Full Length Research Paper

The quality of Nigerian kola nuts

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Dried mature red variety nuts of Cola nitida and Cola acuminata were purchased from three major markets noted for their wholesales with a view to ascertaining the quality of the nuts and generating a baseline data towards elaborating standards for the nuts. Dirt's and other extraneous materials were removed from the sampled nuts and oven dried at 80°C for 16 h. The nuts were physically characterised and pulverised to powder. The proximate, chemical and mineral composition of the nuts was determined in addition to the microbal load and aflatoxin content. The shape of the two species of kola nut varied from flat to oval to spherical. In addition C. nitida had two big cotyledons while C. acuminata had on the average, 3 to 6 cotyledons. The mean length, breadth and thickness of C. nitida were 3.0, 2.0 and 1.8 cm, while that of C. acuminata were 3.4, 2.73 and 2.31cm respectively. The colour of the two species of kola nuts was found to vary between white, red and pink while the proximate, chemical and mineral contents of C. nitida were higher than C. acuminata thus justifying its description as the true kola of commerce. The study further showed that kola nuts contain some active principles found in coffee and cocoa that have important pharmacological properties. The microbial load of the nuts were also within the permissible limit specified by the International Commission on Microbiological Specifications for foods and only two moulds species; Aspergillus flavus and Rhizopus arrhizus were isolated from the kola nuts while the aflatoxin content was found to be less than 2 µg/kg.

Key words: Kola nut, physical characterization, proximate, chemical and mineral composition, microbial load.

INTRODUCTION

The genus Cola belongs to the family Sterculiaceae (from which the nuts are obtained) is indigenous to tropical Africa and has its greatest diversity in West Africa (Asogwa et al., 2006). It grows into a tree form and it is cultivated to a large degree in Nigeria, Ghana, Ivory Coast, Brazil and the West Indian Islands (Ejinatten, 1973; Opeke, 2005). About fifty kola species have been described out of which only seven have edible nuts and only two have been commercially exploited: *Cola acuminata* (Beauvoir) Schott and Endlicher ("abata") and *Cola. nitida* (Ventenat) Schott and Endlicher ("gbanja") (Quarcoo, 1973; Daramola, 1978). In the forest areas of West Africa, kola is perhaps second in importance to the palm tree as an indigenous cash crop (Mokwunye, 2009). Kola is an important economic cash crop to a significant

proportion of Nigerian population who are involved in kola farming, trading and industrial utilisation. The cultivation of *C. nitida* in Nigeria actually began in the 19th century (Asogwa et al., 2006) and it is estimated that the country produces about 88% of the world's kola nuts (Mokwunye, 2009) with an annual production of 200,000 metric tonnes mostly from South Western Nigeria. While the demand is rising, the production remains low because many of the trees in Nigeria are unfruitful or have very low yield due to self and cross incompatibility among trees, partial and total sterility, inefficient natural pollination, old age, field and storage pests and diseases (Asogwa et al., 2006). About 90% of the kola nuts produced in Nigeria is consumed in the country while the remaining 10% is exported as sun-dried nuts to other parts of Africa especially neighbouring West African countries (Ogutuga, 1975; Akinbode, 1982) where they are used as stimulants or as sources of colorants for cloth dyeing. C. acuminata is frequently used for social and religious ceremonies in Southern and Middle-belt Nigeria while C. nitida which is

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referred to as "the true kola of commerce" has featured in the internal trade of West Africa for a number of centuries (Jaiyeola, 2001).

The crop is important because of its nut ("Obi") that has important pharmacological properties (Atawodi et al., 2007) and also contains some active principles found in coffee and cocoa (caffeine, theobromine, kolatin) (Opeke, 2005) which prevents sleep, thirst and hunger and also acts as an anti-depressant (Mokwunye, 2009). The nuts are the cotyledonous seeds in which the purplish, cartilaginous testa has been removed. The irregular seeds, due to close nesting in the follicle have a compressed triangular shape which have a bitter astringent taste when wet and a faintly aromatic taste when dry. There are three varieties of kola nuts (white, pink and red); the most predominant being the red variety, while the white variety is not common. The three varieties are normally produced by the same species and often occurring in the same pod. Kola nuts are a common sight in the markets of African villages and cities where they are sold by street vendors at bus stops and train depots. They also have industrial usage for the production of drugs, soft drinks, wines, candies and beverages (Ogutuga, 1975; Jaiyeola, 2001). Despite the importance of kola nuts to the Nigerian economy and the world at large, the quality of the nuts have not been standardised and there are only few published reports on the chemical composition of C. nitida (Eka, 1971; Ogutuga, 1975, Jaiyeola, 2001) and C. acuminata (Adeveve and Avejuyo, 1994; Martins et al., 2011). Thus this is the first published report on the quality of Nigerian kola nuts.

