



Cola parchycarpa K. Schum: Chemical Evaluation of Amino Acids, Vitamins and Other Nutritional Factors in Seed, Fruit Mesocarp and Epicarp

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Abstract

Cola parchycarpa is one of the under-utilized monkey kola plants that yield edible tasty fruits. The amino acids, vitamins, mineral elements, proximate and anti-nutrients composition of the white aril, seed and fruit epicarp were evaluated using standard procedures. The total essential amino acids ranged from 31.84-55.70 g/100 g, predominated in lysine, leucine and cysteine. The fruit pulp and epicarp contained substantial amount of ascorbic acid (6.310 and 6.646 mg/100 g) and tocopherols (7.328 and 5.314 mg/100 g), respectively including vitamin B1, B2, B3, B5, B6, B9 and K1. Potassium, zinc and manganese were relatively high in the seed while calcium, magnesium and iron dominated in the fruit epicarp. The proximate analysis data of the fruit pulp and epicarp were similar except in protein and lipid content. Anti-nutritional factors (phytate, oxalates, cyanide and tannins) were below permissible limits. This is the first report on amino acids and detailed vitamins composition of *C. parchycarpa*. These findings indicate the rich nutritional potential of this tasty fruit and further processing into other value added products would encourage conservation and subversion of its impending extinction.

1 Introduction

Cola parchycarpa K. Schum (Malvaceae) is a perennial tree commonly described as monkey kola¹. Monkey kola is a popular nomenclature for the lesser known members of the *Cola* species that yield edible tasty fruits. They are a close relative to the familiar West African kola nuts (*C. nitida* and *C. acuminata*), cultivated for their masticatory and stimulating nuts^{1,2}. In southern Nigeria and the Cameroon, the fruit pulp is eaten by humans as well as some wild primate animals especially monkeys, baboons and other species. The regular cylindrical caulescent follicles of *C. parchycarpa* consist of one to eight nuts which correspond to the fruit length. The follicles are beaked and ribbed with rough and light brown epicarp; seeds (greenish or reddish brown) are obliquely ovate with two flat rough surfaces. The whitish aril (waxy mesocarp) consist the sweet edible portion of the follicle³ (Fig. 1).

Proximate, anti-nutrients, mineral elements analyses and antioxidant activity of *C. lepidota*, *C. parchycarpa* and *C. lateritia* fruits' pulp have been reported⁴⁻⁸. Research has shown that

juice and jam can be developed from the pulp of the monkey kola^{9,10}. Fabunmi and Arotupin¹¹, suggested from their findings that the husk and white shell of slimy kola nut (*C. verticillata*) could serve as a blend in animal feed. *In vivo* studies also revealed that about 50% of kola nut husk meal could replace maize diets of rabbits¹².

Several valuable fruit species in Africa are not yet domesticated. However, substantial economic produce are obtained from their wild or gardens, farms and forest reserves¹³⁻¹⁵. Dearth of scientific research inputs on these indigenous plants have led to concepts such as neglected and underutilized species^{16,17}. As part of the systematic analysis of the poorly studied fruit plants for their nutritional potentials¹⁸, we present the first report on amino acids and detailed vitamins composition of this endangered species of monkey kola; as well as the mineral elements, proximate and anti-nutrients profiles of the aril, seed and fruit epicarp.



Fig 1: *Cola pachycarpa* fruit, fruit pulp and epicarp

2 Materials and Methods

2.1 Sample collection and preparation

The fruits of *C. pachycarpa* were purchased from a local market in Essien Udim Local Government Area, Akwa Ibom State, Nigeria, in July 2015. The plants were identified and authenticated by a taxonomist, M. E. Bassey, Department of Botany and Ecological Studies, University of Uyo, where voucher specimens were deposited. The seed, fruit pulp and epicarp were separated, chopped into small pieces, oven dried at 40 °C to constant weight and stored in air tight containers.

2.2 Extraction and analysis of amino acids

The methods of AOAC¹⁹ and Obreshkova *et al.*²⁰ with slight modifications were employed for this determination. The sample (10 g) was defatted with petroleum spirit in a Soxhlet extractor. The sample was macerated in KOH (30 mL, 1M) and incubated for 48 hr at 110 °C in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to pH 2.5-5.0. The solution was purified by cation-exchange solid phase extraction. The amino acids in purified solutions were derivatized with ethyl chloroformate.

