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Nutritional and Anti-Nutrient Composition of Three Kola Nuts (*Cola nitida*, *Cola acuminata* and *Garcinia kola*) Produced in Benin

Durand Dah-Nouvlessounon[1], Adolphe Adjanohoun[2], Haziz Sina[1], Pacôme A. Noumavo[1], Nafan Diarrasouba[3], Charles Parkouda[4], Yann E. Madodé[5], Mamoudou H. Dicko[6], Lamine Baba-Moussa[1*]

[1]Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, FAST, Université d'Abomey-Calavi, Cotonou, Bénin
[2]Centre de Recherches Agricoles Sud, Institut National des Recherches Agricoles du Bénin, Attogon, Bénin
[3]UFR des Sciences Biologiques, Université Péléforo Gon Coulibaly, Korhogo, Côte d'Ivoire
[4]Département de Technologie Alimentaire, IRSAT/CNRST, DTA, Ouagadougou, Burkina Faso
[5]Département de Nutrition et Sciences Alimentaires, FSA, Université d'Abomey-Calavi, Cotonou, Bénin
[6]Laboratoire de Biochimie Alimentaire, Enzymologie, Biotechnologie Industrielle et Bioinformatique, Université de Ouagadougou, Ouagadougou, Burkina Faso
Email: [*]laminesaid@yahoo.fr

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Abstract

Kola nuts were regularly chewed by West Africans and Beninese in particularly. The aim of this study was to investigate nutritional and anti-nutrient content of three Benin's kola nuts (*Cola nitida*, *Cola acuminata* and *Garcinia kola*). Proximate composition of the three species of kola nuts was assessed using standard analytical AOAC methods. Phenolics and flavonoids contents were determined by Folin-Ciocalteu and aluminum trichloride methods, respectively. Mineral composition was determined by Atomic Absorption Spectrometry method. Free and total amino acids were separated and quantified by HPLC. Protein content of the three kola nuts ranges from 4.95% (*G. kola*) to 10.64% (*C. acuminata*) whereas fat content ranges from 0.2 ± 0.00 (*C. nitida*) to 2.5 ± 0.42 (*G. kola*). Total phenolics abounded (2444.96 ± 81.56 µg Eq AG/100g) in *C. acuminata*, while flavonoids predominated (561.69 ± 22.10 µgEqQ/100g) in *G. kola*. The three species are a good source of magnesium and a copper provider was lowest in *C. nitida* (0.59 ± 0.08 mg/g) and *in C. acuminata* (0.65 ± 0.02 mg/g). The dominant total essential amino acids were threonine (*C. acu-*

[*]Corresponding author.

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***minata*) and methionine (*C. acuminata* and *G. kola*), while the predominant non-essential total amino acids according to species were arginine (*C. nitida* and *G. kola*), proline (*C. acuminata*) and cysteine (*G. kola*). For the anti-nutrients factors, saponins were in great proportion (8.33% ± 0.25%), while the oxalates were in small proportion (0.44% ± 0.04%). The three species have an interesting nutritional composition, but these seeds have the relatively lowest amino acids content.**

Keywords

Kola Nuts, Polyphenolic Compound, Micronutrient, Benin

1. Introduction

Plants are important in human being everyday existence. They provide our foods, produce the oxygen that we breathe, and use as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our buildings and in the manufacture of dyes, perfumes, pesticides and drugs [1]. These plants contain the wild fruits currently used for their fruits, seeds, kernels, flowers, sap and other edible products. Indeed, these elements are important in diets food and could powerfully help to solve or minimize the problems of food insecurity [2] [3]. Wild fruits are an important (quality and quantity) part of the diet, especially for children [4]. Some wild fruits also can be kept for 4 - 5 months during the dry season [5]. It is generally accepted that the concentration of vitamin C in the indigenous wild fruits is higher than that in exotic fruits [6]. The protein content of seeds and kernels of native species is high. The wild fruits are also good sources of carbohydrate, calcium, magnesium and potassium [7] [8].

Kola nut (*Cola* spp.) belongs to the S*teruliacea* plant family with over 20 species native to the Africa tropical rain forest [9]. *Cola nitida* and *Cola acuminata* are the most common *Cola* species used. These species are sources of caffeine in processing and pharmaceutical industries and often chewed by some ethnic's group settings as stimulants [10]. The presence of other chemicals in kola nuts such as kolanin and theobromine also makes them suitable for use in drug preparation [11]. In addition, research has shown some potential uses of kola nut in the production of wine, chocolate and many non-alcoholic beverages [12]. Apart of those two species cited above, there is *Garcinia kola* (angiospermae) belonging to the *Clusiaceae* family [13]. The seeds of *G. kola* are currently used in traditional medicine in many herbal formulations and have potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds [14]-[16].

In Benin the nuts of these three species are regularly chewed and have varied socio-cultural importance. Unfortunately, in the country, very few scientific studies have been carried out on those species [17]. Apart of the biochemical characterization and the nutritional properties of seeds, it is important to investigate the anti-nutrienal factors [18]. Indeed, the anti-nutritional factors have been described as substances that block or inhibit important metabolic pathways, especially digestion [19]. These substances are known to reduce the bioavailability of many nutrients such as proteins, vitamins and minerals. For example, minerals and trace elements are inefficiently and variably absorbed from diet due to many factors including anti-nutrients such as phytates, oxalates, tannins, and cyanides in foods [20]. Against this background the following questions are raised: 1) what is the composition of nutritional compounds of studying Benin's kola nuts? 2) What is the anti-nutritional profile of these three species nuts? In this direction, the essence of this research is to examine the proximate, nutritional and anti-nutrient composition of three species of kola nut collected in Benin.