MATERIALS AND METHODS

Sample collection and treatment

Dried mature red variety nuts of *C. nitida* and *C. acuminata* were purchased from three major markets noted for their wholesales; Oje and Oja-Oba markets in Ibadan (Oyo State) and Isale-Oko market in Sagamu (Ogun State), Nigeria. Kola nuts are often brought from other parts of the country for sale in these markets. The nuts (1kg) were randomly picked from three portions in each market and mixed together to form a composite sample of 3 kg per market. A total of 9 kg nuts were sampled and only consignments that were less than 3 days old in the market were sampled. Dirt's and other extraneous materials were removed from the nuts with a stainless steel knife and the nuts oven dried at 80 °C for 16 h (Oguntuga, 1975). They were pulverized with a ceramic mortar and pestle to powder and the powder used for subsequent analysis or stored at 4 °C until required.

Physical characterisation of kola nuts

Physical attributes such as shape and number of cotyledons of the two varieties of kola nuts were visually determined as well as the colour of the nuts before pulverisation. The length, width and thickness of one hundred randomly selected nuts of each species of kola were measured with a Vernier Calliper and the means

calculated.

Chemicals and standard

All reagents and solvents used were of Analytical grade (AR) grade while aflatoxin B_1 standard was from Sigma Aldrich Chemical Company (St. Louis, MO, USA)

Proximate composition of kola nuts

The proximate analyses were carried out according to the Association of Official Analytical Chemists AOAC (2005). Moisture content was determined by oven drying at $105\,^{\circ}$ C for 2 h to a constant weight, ash by igniting kola in a muffle furnace at $550\,^{\circ}$ C, crude protein by multiplying the Kjeldahl nitrogen with a factor of 6.25, fat by the reflux Soxhlet extraction method with petroleum ether and crude fibre by the Weende Method as described in AOAC (2005).

Chemical composition of kola nuts

The alkaloids and extractable caffeine were determined by the Pearson (1991) method. The alkaloid content of the kola was quantified by multiplying the Kjeldahl nitrogen with a factor of 3.26, while the flavonoids, saponins, antraquinones and phenolics were determined by AOAC, 2005 and read on a Spectronic 21D spectophotometer (AOAC, 2005) at wavelengths of 520, 380, 640 and 735 nm, respectively.

Mineral composition of kola nuts

The phosphorus content was determined by the Vanado-Molybdate colourimetic method as described in AOAC (2005) and the absorbance read at 470 nm on a Spectronic 20 spectrophotometer. Iron and zinc contents were determined by the bulk 200 atomic absorption spectrophotometer while the sodium content was read on a Jenway digital flame photometer (AOAC, 2005).

Microbial counts of kola nuts

The total plate, fungal and coliform counts were determined by the AOAC (2005) method after diluting the samples decimally and pour plating 1ml of each kola species on nutrient agar (Oxoid), malt extract agar (Oxoid) to which (0.01% chloramphenicol had been incorporated to prevent bacterial growth and MacConkey agar No 3 (Oxoid), respectively. The plates were incubated at 30 °C for 48 h, room temperature (28±2 °C) and 37 °C for 24 h, respectively. Fungal colonies were identified by morphological characteristics, spore formation and the production of fruiting bodies (Barnett and Hunter, 2003, Raper and Fennel, 1973) after incubation for 5 to 7 days. Dark red, non-mucoid colonies measuring 0.05 mm or more on uncrowded plates (15 to 150 colonies) were counted as coliforms. Confirmation was by fermentation of lactose and indole at 44 °C after 24 h incubation.

Aflatoxin content of kola nuts

The thin layer chromatography (TLC) method was used for the detection of aflatoxin B_1 AOAC (2005). Aflatoxin B_1 was identified on the basis of co-migration with aflatoxin standard and its characteristic fluorescent (blue) colour under long wave UV light (365 nm).

Table 1. Physical characteristics of Nigerian kola nuts.