The derivatized amino acids were evaluated by Gas Liquid Chromatography on a HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] Software and equipped with a PFPD detector. Separation was performed using a fused capillary column (HP EZ, 10 m x 0.2 mm x 0.25 µm) as stationary phase. The oven temperature was programmed as follows: initial temperature at 110 °C, first ramping at 27 °C/min to 320 °C; second ramping, constant 5 min at 320 °C. The injector and detector temperatures were 250 °C and 320 °C respectively.

The carrier gas was hydrogen and a split ratio of 20:1 was used. The amino acids were identified by comparing their retention times to those of a standard mixture of amino acids and the peak areas were integrated.

2.3 Vitamins determination

The vitamins profile of the samples were analysed by methods of AOAC¹⁹ with slight modifications. The sample (0.1 g) was extracted and concentrated to 1.0 ml for chromatographic analysis.

Chromatographic conditions: Analytical column: 30 m x 0.25 mm x 0.25 µm HP 5; oven program – initial at 50 °C for 2 mins; first ramp at 10 °C/min for 20 mins, maintain for 4 mins; second ramp at 15 °C/min for 4 mins, constant for 2 mins; injector temperature: 250 °C, 20:1 split ratio; temperature of PFPD detector: 320 °C; carrier gas, nitrogen; flow rate: 1.0 ml/min. The vitamins were identified by comparing their retention times to those of a standard mixture of vitamins and the peak areas were integrated.

2.4 Mineral element composition

The minerals were determined after the ground samples were subjected to dry ashing. Triplicate sample of one gram each were weighed into porcelain crucible and placed in muffle furnace. The temperature was raised gradually to 450 °C. The sample was ashed at 550 °C for 5-6 hours. After cooling to room temperature, the ash was dissolved in one millilitre (1 ml) 0.5% HNO₃. The sample volume was made up to 100 mL and the level of mineral elements, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), was analyzed by atomic absorption spectrophotometer (UNICAM 959). Sodium (Na) and potassium (K) were determined using flame atomic emission spectrometer²¹.

2.5 Proximate compositional analysis

Moisture content was obtained from fresh samples; total lipid, protein, ash, crude fibre and carbohydrate were determined from oven-dried powder using standard procedures^{19,22}. The moisture content was obtained by drying in a moisture determination apparatus (Precisa HA60) at 110 °C until circulation was complete; ash, from the incinerated residue was obtained at 550 °C after 3 h; crude protein content was established by the Kjeldahl method with a conversion factor of 6.25; while the crude fat was gravimetrically determined after Soxhlet extraction with petroleum ether. The crude fat was converted into fatty acids by multiplying with conversion factor of 0.8. The total carbohydrate was calculated as 100% - (% moisture+ % ash+ % crude protein+ % fat+ % fibre). The total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 Kcal/g and lipid by the factor of 9 kcal/g. Data points represent mean of three

determinations and proximate values were reported in percentage.

2.6 Determination of anti-nutritional factors

2.6.1 Phytate determination

Extraction and precipitation of phytate were done through phytic acid determination using the procedure described by Lucas and Markaka²³. This entails the weighing of sample (2g) into a 250 mL conical flask. 2% conc. HCl (100 mL) was used to soak the samples in the conical flask for 3 h and then filtered through a double layer filter paper. Sample filtrate (50 mL) was placed in a 250 mL beaker and distilled water (107 mL) added to give/improve proper acidity. 0.3% ammonium thiocyanate solution (10 mL) was added to each sample solution as indicator and titrated with standard iron chloride solution which contained 0.00195 g iron/mL and the end point was signified by brownish-yellow colouration that persisted for 5 min. The percentage phytic acid was calculated.

2.6.2 Tannins determination

Tannin values were obtained by adopting the method of Jaffe²⁴. Each sample (1g) was dissolved in distilled water (10 mL) and agitated, left to stand for 30 min. at room temperature. The samples were centrifuged and the extracts recovered; the supernatant (2.5 mL each) were dispersed into 50 mL volumetric flask. Similarly, standard tannic acid solution (2.5 mL) was dispersed into separate 50 mL flasks. Folin-dennis reagent (1.0 mL) was measured into each flask followed by the addition of saturated Na₂CO₃ solution (2.5 mL). The mixture was diluted to 50 mL in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tannin was calculated.