2. Material and Methods

2.1. Collection and Sample Preparation

Fresh nuts of three plants (*C. nitida*, *C. acuminata* and *G. kola*) were collected in the village of Adjarra (6˚24'0''N, 2˚12'0''E), Oueme region, Benin. The red variety of *C. nitida* and *C. acuminata*, were collected. After collection, the nuts were identified at Benin National Herbarium, University of Abomey-Calavi. Smooth kola powder (nuts dried 25˚C ± 2˚C for 12 days and milled) as well as fresh nut were stored in airtight glassware and kept in darkness at −20˚C until use.

2.2. Nutritional Analysis

2.2.1. Proximate Analysis

Standard methods of the Association of Official Analytical Chemists AOAC [21] were used to determine the moisture, crude fat, crude protein, crude fiber, total ash contents of the nuts. Moisture content was determined by desiccation of 5 g of sample to a constant weight at 105˚C for 3 hours. Crude protein content was determined by Kjeldahl method using 6.25 as conversion constant. Crude fat content was assessed though Soxhlet method using petroleum ether as organic solvent. The total sugars were measured by spectrometry method [22] at 492 nm using D-glucose as standard. Reducing sugars were also measured by spectrometry method described by Miller [23] with a reagent 3.5 dinitrosalicylic acid (DNS) at 546 nm using maltose as standard.

2.2.2. Determination of Total Phenolic Compound

Total phenolics were quantified in extracts made from 1/15 sample (w:v) fresh nuts of each species once ground with mixer grinder using Folin-Ciocalteu's method described by Singleton [24] and adapted to 96 well-plate. At first it was revealed the extraction capacity of six solvents (water, water-HCl 1%; ethanol; ethanol-HCl 1%; methanol and methanol-HCl 1%) with *C. nitida*. Twenty five microlitre of Folin-Ciocalteu's reagent (50% v/v) was added to 10 µl of 1 mg/ml (w/v) of the nuts extract dissolved in solvents. After incubation (5 mn) at room temperature, 25 µl of 20% (w/v) sodium carbonate (Na_2CO_3) and water were added to a final volume of 200 µl per well. Blanks were prepared by replacing the reagent by water to correct for interfering compounds. After incubation (30 min), the absorbence was read at 760 nm using a multiwell plate reader. All the assays were carried out at least in triplicate. Gallic acid (0 - 500 µg/ml) was used as standard and results were expressed as microgram gallic acid equivalent per 100 gram of extract. The solvent that extracts the number of compound was used to determine the total phenolic content of *C. acuminata* and *G. kola* following the same method described above.

2.2.3. Determination of Total Flavonoïds Compound

The total flavonoids of each sample were quantified by aluminum trichloride method described by Yi *et al*. [25] and adapted to 96 well-plates. Hundred microlitre of methanolic $AlCl_3$ (2%) were mixed with 100 µl of appropriate dilution of extracts solution. After incubation (15 min), the absorbence were read at 415 nm with a software "Gen5" using a multi plate Epoch spectrophotometer Biotech connected to a computer against a blank (mixture of 100 µl methanolic extract solution and 100 µl methanol) and compared to a quercetin (0 - 50 µg/ml) calibration curve (R^2 = 0.99). The flavonoid content was expressed as microgram of quercetin equivalents per 100 g of extract.

2.2.4. Determination of Micro-Nutriments

The mineral contents (Zn, Mg, Ca, Cu and Fe) of each sample were determined by Atomic Absorption spectrometry after dry ashing of the samples according to method describe by Pinta *et al*. [26]. Vitamin C was determined by colorimetrie method with 2,6-dichlorophénolindophénol (DIP). For Amino acid profile, separation and quantification of free and total amino acids was accomplished by High Performance Liquid Chromatography (HPLC) with PICO·TAG method described by Bildlingmeyer *et al.* [27]. After drying, redrying, hydrolysis and derivatization, 100 µl syringes is used to inject 40 µl samples into the HPLC injector equipped of waters in + line-degaser AF 600 controller and dual λ absorbance detector waters 2487 id.nr.13300261. Four microlitre of injection volume is analyzed.

2.3. Anti-Nutritional Factors Analysis

Tannin content was determined by spectrometry method as described by Sombié *et al*. [28]. The results were expressed as milligramme of tannic acid equivalents (TAE) per 100 mg of extract (mg TAE/100mg extracts). The content of saponins was determined according to Sathya *et al*. [29] method. The gravimetric method previously described by Mir *et al*. [30] was used in the determination of kola nuts total alkaloid contents. Phytic acid was determined according with Lucas and Markakas [31] procedure.

3. Results and Discussion

3.1. Proximate Analysis

The proximate composition of the three species of kola nut is shown in **Table 1**.

Table 1. Proximate composition of different species of kola nut.