Creation of calo	Colour of mut	No of cotyledon	Length (cm)		Breadth (cm)		Thickness (cm)	
Species of cola	Colour of nut		Mean	Range	Mean	Range	Mean	Range
C. nitida	White, red, pink	1-2	3.00 ^a	1.10-2.50	2.00 ^a	1.10-2.50	1.80 ^a	2.60-3.50
C. acuminata	White, red, pink	3-6	3.40 ^a	3.00-4.50	2.73 ^a	2.00-3.50	2.31 ^a	1.50-3.00

Mean values within a column with same letters are not significantly different (P< 0.05).

Statistical analysis

Data were analyzed with analysis of variance (ANOVA) for multiple comparisons among means which was separated with Duncan's Multiple Range Test and the means considered statistically different at 95% confidence level. In addition, the proximate, chemical, mineral composition and fungal occurrence were reported as percentages.

RESULTS AND DISCUSSION

Table 1 shows some physical characteristics of Nigerian kola nuts. The shape of the nuts varied from flat to oval to spherical. C. nitida has two big cotyledons while C. acuminata has an average of 3 to 6 cotyledons. The length, breadth and thickness of C. nitida ranged from 1.10 to 2.50, 1.90 to 2.50 and 2.60 to 3.50 cm, respectively with a mean of 3.00, 2.00 and 1.80 cm, respectively while that of C. acuminata was 3.00 to 4.50, 2.00 to 3.50 and 1.50 to 30.0 cm, respectively with a mean of 3.40, 2.73 and 2.31cm. The colour of the two species of kola nuts varied between white, red and pink. White kola nuts usually attract higher prices in the market while the red nuts are the most commonly consumed nuts probably because of their higher caffeine content (Mokwunye, 2009). Figure 1 shows the mean proximate composition of Nigerian kola nuts. The moisture content ranged between 7.34 to 7.40% with a mean of 7.40% (C. nitida) and 8.26 to 8.29% with a mean of 8.27% for C. accuminata. The low moisture content of both species of kola nuts is good for their long preservation as it will prevent early spoilage of the nuts. It is also beneficial to the buyers of both species of kola as most sellers sell by volume and not by weight (Adeyeye and Ayejuyo, 1994).

The variations in the moisture content of the two species of kola may be attributed to varietal differences and sizes, as *C. acuminata* nuts are usually bigger in size than *C. nitida*. The higher moisture content of *C.acuminata* which may adversely affect its storability, probably explains why Nigerian kola nut sellers attach very much importance to the save storage of *C. acuminata* than *C. nitida* nuts (Mokwunye, 2009). The protein content ranged between 15.38 to15.47% with a mean of 15.4% for *C. nitida* and 13.27 to 13.29% with a mean of 13.38% for *C. acuminata*. Furthermore, the protein content of *C. nitida* was higher than *C. acuminata*. The fat content ranged from 11.80% to 11.90% with a

mean of 11.90% for *C. nitida* while for *C. acuminata* it was between 10.76 to 10.83% with a mean of 10.80%. The range of the crude fibre content of *C. nitida* was 10.72 to 10.77% with a mean of 10.70% and 9.67 to 9.69% with a mean of 9.68% for *C. acuminata*. Vegetables and fruit fibres have been found to have hypocholesterolmic properties (Adeyeye and Ayejuyo, 1994) hence the species of kola can be exploited for this purpose. The ash content ranged between 4.29 to 4.31% with a mean of 4.30% for *C. nitida* and 3.95 to 3.97% with a mean of 4.00% for *C. acuminata*. The protein, fat and ash contents were higher than 8.06, 0.92 and 2.40% obtained by Ogutuga (1975) for *C. nitida* and 11.95% (crude protein) and 3.95% (ash) obtained by Adeyeye and Ayejuyo (1994) for *C. acuminate*, respectively.

