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Full Length Research Paper

Effect of different botanicals on mineral, proximate, phytochemicals of varying colours and weights (sizes) of kola (*C. nitida*) at 6 weeks after curing

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Abstract

Phytochemicals and mineral compositions of preservative botanicals used in curing *Cola nitida* nut were assayed, carried out in cocoa research institute of Nigeria. Different weights (1-10g, 11-20g and >21g) and colours (pink, red and white) above 21g of weight of *C. nitida* nut cured for 6 weeks were also analyzed for biochemical compositions. *T. grandis* recorded the highest in nitrogen, phosphorus, calcium potassium and manganese while plantain leaves recorded the highest in sodium, magnesium and iron respectively. Result also reveals that *A. indica* recorded significant values in phytochemicals content assayed except for alkaloid, flavonoid and theobromine. Significant increase was detected in phytochemicals minerals and proximate content from the smallest nut weight to the biggest nut weight. The results obtained for different colours shows that the red *C. nitida* nut above >21g of weight recorded the highest in the entire mineral element assayed except for calcium when preserved with botanicals and the result was significant. Similarly, red *C. nitida* nut above 21g recorded the highest in crude fibre, crude fat, ash content and vitamin C while the pink *C. nitida* nut had the highest in crude protein, moisture content, organic carbon, organic matter and carbohydrate cured for 6 weeks. Result obtained for phytochemical content shows that red *C. nitida* nut had the highest in tannin, flavonoid, theobromine, kolatin and polyphenol while that of pink *C. nitida* nut recorded the highest in alkaloid, saponin anthraquinone and caffeine respectively. Phytochemical content showed high quantity of anthraquinone and flavonoids suggesting their antioxidant potentials and justifying their therapeutic uses which could utilized in drug formulation.

Keywords: *Cola nitida*, phytochemicals, proximate and mineral

Introduction

Cola nitida (the kola nut) also known as *cola*, goro nut, cola seed is an important commercial and economic crop for many West African countries (Lovejoy, 1980) and Central Africa (Van Eijnatten, 1969). There are about twenty five species of the genus cola but only five or six species produce edible seeds commonly called 'nuts'. These are *Cola nitida* (vent), *Cola acuminata* Schott and Endl., *Cola vertillata* Thorn, *C. anomala* and *C. ballay* (Russell, 1955). However, only two species; *C. nitida* and *acuminata* are cultivated as plantation crops (Quarcoo, 1973; Daramola, 1978)

Kola nuts are extremely popular due to their high caffeine content. Kola nuts have a bitter taste and contain between 1–1.5% caffeine by weight (Clayton, 2002). They are also a source of antioxidants and contain small amounts of theobromine, d-catechin, L-epicatechin, kolatin, phlobaphens, antioxidant pigment, betaine and protein. Kola extract is popular ingredient in fat loss supplements. It suppresses hunger, aids digestion of food and acts as a diuretic. Kola nuts contain two alkaloids, caffeine and theobromine, which are powerful stimulants that counteract fatigue, suppress thirst and are believed to enhance intellectual activity (Nickalls, 1986; Sundstron, 1966). Due to their unique bitter taste, fresh kola nuts when chewed according to Nickalls (1986) is use to sustain people during long journeys. It features prominently in religious, social and ritual activities. Nuts are used for some ceremonies, such as marriage, child

naming, and funerals and for making sacrifices to various gods and goddesses of Africa Mythology (Opeke, 2005). Kola nuts are offered to visitors as a sign of welcome and appreciation. The gift of kola especially the splitting and sharing of kola nuts between two or more people signifies a special bond of friendship.

Industrially, it is useful for the preparation of kola type beverages namely Coca Cola, Pepsi- Cola, wine and kola chocolate. Also, it is reported by Mokwunye (2009) that kola powder best suited for beverage production could be produced by drying kola nuts at 80^{o}c for 9 hrs. Kola nuts invigorate dental gums and prevent gout and diseases (Opeke, 1992). The kola testa is used in feeding Africa giant land snail raised in a kola plantation (Hamzat, *et al.,* 2002). Kola nuts are a good source of material for dyes in textile and thread industry. Kola provides income and employment for those who are engaged in the production of the crop.