Parameter (% DM)	Content (Mean ± SD)		
	C. nitida	*C. acuminata*	*G. kola*
Moisture	12.46 ± 0.80	10.20 ± 0.80	8.46 ± 0.83
Dry matter (DM) %	87.53 ± 0.80	89.80 ± 0.80	91.53 ± 0.83
Total ash	3.00 ± 0.50	3.50 ± 0.86	2.00 ± 0.50
Fat	0.20 ± 0.00	1.20 ± 0.28	2.50 ± 0.42
Protein	10.06 ± 0.75	10.64 ± 0.50	4.95 ± 0.25
Total sugars (TS)	5.18 ± 0.56	5.57 ± 0.12	6.27 ± 0.61
Reducing sugars (% TS)	8.30 ± 0.78	15.22 ± 0.34	6.48 ± 0.36
Energizing value ($Kcal \cdot g^{-1}$)	85.58 ± 0.12	98.98 ± 0.57	93.49 ± 0.17
Crude fibre	4.31 ± 1.02	7.35 ± 1.45	1.35 ± 0.12

Moisture

The moisture content of the three species ranged between 8.46 - 12.46, with *C. nitida* having the highest (12.46 ± 0.80) moisture content and *G. kola* the least (8.46 ± 0.83). The values obtained are on the one hand below those found by Ajai *et al.* [32], which are between 20.62 - 22.50 for the three species, as well as those found by Odebumi *et al*. [33] for *C. nitida* (66.40 ± 0.08) and *G. kola* (60.48 ± 0.06). On the over hand, these values are above those obtained by Dewole *et al*. [18] which obtained 9.73 ± 0.02 for *C. acuminata* and 9.81 ± 0.01 for *C. nitida* and 7.2 ± 0.08 for *G. kola* [1]. This variation of moisture content in the different studies would be related to drying time of the samples before analysis because Lowor *et al*. [34] showed in their study that the moisture content of a sample varies by drying time of the latter. The high moisture content is an index of spoilage. Too much of moisture in any food sample can make the sample viable for microorganisms growth. This accounts for most of the biochemical and physiological reactions in the plant [35]. Indeed, the low moisture content of the three species of kola nuts is good for their long preservation as it will prevent early spoilage of the nuts. It is like this beneficial to the buyers of three species of kola as most sellers sell by volume and not by weight [36].

Protein

Protein content of the three species ranges from, 4.95 (*G. kola*) to 10.64% (*C. acuminata*) but the content of *C. nitida* (10.06% ± 0.75%) is near of those obtains with *C. acuminata*. Other researches were also showed the proximity between *C. nitida* and *C. acuminata* protein content. For the both species, our results were on the one hand higher of those obtained by Arogba [37] with *C. nitida* (3.5%) and Ajai *et al*. [32] in Niger which obtained 8.68% for *C. nitida* and 8.65 for *C. acuminata*. On the other hand, the results of this study were lower than those obtain Adeyeye *et al*. [36] concerning *C. acuminata* (11.95%) and also those obtained by Dewole *et al*. [18] in Nigeria which obtained 15.24% ± 0.58% with *C. nitida* and 19.14% ± 0.25% with *C. acuminata*. The difference between these studies may be due to pedology and ecological conditions because the water deficit influences the nitrogen supply in the plant [38]. Moreover physiological characteristics of a plant which confers a greater resistance to the nitrogen supply to water deficit are also genetically variable [39]. The protein content relatively high could complement the body's need of these essential nutrients for growth and development because proteins, another class of food often times referred to as the "Nitrogen-containing natural product", has been proved to be essential for the survival of human beings and animals [40]. Likewise concerning *G. kola*, we note that our data were higher than those of Adesuyi *et al*. [1] which obtained 1.85% ± 0.15%. On the contrary Ajai *et al*. [32] were obtained in their study the content twice higher than ours.

Fat and Ash

G. kola presents the higher content (2.5% ± 0.42%) in fat while *C. nitida* have the lowest (0.2% ± 0.00%). For *C. nitida*, this result can be comparable to 0.87% obtains by [32] Ajai *et al.* (2012). Contrary to our study, Odebunmi *et al*. [33] have showed that the fat content of *G. kola* seeds (4.51% ± 0.56%) was low to those obtained with *C. nitida* (5.71% ± 0.74%). The low lipid content of these nuts is predictable because these seeds are not oleaginous. For the three species, the ash content is higher than which obtained by Dewole *et al*. [18], but the comparable results were obtained by Ajai *et al*. [32], Agbeniyi and Ayodele [41] and Adeyeye and Ayejuyo [36] for *C. acuminata*.

Carbohydrates and Energy Content

Among the three species, *G. kola* has high content of total sugar (6.27% ± 0.61%) and also has the lowest content of reducing sugar (6.48% ± 0.36%). *C. nitida* and *C. acuminata* have similar total sugar content while *C. acuminata* has highest content of reducing sugar (15.22% ± 0.34%). Agbeniyi and Ayodele [41] reported in their study high content of carbohydrate. Furthermore, the results show that *G. kola* has the highest energy value (93.49 ± 0.17 Kcal/g) while *C. nitida* has the lowest (85.58 ± 0.12 Kcal/g).

The varying composition reported by different authors may imply that the proximate composition of these nuts varies with season, environment and/or condition or time of evaluation.

3.2. Polyphenolic and Flavonoïds Content

Figure 1 presents the solvent ability to total phenolic compound extraction.

The best phenolics compound extraction solvent is mixture methanol 1% chlorydric acid. From the starting three solvents (water, ethanol and methanol), the extraction of the phenolic compounds is important when the dipole moment of the solvent is low. The extraction was done therefore according to the polarity of involved solvents. Furthermore, chlorhydric acid addition seems not define the same extraction pattern, but rather revealed a beneficial synergistic effect of extraction ability of each solvent with chlorhydric acid. This effect will vary depending on the solvent and is more beneficial to methanol. Similar results were obtained by Dicko *et al.* [42] on *Sorghum bicolor*.