This may be due to variation in climatic, edaphic and disease factors of the kola nuts (Olunloyo, 1979 and Odebode, 1984). They further reported that these factors could cause serious variations in the nutritional properties of kola nuts. Figure 2 shows the mean chemical composition of kola nuts. The saponin content was between 0.49 and 0.52% with a mean of 0.5% for C. nitida while it was between 0.36 to 0.39% with a mean of 0.40% for C. acuminata. Saponin has a cleansing effect and is mainly used as a tonic for the liver as it enhances the function of the liver and gall bladder (Mokwunye, 2009) thus kola nuts could be an important cleansing agent of the body. Anthraquinone was found in the range of 0.076 to 0.081% with a mean of 0.079% for C. nitida and 0.054 to 0.057% with a mean of 0.06% for C. acuminata. Anthraquinones have a mild laxative effect, which may be effective in body weight control. The range of flavonoids was between 0.055 to 0.058% with a mean of 0.050% and 0.031 to 0.037% with a mean 0.034% for C. nitida and C. acuminata, respectively.

Flavonoids are non-toxic and are found in orange and lemon rinds as well as the colouring of plants thus making kola nut a potential source of food colorant. The range of tannin was between 0.046 to 0.48% with a mean of 0.47% for *C. nitida* and between 0.38 to 0.42% with a mean of 0.40% for *C. acuminata*. Similarly, the caffeine (1, 3, 7-trimethylxanthine) content of *C. nitida* was between 1.06 and 1.13% with a mean of 1.09% and 0.98 to 1.04% and with a mean of 1.01% for *C. acuminata*. These values were lower than the 1.5% obtained by Ogutuga (1975) and Jaiyeola (2001) for *C. nitida*.

Caffeine occurs partly free and partly combined in an

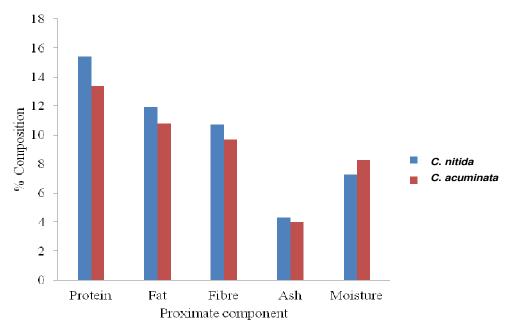


Figure 1. Mean proximate composition of Nigerian kola nuts.

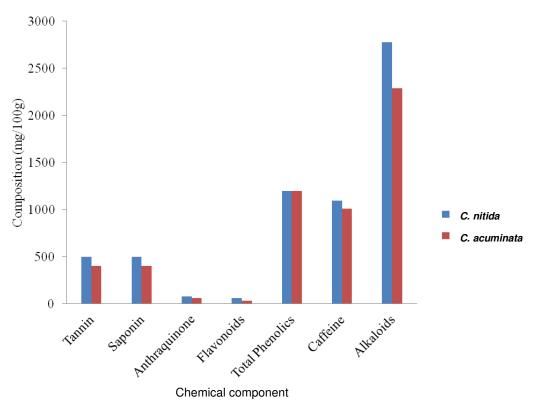


Figure 2. Mean chemical composition of Nigerian kola nuts.

unstable complex known as kolatin (tannin) and caffeine glycosides in kola. Pure caffeine is colourless and has a distinctively bitter taste at the temperature, pH and salt concentrations normally encountered in food processing

(Graham, 1978). It is also known to produce a variety of biological effects. Thus, caffeine is widely used for its stimulant properties in dietary beverages, self medication with over the counter drugs and in a number of

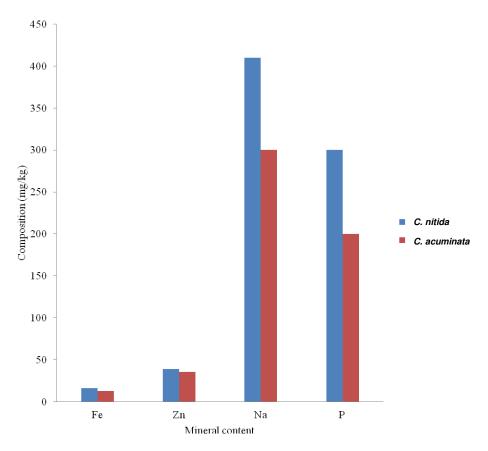


Figure 3. Mean mineral composition of Nigerian kola nuts.