The kola pod husk has also been utilized for the production of liquid soap. The most recent and remarkable advancement in kola by-product utilization is the use of kola pod husk in the replacement of up to 60% of the maize used in poultry feed formulations (Yahaya *et al*., 2001; Hamzat, 2001; Hamzat and Babatunde, 2001; Hamzat and Longe, 2002; Hamzat *et al*., 2000; 2002a; 2002b; Olubamiwa *et al*., 2002).

Medicinally in the traditional circle, the leaves, twings, flowers, fruits follicle and the bark of both *C. nitida* and *C. acuminata* were used to prepare a tonic as a remedy for dysentery, cough, diarrhea, vomiting and chest complaints (Irvine, 1961 and Ayensu, 1978).

Kola has a wide application in the food and pharmaceutical industries where it is used as sources of caffeine in foods and pharmaceutical products. Jayeola (2004) reported the possible use of kola nut for the production of soft drinks (Eleyimi, *et al.,* 2006). Kola nut also contain traces of essential minerals like K, Ca, Mg Na, Fe Zn, Mn, and P (Williams, 1979). Some of these minerals act as sources of macro and micro nutrients needed for growth and development and metabolic activities by man. Calcium is essential for the development of bones, sodium relevant for acid base balance and osmotic regulation of the body fluids and the transmissions of nerve impulses. Deficiency leads to lowering of osmotic pressure and reduces utilization of digestive proteins (*Macrae et al*., 1997). Iron is relevant for metabolic processes involving oxygen transport, storage as well as oxidative metabolism and circular growth (AOAC, 2000). Deficiency of iron in the body leads to anemia, fatigue and palpitation, depressed growth in children, anorexia and resistance to infection. Potassium also influences osmotic equilibrium and the maintenance of acid-base balance in the body. It also facilitates the amino acids uptakes by cells and influences carbohydrate metabolism in cells. It is also required for normal tissue protein synthesis and functioning of the heart and kidney muscles.

The demand of kola for safety in food and health care products has increased over the years. This has called for equally higher quality standards for product ingredients. The biochemical modifications, especially the changes in polyphenol, phytochemicals mineral contents have not been well studied and exploited in assessing kola quality, thus the aim of the study is to determine the level of polyphenol, phytochemicals and mineral content on kola nut cured for 6 weeks in assessing kola quality.

Materials and methods

This study was carried out in Cocoa Research Institute of Nigeria (CRIN) Headquarters, Idi-Ayunre, Ibadan. Fresh *C. nitida* nut with three different weights and colours was purchase from "Ogunmakin" village market, Ogun State. The nuts were classified into weight as follows: 1-10g, 11-20g and 21-40g and into colours: red, pink and white. The nuts of the different weights and colours were cured in a basket lined up with different botanicals for 6 weeks. This was later crushed separately into smaller particle sizes using perforated grater and stored in a capped container until they were needed for analysis.

Phytochemical, nutritional, proximate and anti-nutrients analyses of *Cola. nitida*:

Proximate composition of kola nuts

The nuts was washed, chopped into pieces and dried in oven at 70 ^{0}C for 24h. After drying, the nuts was grounded into a fine powder using mortar and pestle and stored in a well labeled air-tight container for analysis. The proximate analyses was carried out according to the Association of Official Analytical Chemists AOAC (2000). Moisture content was determined by oven drying at 105°C for 2 h to a constant weight, ash by igniting kola in a muffle furnace at 550°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 (2000). Total carbohydrate was obtained by difference.

Mineral composition of kola nuts

The phosphorus content was determined by the Vanado-Molybdate colorimetric method as described in AOAC (2000) and the absorbance read at 470 nm on a Spectronic 20 spectrophotometer. Iron and zinc contents was determined by the bulk 200 atomic absorption spectrophotometer while the sodium and potassium content was read on a Jenway digital flame photometer (AOAC, 2000). Phytates was determined by the method of Early and DeTurk (1944) modified by Thompson and Erdman (1982). Total oxalate was determined by the method described by Ukpabi and Ejidoh (1989) and trypsin inhibitors by the method described by Kakade *et al.* (1974)

Quantitative Screening of phytochemicals

Chemical tests was carried out on the aqueous extract and on the powdered samples using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). Alkaloids, tannin, saponin, anthraquinone, flavonoid, was determined using the methods above.

Statistical analysis

The average data obtain for the physiological and growth parameters for the experiments were analyzed using ANOVA with an F-test. The treatment means was compared using a Duncan Multiple Range Test at the 5% probability level (Gomez and Gomez, 1984).