2.6.3 Cyanogenic glycoside determination

The alkaline picrate method²⁵ was used for cyanogenic glycoside determination. The samples (5 g each) in conical flasks were added distilled water (50 mL) and allowed to stand overnight. Alkaline picrate (4 mL) was added to sample filtrate (1 mL) in a corked test tube and incubated in a water bath for 5 min. A colour change from yellow to reddish brown after incubation for 5 min in a water bath indicated the presence of cyanides. The absorbance of the samples was taken at 490 nm and that of a blank containing distilled water (1 mL) and alkaline picrate solution (4 mL) before the preparation of cyanide standard curve.

2.6.4 Oxalates determination

The oxalates content of the samples was determined using titration method²⁶. The samples (2 g each) were placed in a 250 mL volumetric flask suspended in distilled water (190 mL) for soluble oxalate determination; 6 M HCl solution (190 mL) was added to the samples (2 g each). The suspensions were

digested at 100 °C for 1h. The samples were then cooled and made up to 250 mL mark of the flask. The samples were filtered, triplicate portions of the filtrate (50 mL) were measured into beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH₄OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90 °C, cooled and filtered to remove the precipitate containing ferrous ion. The filtrates were again heated to 90 °C and 5% CaCl₂ (10 mL) solution was added to each of the samples with consistent stirring. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatant were decanted and the precipitates completely dissolved in 20% H₂SO₄ (10 mL). The total filtrates resulting from digestion of the samples (2 g each) were made up to 200 mL. Aliquots of the filtrate (125 mL) were heated until near boiling and then titrated against 0.05 M standardized KMnO₄ solution to a pink colour which persisted for 30 sec. The oxalate contents of each sample were calculated. All determinations were performed in triplicates and presented in mg/100 g.

3 Results and Discussions

The amino acids content of the seed, fruit pulp and epicarp of *C. pachycarpa* is shown in table 1. Results indicated that the dominant essential amino acids are lysine (5.07-14.64 g/100 g), leucine (7.17-9.29 g/100 g) and cysteine (0.54-9.19 g/100 g); total essential amino acids ranged from 31.84-55.70 g/100 g. Glutamic acid (5.59-14.74 g/100 g) and aspartic acid (7.40-10.37 g/100 g) were the major non-essential amino acids identified. The aril contained relative higher amount of the essential amino acids while both seeds and fruit pericarp showed predominance in the non-essential components in comparative amount. Eleyinmi *et al.*²⁷ reported that lysine, leucine and valine in the seed, and valine, leucine and lysine in the hull of *Garcinia kola* were the dominant essential amino acids. The samples in this study contained higher amount of essential amino acids than *C. acuminata* and *G. kola* (356.24 mg/g and 112.90 mg/g respectively) and *G. kola* seed and hull (11.10 g/kg and 28.0 g/kg respectively)^{27,28}. Amino acid profile is significant in two aspects, namely nutrition and flavour²⁹. Glycine and alanine bestow sweetness, while valine is bitter and glutamine furnish umami³⁰. This may account in part for the relative low content of valine (1.98 g/100 g) in the sweet pulp of *C. pachycarpa* in this study compared with the seed and fruit epicarp (4.4 and 4.56 g/100 g respectively). The essential amino acids (EAA) to non-essential amino acids (NEAA) ratio was 1.62, 0.65 and 0.79 respectively for aril, seed and fruit epicarp; this is higher than sea urchin, *Paracentrotus lividus*, EAA:NEAA, 0.58. Usually in marine foods, EAA:NEAA greater than 0.5 indicates a useful source of dietary proteins³¹.

The white aril of *C. pachycarpa* contained higher amount of vitamins compared to the seed and fruit pericarp, except the

ascorbic acid content of fruit pulp and pericarp which were relatively similar (6.310 and 6.646 mg/100 g respectively) (Table 2). Lower concentrations of vitamin B1 and B2 (0.01 mg/100 g) are shown for juice obtained from *C. parhycarpa* pulp⁹. Processing may account for the observed differences in vitamins content of *C. parhycarpa* pulp juice and unprocessed pulp. Vitamins are relatively labile and can be destroyed during processing and storage of food³². The juice obtained from the yellow pulp specie was also reported to contain higher levels of vitamins than the white pulp⁹. Vitamins are a broad class of organic compounds that are minor, but significant components of food required for normal growth, self-maintenance, and functioning of human and animal systems. They play diverse specific and indispensable functions in metabolism, and their deficiency produces specific ailments³².