prescriptions drugs containing a combination of acetylsalicylic, phenacetin, caffeine and in Darvon (Graham, 1978). Furthermore, definitive tests have shown that caffeine is not adaptive as regular consumption does not diminish its stimulant effects and its consumption in high amounts may become toxic (Graham, 1978). The alkaloid content also ranged between 2.75 to 2.81% with a mean of 2.8% for C. nitida and between 2.26 to 2.31% with a mean of 2.3% for C. acuminata. The percentage of kolanin in kola nuts is usually 5 to 10% and is made up of cathechol and epicatechol. This complex oxidises and hydrolyses to form kola-red and free caffeine under the influence of enzymes when the nuts are drying out (Adeyeye and Ayejuyo, 1994). In addition kola nuts have also been reported to contain very small quantities of (0.02 to 0.08%) theobromine (3,7-dimethylxanthine) and theophylline (1, 3- dimethylxanthine) (Adeyeye and Ayejuyo, 1994). The alkaloid compounds found in kola nuts can therefore be useful in pharmaceutical preparations (Olunloyo, 1979). Figure 3 shows the mean mineral composition of Nigerian kola nuts.

The iron content of *C. nitida* was found to be between 15.8 and 16.2 mg/kg with a mean of 16.0 mg/kg while it ranged between 12.6 to 12.7 mg/kg with a mean of 12.65 mg/kg for *C. acuminata*. The recommended iron intake for men and menopausal women is 8 and 18 mg/kg for

pre-menopausal women (Mokwunye, 2009). Iron is an essential component in the transfer of oxygen as it is a component of cytochromes and is the element most closely associated with anaemia. The zinc content was between the range of 38.75 to 39.20 mg/kg with a mean of 38.98 mg/kg for C. nitida while it was between 35.2 and 35.6 mg/kg with a mean of 35.4 mg/kg for C. acuminata. Zinc has been associated with enzyme systems, particularly oxidation processes (Adeyeve and Aveiuvo, 1994). The phosphorus content was also found to be between 250 and 280 mg/kg with a mean of 260 mg/kg for C. nitida and 170 mg/kg to 200 mg/kg with a mean of 180 mg/kg for *C. acuminata*. These values were however lower than the range of 0.48 to 0.94, 0.68 to 1.35, and 54.45 to 129.85 mg/100 g, respectively recorded for Fe, Zn and Ph by Martins et al. (2011) but can serve as supplementary sources of minerals to consumers of kola.

Similarly, the sodium content ranged from 390 to 430 mg/kg with a mean of 410 mg/kg for C. nitida and 310 to 340 mg/kg with a mean of 320 mg/kg for C. acuminata. Table 2 shows the mean colony counts of Nigerian kola nuts. The mean total plate count was 9.20×10^3 cfu/g for C. nitida while it was 7.67×10^3 cfu/g for C. acuminata. The mean fungal count was 1.20×10^3 cfu/g for C. nitida while it was 0.45×10^3 cfu/g for C. acuminata. The mean

Table 2. Mean colony count of Nigerian kola nuts (cfu/g).

Cola species	Total plate count (x10 ³)	Fungal count (x10 ³)	Coliform count (x10 ²)
C. nitida	9.20±0.30 ^a	1.20±0.38 ^a	8.00±0.33 ^a
C. acuminata	7.67±0.52 ^a	0.45±0.22 ^b	1.60±0.24 ^b

Mean values within a column with same letters are not significantly different (p < 0.05).

Table 3. Percentage frequency of occurrence of fungi isolated from Nigerian kola nuts.

Cola species	Fungal isolate	Fungal frequency of occurrence (%)
C. nitida	Rhizopus arrhizus	40
	Aspergillus flavus	42
C. acuminata	Rhizopus arrhizus	50
	Aspergillus flavus	48

coliform count was 8.00 x102 for C. nitida and 1.60 x 10²cfu/g for *C. acuminata*. Although, the fungal and coliform counts of C. nitida were significantly higher (p<0.05) than that of C. acuminata, they were still within the permissible limits of 10⁵, 10⁴ and 10² cfu/g recommended by the International Commission on Microbiological Specification of Foods (ICMSF, 1974). Table 3 shows the list of moulds isolated from the kola nuts and their percentage frequency of occurrence. Only two types of moulds R. arrhizus and A. flavus were isolated in this study. Similarly, the aflatoxin B₁ content of the nuts was found to be less than 2 µg/kg (the detection limit of TLC). The present study has shown that kola nuts contain some active principles found in coffee and cocoa that have important pharmacological properties. It also shows that the proximate, chemical and mineral contents of C. nitida are higher than C. acuminata thus justifying its description as "the true kola of commerce". The values obtained can also be used as a baseline data towards elaborating standards for the nuts.

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