Table 2 shows the kola nuts total polyphenolic content determined with methanol chlorydric acid (1%) and flavonoids content of three species.

The three species have a high content of total phenolic compound but which varies according to the plant species. *C. acuminata* has a high concentration while *C. nitida* presents a low concentration. Since the three species are regularly chewed, these results are interesting because phenolics can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. It has been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations [43] [44]. Polyphe-

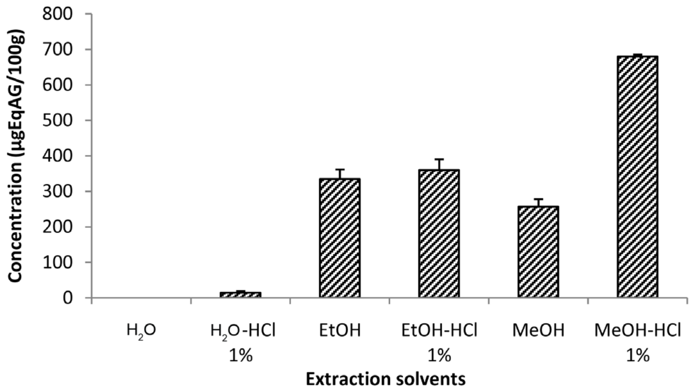


Figure 1. Variation of *C. nitida* total phenolics compound according to different solvents. H_2O: Water; HCl: Chlorydric acid; EtOH: Ethnol; MeOH: Methanol.

Table 2. Polyphenolic and flavonoïds content of three species of kola nut.

Plant species	Parameters	
	Total phenolic (µgEqAG/100g)	Total flavonoïds (µgEqQ/100g)
C. nitida	679.87 ± 5.22	374.60 ± 80.75
C. acuminata	2444.96 ± 81.56	537.38 ± 92.99
G. kola	1970.99 ± 35.85	561.69 ± 22.10

nolic compounds in plant, exert anti carcinogenic, anti mutagenic and cardio protective effects linked to their free radical scavenging [44] [45]. It has been reported that to be chemopreventive agents by lowering cholesterol and roughly limit cell damage [46]. *G. kola* has the highest total flavonoids content (561.69 ± 22.10 µgEqQ/100g). These results show that the total flavonoids represent a large portion in *G. kola* total phenolics composition. Terashima *et al.* [47] and Adesuyi *et al.* [1] reported also that *G. kola* can be a good dietary source of flavonoids. Indeed, among the three kola nuts, the consumption of *G. kola* nut would be more advantageous than those of *C. nitida* and *C. acuminata* because, flavonoids which are generally found in a variety of foods, such as oranges, tangerines, berries, apples and onions have protective effects including anti inflammatory, anti-oxidant, antiviral, and anti carcinogenic properties [48].

3.3. Micronutrient Content

The mineral and vitamin C composition of kola nut is shown in **Table 3**.

The mineral content in the three species of kola nut varied according to species. Calcium (Ca) value ranged between 1.33 - 4.3 mg/g and *C. nitida* had the highest concentration. *C. acuminata* and *G. kola* had the similarly value. However calcium content have not inconsiderable because the level is relatively high compared to the level reported for African *Adansonia digitata* kernel (0.43 - 3.76 mg/100g) by Ajayi *et al.* [49] and Nnam and Obiakor [8]. These elements support human biochemical processes by serving structural and functional roles as electrolytes [50].

The zinc (Zn) content is relatively low for the three species, but *C. acuminata* present the highest concentration (1.36 ± 0.27 mg/g). Despite the relatively lowest concentration of Zinc, his presence in these nuts is important because zinc has been associated with enzyme systems, particularly oxidation processes [36]. It has a structural and functional role in a large number of macromolecules and is required for over 300 enzymic reactions. Zinc ions (Zn^{2+}) participate in all aspects of intermediary metabolism, transmission, and regulation of the expression of genetic information, storage, synthesis, and action of peptide hormones and structural maintenance of chromatin and biomembranes [51]. It is estimated that up to 1% of the human genome codes for zinc finger proteins. In the central nervous system, zinc has an additional role as a neurosecretory product or cofactor [52].

Among the three species, the results shown that *C. nitida* present the highest concentration of iron (4.37 ± 0.53 mg/g) while *G. kola* displayed the lowest (1.37 ± 0.17 mg/g). These values differ from earlier reports in other works [53] [41]. The presence in these nuts of this element is good because iron plays an important role for human health [54]. The body requires iron for the synthesis of oxygen transport proteins, in particular hemoglobin and myoglobin, and for the formation of enzymes involved in electron transfer [55].

Magnesium is the most abundant mineral element in the three kola nuts. The similarly trend was observed by Agbeniyi and Ayodele [41] and Odebunmi *et al.* [33] in relation to the studied mineral elements. The magnesium values ranged from 10 ± 0.23 mg/g (*C. acuminata*) to 11.48 ± 0.05 mg/g (*C. nitida*). This account observed in the kola nuts is interesting because, Magnesium is used now, as adjunct to tocolytic therapy, or alone, to prevent premature uterine contractions and preterm births [56]. In addition, magnesium is essential, especially within cells, being the second most common intracellular cation after potassium, with both these elements being vital for numerous physiological functions [57].

