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Effect of Varying Sizes, Colours and Time of Cured *c.nitida* Nuts on Phytochemical Content and Enzyme Activities Using Botanicals as a Preservative Material

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ABSTRACT

Cola is a tropical tree crop that belongs to the family Sterculiaceae. Phostocin is a preservative chemical used by farmers to store *Cola nitida* nuts which is considered unsafe to human health when consumed. Fresh *C. nitida* nuts of different weights (1-10g, 11-20g and above 21g) and colours (pink, red and white) were obtained from Cocoa Research Institute of Nigeria, Ibadan Oyo State. The Nuts were cured for 12weeks with botanicals. These Nuts were oven dried at 70^0C for 2 days, ground into powder for phytochemical and enzymatic analysis. Data were subjected to Analysis of variance. The leaf of *T. grandis* had highest values in alkaloid (4.76 g/100g), flavonoid (0.41 g/100g) and theobromine (0.004g/100g) while *M. paradisiaca* recorded the lowest values for phytochemical analyzed. *Cola nitida* nuts recorded the highest values in tannin (3.49g/100g), saponin (3.45g/100g), flavonoid (2.46g/100g), anthraquinone (5.19g/100g), caffeine (4.29g/100g) and polyphenol (1.65g/100g) when preserved with *T. grandis* and the least was obtained for phostocin in red *C. nitida* nut above 21g. Also, red *C. nitida* nut above 21g preserved with *T. grandis* recorded the highest values in catalase (0.154 mg/$NaBO_3$.$4H_2O$/min/g protein), cellulase (0.125 mg/glucose/min/g protein), total amylase (0.141 mg/glucose/min/g protein) and proteinase (0.121 mg/tyrosine/min/g protein) and lowest values obtained in phostocin. Decreased in enzymes activities and phytochemical content were observed in different colours as the number of weeks increases. In conclusion, the use of botanicals as a preservative material to cure kola nuts is better and safe for consumption than phostocin which the farmers are currently using.

Introduction

Plants are a primary source of medicines, fibre, food, shelters and other items in everyday use by humans. The roots, stems, leaves, flowers, fruit and seeds provide food for humans (Oyenuga and Fetuga, 1975). Plants serve as an indispensable constituent of human diet supplying the body with mineral salts, vitamins and certain hormone precursors, in addition to protein and energy (Oyenuga and Fetuga, 1975). Seeds have nutritive and calorific values which make them necessary in diets (Odoemelam, 2005). Among these plants are the nuts of *Cola nitida* which can also be eaten for their special taste and flavour.

Cola is a tropical tree crop that belongs to Sterculiaceae. Fifty species of this genus have been described in West Africa (Adebola, 2003). Three species were later added (Cheek, 2002). Of these, only a few are fruit bearing while majority are woody species of economic importance. The most commonly used species are *Cola nitida* [(Vent) Schott and Endlicher], *Cola acuminata* [(pal de. Beuav) Schott and Endl] and *Cola anomala* (Schott and Endlicher) know in Cameroon as non-timber forest products.

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). Due to their unique bitter taste, fresh kola nuts when chewed according to Nickalls (1986) is used 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^oc 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 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 *et al.*, 2000; 2002a; Hamzat

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and Longe, 2002; Hamzat *et al*., 2002; 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). It contains in substantial amount active ingredients of caffeine, theobromine and kolatin.

Fresh kola nuts are highly hydrated, have very bright colours and are astringently bitter. They contain a wide array of complex secondary plant metabolites and polyphenolics known to be mainly responsible for the astringency and bitter taste in the fruits. Several curing and preservation methods have been tried to remove the astringency, bitterness and high moisture content to make the nuts palatable and acceptable (Osei Bonsu *et al.,* 1977; Takrama *et al.,* 2000). Curing usually involves the storage of the fresh *cola* nuts for months or years in cane baskets properly lined with either banana or *Mitragygna stipulosa* leaves. During curing, polyphenols are subjected to biochemical modifications through polymerization and complexing to proteins and hence decreasing solubility and astringency (Bonhevi and Coli, 1997). The amount of polyphenols is also substantially reduced by enzymatic browning caused by polyphenol oxidase (PPO) (Wollgast and Anklam, 2000). High levels of polyphenol oxidase responsible for enzymatic browning have also been reported in *C. nitida* (Prohp *et al.*, 2009). Cured kola nuts that are not slimy, not astringently bitter in taste but palatable and crispy are normally considered to have the desirable characteristics of good quality (Quarcoo, 1973). The most important factors that determine the value of kola nuts at the local markets are size, flavour, colour and keeping qualities of the nuts (Quarcoo, 1973). While size and colour can be physically determined; flavour and keeping quality are ultimately determined by the method of curing and preservation.