Results and Discussion

Table 1 shows mineral composition (mg/100g dry matter) screened for botanicals used in storing kola nut. The result showed that *T. grandis* had the highest content of nitrogen, phosphorus, calcium, potassium and manganese when compared to other botanicals and these were significant. *A. indica* followed with respect to these nutrients and the least were obtained for plantain leaves. Plantain leaves had the highest contents of sodium, magnesium and iron, followed by *A. cordifolia* and the least was detected in *A. indica.* Potassium (K) was the highest of all the mineral elements analyzed and the differences were significant.

Table 1: Mineral composition (mg/100g dry matter) screened for botanicals used in storing kola nuts

Botanicals	N	P	Ca	K	Na	Mg	Fe	Mn
T. grandis	1.46^{a}	8.75^{a}	6.25^{a}	84.80^{a}	5.99^{c}	3.23^{c}	2.92^{c}	2.13^{a}
A. indica	1.35^{b}	7.83^{b}	5.15^{b}	70.85^{b}	4.91^{d}	2.76^{d}	2.87^{d}	1.65^{c}
A. cordifolia	1.27^{c}	6.85^{c}	4.93^{c}	64.91^{c}	7.96^{b}	3.80^{b}	3.13^{b}	1.84^{b}
Plantain leaves	1.15^{d}	5.87^{d}	4.87^{d}	52.67^{d}	8.53^{a}	4.14^{a}	3.92^{a}	1.87^{b}

Means followed by the same letters on the same column are not significant different from each other at 5% level of probability

Table 2 shows the phytochemical screening of botanicals used in preserving kola nut. From the result, alkaloid was the highest of all the phytochemical content assayed using *T. grandis* as preservative materials, followed by saponin in *A. indica* and the least was observed for theobromine in plantain leaves. The range of values included; alkaloids, 3.75g/100g in plantain leaves to 4.76g/100g in *T. grandis*, saponin, 1.48g/100g in plantain leaves to 3.83g/100g in *T. grandis*, theobromine, and 0.001g/100g in plantain leaves to 0.004g/100g in *T. grandis*. Other values included; tannin,0.27g/100g in plantain leaves to 0.37g/100g in *A. indica*, polyphenol, 0.22g/100g in plantain leaves to 0.28g/100g in *A. indica*, anthraquinone, 0.11g/100g in plantain leaves to 0.58g/100g in *A. indica*, flavonoid, 0.25g/100g in plantain leaves to 0.41g/100g in *T. grandis*, caffeine, 0.010g/100g in plantain leaves to 0.0016g/100g in *T. grandis* and kolatin, 0.002g/100g in plantain leaves to 0.006g/100g in *A. indica* respectively.

Table 2: Phytochemical content (g/100g) screened for botanicals used in storing kola nuts

Botanicals	Alkaloid	Saponin	Tannin	Polyphenol	Anthraquinone	Flavonoid	Caffeine	Kolatin	Theobromine
T. grandis	4.76^{a}	3.83^{b}	0.35^{b}	0.24^{b}	0.32^{b}	0.41^{a}	0.016^{b}	0.003^{b}	0.004^{a}
A. indica	4.40^{b}	4.85^{a}	0.37^{a}	0.28^{a}	0.58^{a}	0.35^{b}	0.110^{a}	0.006^{a}	0.003^{b}
A. cordifolia	4.12^{c}	2.34^{c}	0.29^{c}	0.23^{c}	0.14^{c}	0.34^{c}	0.012^{c}	0.002^{c}	0.002^{c}
Plantain leaves	3.75^{d}	1.48^{d}	0.27^{d}	0.22^{d}	0.11^{d}	0.25^{d}	0.010^{c}	0.002^{b}	0.001^{d}

Means followed by the same letters on the same column are not significant different from each other at 5% level of probability according Duncan Multiple Range Test