Table 3. Micronutrient composition of three species of kola nuts

Elements	Sample value mg/g (Mean ± SD)		
	C. nitida	*C. acuminata*	*G. kola*
Ca	4.33 ± 1.41	1.66 ± 0.47	1.33 ± 0.00
Zn	0.69 ± 0.19	1.36 ± 0.27	0.39 ± 0.15
Fe	4.37 ± 0.53	2.75 ± 0.35	1.37 ± 0.17
Mg	11.48 ± 0.05	10.00 ± 0.23	10.73 ± 0.66
Cu	0.59 ± 0.08	0.65 ± 0.02	1.47 ± 0.35
Vit C (mg/100ml)	6.26 ± 0.46	0.53 ± 0.61	1.20 ± 0.61

The cooper content in the three kola nuts decreases as follows *G. kola* > *C. acuminata* > *C. nitida*. Whereas *C. nitida* present the highest value of Vit C (6.26 ± 0.46 mg/100 ml) what is not negligible. The vitamin C protects organism against the free radicals because it is the most effective antioxydant. It takes part in several metabolic processes, like the biosynthesis of collagen in conjunctive, and as neurotransmettor. Vit C increases the absorption of calcium and the biodisponibility of iron and is link to the prevention of many degenerative diseases [58].

3.4. Amino Acids Content

Figure 2 shows the auto-scaled chromatogram profile of standard, total and free amino acids while **Table 4** shows their amount.

The results show that 52.94% (9/17) of amino acids search was obtained in the kola nuts. The total amino acids were more measure than free. In addition, 66.66% of these free amino acids measured were non-essential. For total amino acids, the dominant essential amino acids are threonine (*C. acuminata*) and methionine (*C. acuminata* and *G. kola*), while the predominant non-essential amino acids according to species are arginine (*C. nitida* and *G. kola*), and proline (*C. acuminata*). Beside threonine and lysine are the free essential amino acids measured, therefore the amino acids content varied according to the species. Indeed, 100% of total amino acids measured are present in *C. nitida* against 90% in *C. acuminata* and 77.77% in *G. kola*. Whereas proline is the free amino acids more concentred in the three Cola species but the highest concentration (0.69 ± 0.06 w/w % dry weight) is obtained with *C. nitida* while *C. acuminata* and *G. kola* displays the lowest (0.61 ± 0.01 w/w % dry weight). However the three species have not great amino acids content, Eleyinmi *et al*. [59] were reports the relatively low concentrations of amino acids while Adeyeye *et al.* [60] report the high concentration of amino acids. The difference of results could be explained by the nuts pretreatment before analysis.

3.5. Quantitative Anti-Nutrient Screening of Kola Nut

The **Table 5** shows the anti-nutrient content of three kola nuts. The result shows that *C. acuminata* has high value (8.33% ± 0.25%) of these nuts anti-nutrient (saponin) content while *C. nitida* has the lowest (0.44% ± 0.04%) with the oxalates.

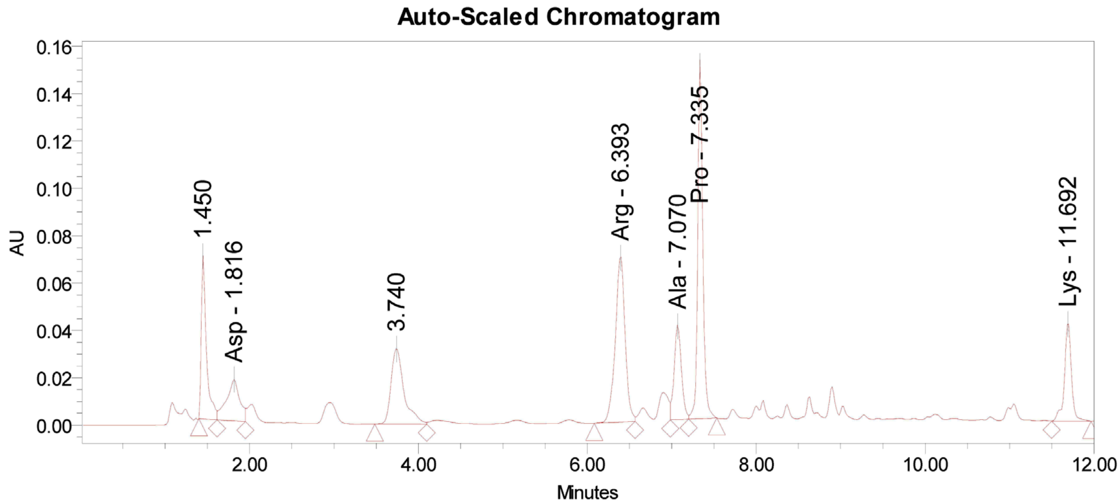
Table 4. Total and free amino acids concentration of three species of kola nut w/w% (dry weight).

