg) predominate in the hull. With the exception of tyrosine and methionine, essential amino acid is significantly higher (2-fold) in the hull compared with the seed. The same pattern was observed with non-essential amino acids. The proportion of essential amino acids in the total amino acid is 44.52% compared with 35.81% in the seed. The presence of essential amino acids in appreciable amounts in the hull (in relation to its seed) suggests that it may find use in food / feed formulations. However, the quality of the proteins needs to be further evaluated through *in-vivo* studies to ascertain their nutritional quality and ability to support growth.

CONCLUSION

The presence of nutritionally valuable components in the seed and hull of *Garcinia kola* suggests that it may find further use in food and feed formulation. It also adds value to the hull which, hitherto is discarded, as a potential source of nutritionally valuable nutrients and industrial raw materials.

TABLE 5. Amino acid composition (g/kg) of *Garcinia kola* seeds and hulls.

Amino acid	*Garcinia kola*	
	Seed	Hull
Threonine	1.10[a]	3.70[b]
Tyrosine	0.60	1.00
Methionine	0.40	0.70
Valine	1.70[a]	7.10[b]
Phenylalanine	1.40[a]	2.80[b]
Isoleucine	1.60[a]	3.80[b]
Leucine	1.90[a]	4.80[b]
Lysine	2.40[a]	4.10[b]
Cysteine	ND	ND
Tryptophan	ND	ND
Total Essential Amino Acids	11.10	28.00
Arginine	5.50b	3.30a
Aspartic acid	2.40[a]	7.50[b]
Glutamic acid	6.80[a]	8.10[b]
Serine	1.20[a]	4.30[b]
Histidine	0.60[a]	1.70[b]
Proline	ND	ND
Glycine	1.80[a]	5.30[b]
Alanine	1.60[a]	4.70[b]
Total Non-Essential Amino Acids	19.90	34.90

Values are means of three replicate readings. Means followed by different superscript in the same row are significantly different ($p<0.05$). ND: Not determined

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