Table 1: Amino acids composition (g/100 g) of *Cola parhycarpa*

Amino acid	Fruit pulp	Seed	Fruit epicarp
Lysine	14.64450	5.07578	6.93420
Threonine	3.08649	1.22664	3.96042
Cysteine	9.19446	0.53643	0.32101
Valine	1.97742	4.40194	4.56269
Methionine	3.42068	0.50968	0.76437
Isoleucine	6.84774	4.38702	3.62706
Leucine	9.28887	7.95312	7.16841
Tyrosine	3.62786	2.41878	3.25083
Phenylalanine	2.63233	4.04416	4.80659
Tryptophan	0.97738	1.29065	0.51005
Total (EAA)	55.69773	31.84420	35.90563
Histidine	2.50188	2.28163	4.34090
Arginine	5.26105	4.36494	6.02845
Aspartic acid	7.40121	10.36936	8.11549
Serine	2.40693	5.95879	2.78044
Glutamic acid	5.59426	14.74402	11.67527
Proline	3.54296	4.53876	4.52287
Glycine	3.05760	3.61983	3.13948
Alanine	4.66057	3.40487	4.70192
Total (NEAA)	34.42646	49.28220	45.30482
Total Amino Acids	90.12420	81.12640	81.21045
EAA/NEAA	1.62	0.65	0.79

EAA = essential amino acids; NEAA = non-essential amino acids

Table 2: Vitamins composition (mg/100 g) of *Cola parhycarpa*

Vitamin	Fruit pulp	Seed	Fruit epicarp
Retinol (vitamin A)	0.000288	0.004588	0.000212
Thiamine (vitamin B1)	0.192687	0.117670	0.119610
Riboflavin (vitamin B2)	0.091560	0.050766	0.051396
Nicotinamide (vitamin B3)	0.966717	0.050008	0.717315
Pantothenic acid (vitamin B5)	0.000004	0.448437	0.000003
Pyridoxine/pyridoxal hydrochloride (vitamin B6)	0.264659	0.111234	0.203060
Folic acid (vitamin B9)	0.133600	0.000035	0.096790
Ascorbic acid (vitamin C)	6.309850	0.557099	6.646090
Tocopherol (vitamin E)	7.328250	3.170090	5.313950
Phylloquinone (vitamin K1)	0.000009	0.001249	0.000006

The mineral elements content of *C. parhycarpa* aril, seed and fruit pericarp are presented in Table 3. High amount of potassium (4005 and 6285 mg/Kg) in the fruit pulp and seed were identified compared with the sodium content. The concentration of sodium (1430 mg/Kg) in the fruit pulp was relatively lower compared to the seed and fruit pericarp. Interestingly, the fruit pulp is cherished as food which is advantageous due to the direct correlation of sodium intake with hypertension in human³³. The seeds have a relative high content of potassium, zinc and manganese whereas calcium, magnesium and iron were predominant in the fruit pericarp. The relative high content zinc in the seed could be implicated in the management of diabetes, which results from insulin malfunctioning. Zinc is significant for the production of insulin, a hormone and carbonic anhydrase³⁴. This observed high calcium content in the fruit pericarp could be implicated in the maintenance of fruit firmness³⁵ and is required in fruits to enhance cell wall and membrane stability³⁶. The rich Ca and Mg content of *C. parhycarpa* fruit epicarp could be exploited in animal feed blends with nutrient requirements in Ca and Mg. Eneobong *et al.*⁸ reported that calcium and magnesium were the most abundant minerals in the fruit pulp of *C. parhycarpa* and *C. lepidota*. High amount of some essential minerals in the endocarp of *C. lepidota* relative to the exocarp was also documented by Osabor *et al.*⁷.