Amino acids	*C. nitida*		*C. acuminata*		*G. kola*	
	Total	Free	Total	Free	Total	Free
Aspartic acid	0.53 ± 0.28	0.53 ± 0.28	-	-	-	-
Arginine	1.06 ± 0.06	0.69 ± 0.06	0.86 ± 0.01	0.61 ± 0.01	0.61 ± 0.02	0.61 ± 0.02
Threonine*	0.39 ± 0.01	-	0.33 ± 0.04	-	-	-
Alanine	0.50 ± 0.01	0.20 ± 0.01	0.42 ± 0.09	0.23 ± 0.07	0.28 ± 0.12	0.19 ± 0.00
Proline	0.83 ± 0.04	0.53 ± 0.04	1.83 ± 0.23	1.37 ± 0.23	0.10 ± 0.13	-
Valine*	0.35 ± 0.01	-	0.30 ± 0.02	-	0.33 ± 0.08	-
Methionine*	0.22 ± 0.00	-	0.48 ± 0.03	-	0.43 ± 0.26	-
Cysteine	0.27 ± 0.00	-	0.34 ± 0.01	-	0.19 ± 0.27	-
Lysine*	0.30 ± 0.02	0.16 ± 0.02	0.30 ± 0.01	0.16 ± 0.00	0.31 ± 0.01	0.18 ± 0.13

With*: Essential amino acids; Without*: Non essential amino acids.

Table 5. Anti-nutrient composition of different species of kola nut.

Parameters	Kola nuts content (Mean ± SD)		
	C. nitida	*C. acuminata*	*G. kola*
Alkaloids (%)	2.06 ± 1.50	1.80 ± 0.52	4.66 ± 1.15
Saponin (%)	4.66 ± 0.03	8.33 ± 0.25	5.83 ± 0.01
Oxalates (%)	0.44 ± 0.04	0.71 ± 0.03	0.74 ± 0.02
Phytic acid (%)	0.87 ± 0.07	0.60 ± 0.01	1.09 ± 0.10
Tanin (mg TAE/100mg)	5.66 ± 1.02	7.08 ± 0.11	3.54 ± 0.21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3.740
Arg - 6.393
Ala - 7.070
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Lys - 11.692
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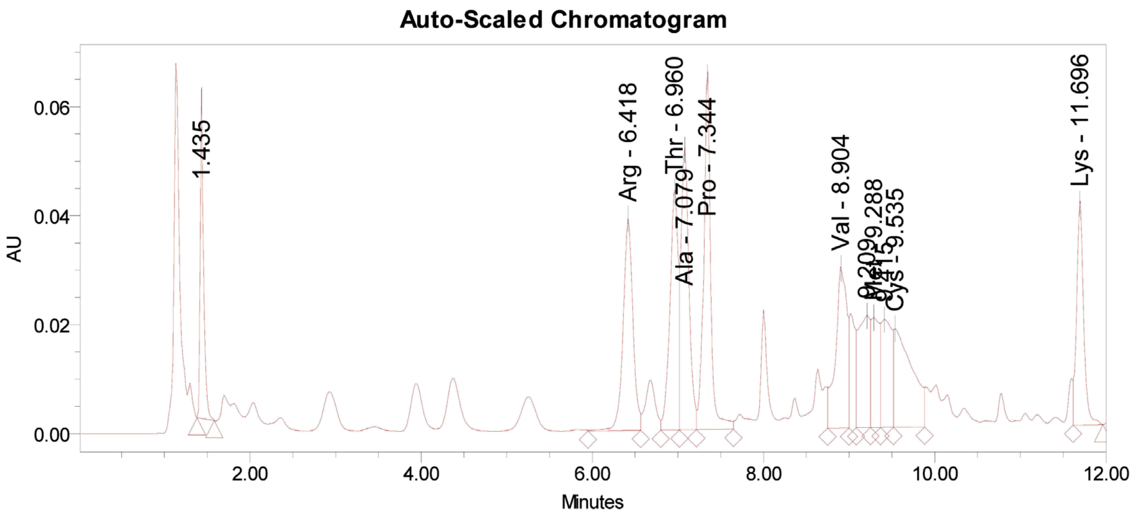
(a)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Val - 8.904
Cys - 9.535
Lys - 11.696
2.00
4.00
6.00
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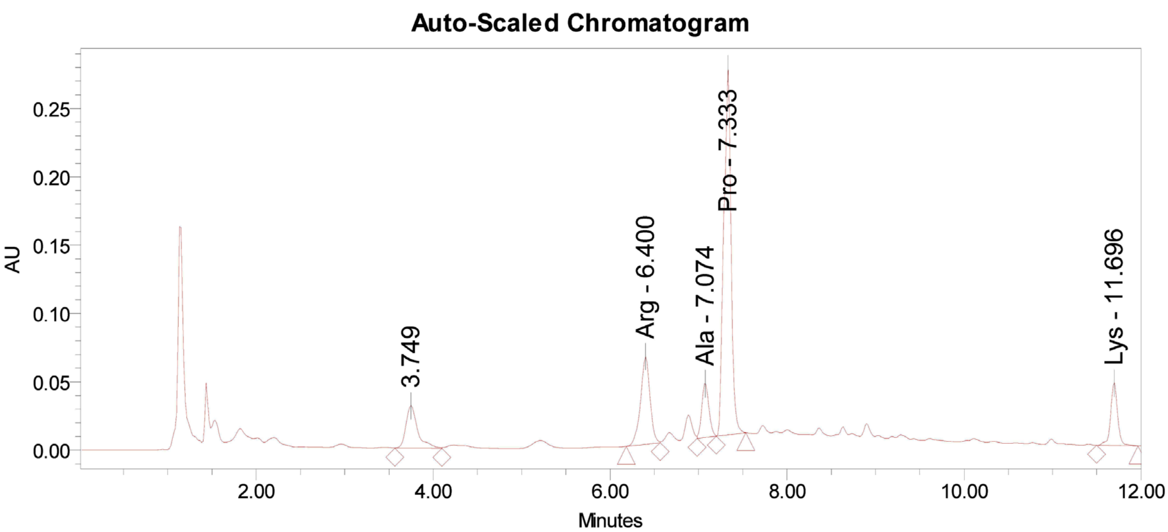
10.00
12.00
Minutes