Table 3 shows the effect of preserving botanicals on mineral composition (mg/100g dry matter) of C. nitida nut at 6weeks after curing of different weights. A common trend of increasing quantities from the lowest nut weight to the highest nut weight with the preponderance of potassium and the result was significant. Similar work carried out by Oladokun, 1989 shown that heavier nut contains more nutrients compared to small and medium nut sizes. This result obtained from this study compared favorably with that reported by other Workers in their study (Jayeola, 2004 Odebunmi *et al*., 2009). Table 4 shows that red *C. nitida* nut above 21g of weight recorded the

highest in the entire mineral element assayed except for calcium with potassium having the highest value. Similar result was detected in proximate content with increasing quantities from the lowest nut weight to the highest nut weight except for carbohydrate with the preponderance of moisture content. (Table 5). Red *C. nitida* nut above 21g recorded the highest in crude fibre, crude fat, ash content and vitamin compared to the pink and white *C. nitida* nut and the result was significant. Pink nut recorded the highest in crude protein, moisture content, organic matter, organic carbon and carbohydrate (Table 6). The nutrient composition of kola nut differs relatively from what has been reported by other workers. Jaiyeola (2001) had earlier reported 8.90% protein, 0.92% fat and 2.40% ash in the fresh nut of kola and Ogutuga (1975) also reported a protein content of 8.0%. Other workers also reported 69% carbohydrate 18% crude fat and 3.1% ash by weight of dried powdered sample of kola nut (Arogba, 1999). All of these are quite different from what had been reported in this study. The vary composition reported by various workers may implies that the nutrient composition of these nuts vary with season, environment and/or condition or time of evaluation.

Table 3: Mineral content (mg/100g dry matter) of *C. nitida* nut at 6weeks after curing of varying weights

Nut weights(g)	Ca	K	Na	P	Mg	Fe	Zn	Mn
21-40	**3.30^{a}**	**12.70^{a}**	**4.82^{a}**	**6.49^{a}**	**4.81^{a}**	**3.82^{a}**	**3.16^{a}**	**4.01^{a}**
11-20	2.89^{b}	12.03^{b}	4.07^{b}	5.68^{b}	4.00^{b}	3.21^{b}	2.60^{b}	3.49^{b}
1-10	2.45^{c}	10.01^{c}	2.70^{c}	4.45^{c}	2.67^{c}	2.23^{c}	1.93^{c}	2.52^{c}

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.

Table 4: Mineral content (mg/100g dry matter) of *C. nitida* nut at 6weeks after curing of varying colours above 21g of weight

Nut colours	Ca	K	Na	P	Mg	Fe	Zn	Mn
Pink (>21g)	3.36^{a}	11.86^{b}	3.20^{c}	5.56^{b}	3.57^{b}	3.11^{b}	2.32^{b}	3.18^{b}
Red (>21g)	2.86^{b}	12.01^{a}	4.66^{a}	6.10^{a}	4.60^{a}	3.28^{a}	3.02^{a}	3.88^{a}
White(>21g)	2.59^{c}	10.64^{c}	3.65^{b}	4.96^{c}	3.21^{c}	2.92^{c}	2.34^{b}	2.96^{c}

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.

Table 5: proximate composition of *C. nitida* nut at 6 weeks after curing of varying weights

Nut weights (g)	% CP	%CF	%C Fat	Moisture content	Organic carbon	Organic matter	%Ash content	CHO	Vit C
21-40	4.05^{a}	6.70$_{a}$	2.95^{a}	91.32^{a}	6.14^{a}	10.58^{a}	3.59^{a}	82.76^{c}	5.74^{a}
11-20	3.61^{b}	5.16^{b}	2.57^{b}	90.31^{b}	5.67^{b}	9.76^{b}	2.96^{b}	85.68^{b}	5.12^{b}
1-10	2.80^{c}	3.90^{c}	2.32^{c}	83.09^{c}	5.11^{c}	8.80^{c}	2.35^{c}	88.88^{a}	4.28^{c}

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.
NOTE: CP crude protein, CF: crude fibre, CHO: carbohydrate and Vit C: Vitamin C

Table 6: proximate composition of *C. nitida* nut at 6 weeks after curing of varying colours above 21g of weight

Nut colours	% CP	%CF	%C Fat	Moisture content	Organic carbon	Organic matter	%Ash content	CHO	Vit C
Pink (>21g)	3.66^{a}	5.00^{b}	2.57^{b}	89.30^{a}	5.83^{a}	10.11^{a}	2.56^{c}	86.43^{a}	4.87^{b}
Red (>21g)	3.30^{c}	5.76^{a}	2.87^{a}	86.29^{b}	5.44^{c}	9.37^{c}	3.52^{a}	84.64^{b}	5.90^{a}
White(>21g)	3.49^{b}	4.99b	2.40^{c}	90.30^{a}	5.62^{b}	9.68^{b}	2.81^{b}	86.25^{a}	4.37^{c}