Table 3: Mineral elements composition (mg/Kg) of *Cola parchycarpa*

Mineral	Fruit pulp	Seed	Fruit pericarp
Na	1430	2965	1580
K	4005	6285	1430
Ca	2800±0.013	3.00±0.028	136250±0.029
Mg	140850±0.021	73800±0.026	151200±0.019
Zn	1550±0.019	6000±0.075	60.0±0.102
Cu	19.0±0.014	56±0.002	10.5±0.006
Mn	50.5±0.009	62.5±0.490	38.5±0.045
Fe	198±0.012	267±0.105	289±0.102

Results are mean ± standard deviation of three determinations

Table 4 is a presentation of the proximate analysis of *C. parchycarpa* seed, aril and fruit epicarp. Moisture content is highest in the pulp (65.05%) and ash content in both pulp and fruit epicarp which indicates the presence of some nutritionally important mineral elements. Ogbu *et al.*⁴ showed that *C. parchycarpa* waxy aril contain moisture (80.15 g/100g) and ash (1.76 g/100 g). The yellow aril and seed of *C. lepidota* contain moisture (10.14 & 6.08%) and ash (3.87 & 2.48%) respectively³⁷. Research has shown that the moisture content of plant foods are related to factors such as, harvesting time, plant maturity, environmental, and storage conditions³⁸. The result also revealed a relative high carbohydrate content (67.79%) in the seeds, lipid (2.73%) in the fruit pulp and protein (14.88%) in the fruit epicarp. Calculated energy values for the samples varied from 321.28-349.81 kCal/100 g. Lipid (free fatty acids, tri-, di- and monoglycerides, phospholipids, tocopherols, sterols and derivatives) are isolated from these plant parts as crude fat³⁸. Nwisiator *et al.*³⁷ showed that proximate contents were higher in the yellow arils compared to the seeds of *C. lepidota*, except for fats and carbohydrates. A relative lower proximate content is also documented for juice developed from *C. parchycarpa* and *C. lepidota* pulp⁹ compared to the unprocessed fruit pulps.

The anti-nutrients analysis revealed the presence of tannins (6.81-17.82 mg/100 g), cyanides (1.76-8.32 mg/100 g), phytates (1.14-9.69 mg/100 g), and total oxalates (14.52-42.24 mg/100 g). A relative higher content of cyanide and phytates were found in the seed, however with lower tannins and oxalate values (Table 5). Osabor *et al.*⁷ reported lower levels of cyanide in the fruit endocarp and exocarp of *C. lepidota* and our studies revealed low phytate content compared with the yellow pulp specie of monkey kola. The levels of anti-nutrients in the seeds, aril and fruit epicarp of *C. parchycarpa* in this study, were below

the permissible toxic levels³⁹ and indicate probable lack of interference with the availability of mineral elements.

Table 4: Proximate composition of *Cola parchycarpa*

Parameter	Fruit pulp	Seed	Fruit epicarp
Moisture (%)	65.05±0.02	18.80±0.01	15.50±0.15
Ash (%)	12.84±0.028	8.58±0.010	12.97±0.007
Fibre (%)	7.55±0.015	4.73±0.00	7.67±0.021
Protein (%)	9.10±0.495	7.70±0.400	14.88±0.247
Lipid (%)	2.73±0.028	0.61±0.014	0.76±0.003
Carbohydrate s (%)	67.79±0.544	78.38±0.495	63.49±0.120
Caloric value (kCal/100 g)	332.11±0.05	349.81±0.12	321.28±0.18
	7	7	4

All values are the mean of triplicate determinations expressed in dry weight basis ± standard deviation.

Table 5: Anti-nutrients composition (mg/100 g) of *Cola parchycarpa*

Anti-nutrient	Fruit pulp	Seed	Fruit epicarp
Cyanide	4.31±0.091	8.32±0.001	1.76±0.028
Phytates	1.14±0.013	9.69±0.086	1.52±0.031
Tannins	13.35±0.014	6.81±0.016	17.82±0.049
Oxalates	42.24±1.20	14.52±0.620	38.28±0.80

All values are the mean of triplicate determinations expressed in dry weight basis ± standard deviation

4 Conclusions

Cola parchycarpa aril, seeds and fruit epicarp contain substantial amount of amino acids, vitamins and mineral elements required for nutrition; the fruit pulp showed high amount of these nutrients. The anti-nutritional factors were below permissible toxic levels to allow for bioavailability of mineral elements. The results also provide value-added potential to the seeds and fruit epicarp of *C. parchycarpa* (for exploitation in the formulation or fortification of animal feeds) which hitherto were less appropriated compared to the white aril. Furthermore, intensified scientific research on this underutilized, economic and nutritional viable plant would serve as a necessary step towards its conservation and revert the likelihood of its extinction.

5 Conflicts of Interest

The authors declare no conflict of interest.

6 Author Contributions

EEE and IIU conceived, designed and performed the experiments, and wrote the manuscript.

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