(b)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AU
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0.20
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3.749
Arg - 6.400
Ala - 7.074
Pro - 7.333
Lys - 11.696
2.00
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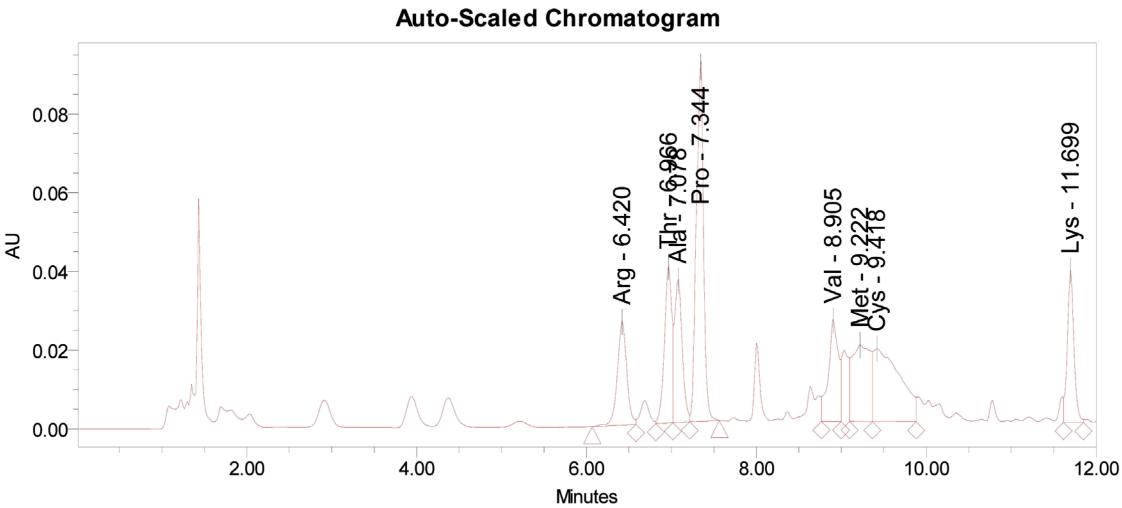
Minutes

(c)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AU
Minutes
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0.08
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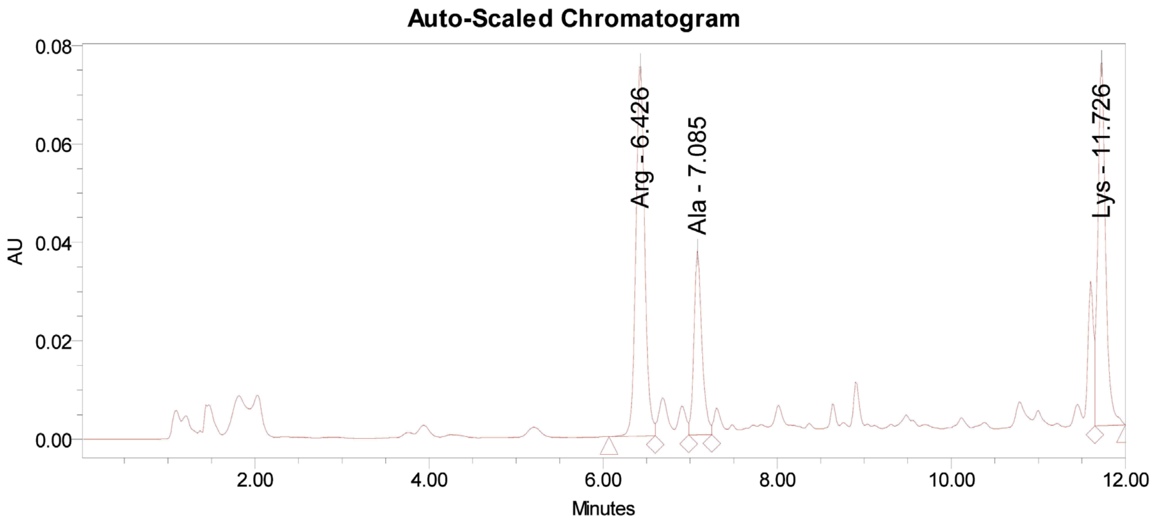
Pro - 7.344
Val - 8.905
Lys - 11.699