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.
NOTE: CP crude protein, CF: crude fibre, CHO: carbohydrate and Vit C: Vitamin C

Quantitative phytochemical content of different weights at 6 week after curing treated with botanicals reveals a common trend of significant increase from the lowest nut weight to the highest nut wit anthraquinone having the highest value (Table 7). The percentage of kolanin in kola nuts is usually 5 to 10% and is made up of catechol and epicatechol. This complex oxidizes 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 in this study contain

considerable amount of theobromine which ranged from 1.65 for the smallest nuts to 2.17 for the biggest nuts which is contrary to (Adeyeye and Ayejuyo, 1994) who reported that theobromine contain very small quantities of (0.02 to 0.08%) theobromine (3,7-dimethylxanthine) and theophylline (1, 3- dimethylxanthine). This could be as a result of the preservative botanicals used in curing the nut. Curing usually involves the storage of the fresh kola nuts for months or years in cane baskets properly lined with plantain leaves and botanicals. During curing, polyphenols are subjected to biochemical modifications through polymerization and complexing to proteins and hence decreasing solubility and astringency (Bonhevi and Coli, 1997). Red nut recorded the highest in tannin, flavonoid, caffeine, theobromine, kolatin and polyphenol compared to pink and white nut (table 8). The implication of this is that red nut above 21g of weight tend to brown the teeth more than the pink and white nut because of the presence of polyphenol, responsible for teeth browning. The presence of secondary metabolites in the kola nuts could be responsible for its antioxidant activity. For example, flavonoid and other phenolic constituents have been shown to play a preventive role in the development of cancer and heart diseases, potential sources of antioxidant compound have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, bark and roots, spices and herbs and crude plant drugs (Pourmorad *et al*., 2006).

Table 7: Phytochemical content (mg/100g) of *C. nitida* nut at 6 weeks after curing of varying weights

Nut weight (g)	Alkaloid	Tannin	Saponin	Flavonoid	Anthraquinone	Caffeine	Theobromine	Kolatin	Polyphenol
21-40g	2.05[a]	4.31[a]	4.18[a]	3.39[a]	6.24[a]	5.14[a]	2.17[a]	4.56[a]	1.98[a]
11-20g	1.94[b]	3.91[b]	3.62[b]	2.95[b]	5.28[b]	4.32[b]	1.96[b]	4.16[b]	1.81[b]
1-10g	1.51[c]	2.88[c]	2.74[c]	2.06[c]	3.79[c]	3.50[c]	1.65[c]	3.31[c]	1.32[c]

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.

Table 8: Phytochemical content (mg/100g) of *C. nitida* nut at 6 weeks after curing of varying colours above 21g of weight

Nut colour	Alkaloid	Tannin	Saponin	flavonoid	anthraquinone	caffeine	Theobromine	Kolatin	polyphenol
Pink (>21g)	1.89[a]	3.18[c]	3.97[a]	2.51[b]	5.50[a]	4.47[a]	2.05[b]	4.22[b]	1.44[c]
Red (>21g)	1.69[b]	4.17[a]	3.19[b]	3.01[a]	5.17[b]	4.42[a]	2.28[a]	4.49[a]	2.10[a]
White(>21g)	1.91[a]	3.76[b]	3.07[c]	1.89[c]	4.65[c]	4.08[b]	1.44[c]	3.32[c]	1.57[b]

Means of the same letter on the same column are not significantly different from each other at 5% level of probability according Duncan Multiple Range Test.

Conclusion

- Large *C. nitida* nut above 21-40g recorded the highest in mineral composition and phytochemical content when compared to medium and small nut.
- Red colour of *C. nitida* nut recorded the highest value in the entire mineral element assayed for fresh *C. nitida* nut.
- Varying colours of *C. nitida* nut above 21g of weight recorded the highest values in all the nutritional composition when preserved with botanicals compared to justocin.
- Red *C. nitida* nuts tend to brown the teeth more than the pink and white nut due to the presence of polyphenol, responsible for teeth browning.
- The presence of phytochemicals (secondary metabolites) in *C. nitida* nut justifies their therapeutic functions.

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