(d)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AU
Minutes
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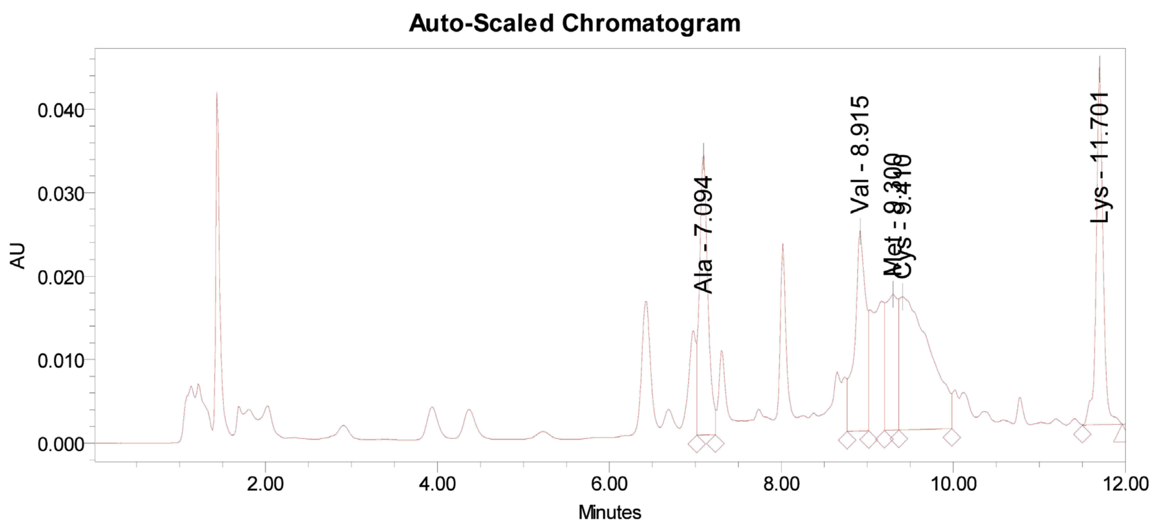
8.00
10.00
12.00
Arg - 6.426
Ala - 7.085
Lys - 11.726

(e)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AU
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0.020
0.030
0.040
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6.00
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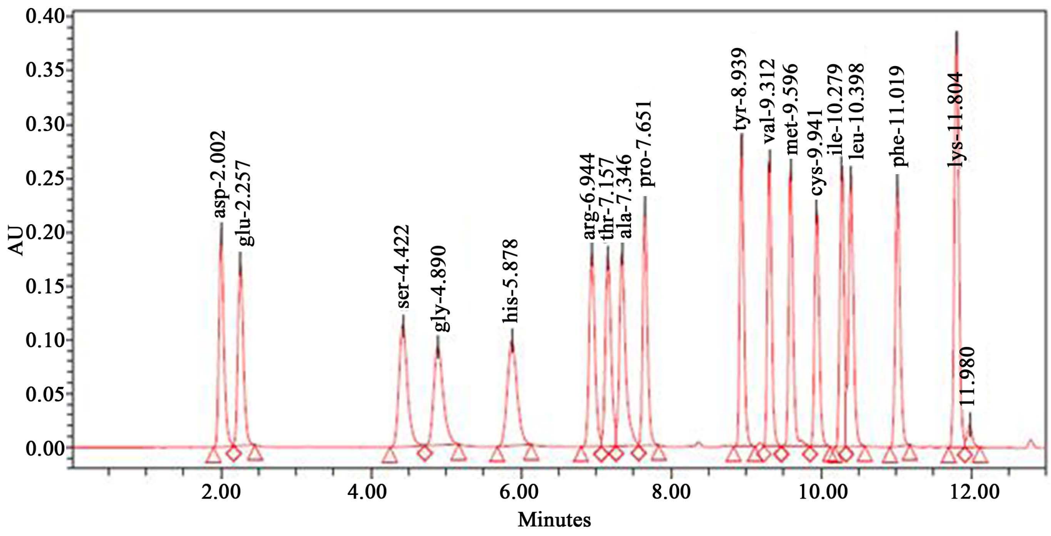
10.00
12.00
Ala - 7.094
Val - 8.915
Lys - 11.701

(f)



(g)

Figure 2. Total, free and standard amino acids auto-scaled chromatogram profile. (a) *C. nitida* free amino acids; (b) *C. nitida* total amino acids; (c) *C. acuminata* free amino acids; (d) *C. acuminata* total amino acids; (e) *G. kola* free amino acids; (f) *G. kola* total amino acids; (g) Pierce H Standard.

The high value of saponin was foreseeable with the three species because these compounds are glycosides containing a polycyclic aglycone moiety either C_{27} steroid or C_{30} triterrpenoid attached to carbohydrate and are characterised by a bitter taste and foaming properties [61]. The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins [62]. They also cause haemolysis of red blood cells [63], and have been demonstrated to have anti-spermal effects on human spermatozoa [64]. Specifically *G. kola* has the high content of alkaloids (4.66% ± 1.15%) and phytic acid (1.09% ± 0.10%) than those of the two other species. Similarly, among the three kola nuts, *C. acuminata* shows the highest value of tannin (7.08 ± 0.11 mg TAE/100mg). Tannins are water soluble phenolic compounds with reported antinutritional effects and may be by their ability to form complex with proteins. Indeed, tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes [65]. Besides, in ruminants, dietary condensed tannins (2% - 3%) have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of a protein-tannin complex [66]. The low value of oxalates and phytic acid in the three species was good because oxalates and phytates bind minerals like calcium and magnesium and interfere with their metabolism to cause muscular weakness and paralysis [67].

4. Conclusion

At the end of this study, it appears that the three selected species of kola nuts present an interesting nutritional composition but it varied according to species. The lipid content of the three kola nuts is very low while the protein content is relatively high for *C. nitida* and *C. acuminata*. They also have a high energy value. Similarly, the mineral composition of the three species is useful with a high vitamin C content of *C. nitida* nut in comparison to the two other species (*G. kola* and *C. acuminata*). Besides, these three kola nuts contain small quantities of total and free amino acids. In the same way, anti-nutrients factors in these nuts were in low proportion. Thus the nuts of those three kola species can be a good alternative to increase the food security in low income countries.

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