The demand for kola nuts as well as its seedlings in the domestic and international markets by food and pharmaceutical industries has increased over the years. (Ayensu, 1978). Traditionally, kola farmers use mainly phostocin to preserve their kola nut which is not safe for notable consumers with little or no knowledge on the use of preservative botanicals.

The scientific basis for using these botanicals as preservative materials in terms of biochemical modification especially the changes in polyphenols have not be well studied and exploited in assessing kola quality. Therefore, the objectives were to:

- ❖Determine the effect of botanicals as preservative on the phytochemical content of cured *C. nitida* nut
- ❖Determine the effect of preservative botanicals and phostocin on phytochemical content of cured *C. nitida* nut.
- ❖Determine the effect of preservative botanicals and phostocin on enzymatic activities of cured *C. nitida* nut.
- ❖Determine the effect of preservative botanicals on the length of storage of kola nuts

The study area

This study was carried out at Cocoa Research Institute of Nigeria (CRIN) Headquarters, Idi- Ayunre, Ibadan. CRIN headquarters is situated in the derived savannah zone of Nigeria (latitude7°25'N, 3° 25'E., altitude 122 m above sea level. The rainfall is between 1250-1500 mm per annum and average temperature of 30°C.

Collection of samples

Cola nitida nuts and botanicals were obtained from Cocoa Research Institute of Nigeria (CRIN), Oyo State. The nuts collected were classified into class of weight as follows: 1-10 g, 11-20 g and 21 g and above, and colours such as red, pink and white. A total of 250 nuts were used for this experiment

Effect of preservative materials, methods and length of storage on nut quality

The preservative materials used in the experiment were chemical and botanicals. The chemical used was phostocin while the botanicals were: *Alchornia cordifolia* (local name: Esin) (family: *Euphorbiaceae*), *Azadirachta indica (local name:* Dongoyaro) (family *Malvaceae*), *Musa paradisiaca* and *Tectonia grandis* used for the experiment. Fresh leaves of the botanicals used were obtained from CRIN. Eighteen (18) baskets were used for the research work and were obtained from CRIN. The storage baskets were lined up first with thin transparent nylon sheet followed by a layer of the leaves of the botanicals listed above placed with the ventral (upper) surface facing downwards in the basket thereby exposing the dorsal (back) surface of the leaves to the nuts. The nuts were carefully placed inside, layer by layer and after each layer leaves were spread out evenly on top of the nuts before finally sealing up the whole thing with the first layer of polythene sheet. The baskets were stored under normal room temperature and relative humidity. The leaves and polythene sheets kept the nuts in an air tight condition and prevent desiccation of the nuts. For the control, no treatment was applied. The nuts were also stored in basket lined with thin transparent polythene sheet, which was in turn lined with a layer of paper. The nuts were inspected every eight days during storage. Any defective or infested nuts were sorted out during the inspection period. This periodical inspection also prevents the overheating of the nuts. Furthermore, the top leaves that have dried and shrunk, was changed with time. The kola nuts were preserved for a period of 3 months. Phytochemical and enzyme activities of the nuts were determined at 3 weeks interval for a period of three (3) months as follows: 0, 3, 6, 9 and 12.

Quantitative analysis of phytochemicals

Determination of alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne (1973). A known weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at 28°C. It was later filtered via whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonium solution and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Flavonoids

This was done according to the method of Harborne (1973). Five gram of the sample was boiled in 50 ml of 2 M HCl solution for 30 mins under reflux, allowed to cool and then filtered through whatman No 42 filter paper. A known volume of the extract was treated with equal volume of ethyl acetate starting with drops. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight differences gave the weight of flavonoid in the sample.

Tannin

Tannin content was determined by the Folis- Dennis colorimetric method described by Kirk and Sawyer (1998). Five gram of the sample was dispersed in 500 ml of distilled water and shaken. The mixture was allowed to stand for 30 mins at 28°C before it was filtered through whatman No 42 grade of filter paper. 2 ml of the extract was dispersed into a 500 ml volumetric flask. Similarly, 2 ml standard tannin solution (tannic acid) and 2 ml of distilled water was put in separate volumetric flask to serve as the standard. Reagent was added to each of the flasks and the 2.5 ml of saturated $NaCO_3$ solution added. The content of each flask was made up to 50 ml with distill water and allowed to incubate at 28°C for 90 mins. Their respective absorbance was measured on a Spectrophotometer at 260 nm using the reagent blank to calibrate the instrument to zero

Saponin

Quantitative determination of saponin was done according to Obadoni and Ochuko (2001). Twenty gram of each powdered sample was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The sample was heated over a water bath for 4 h at 55°C. The mixture was filtered and the residue re- extracted with another 200 ml of 20% aqueous ethanol. The combined extract was reduced to approximately 40 ml over bath at 90°C. The concentrate was transferred into 250 ml separatory funnel, extracted twice with 20 ml diethyl ether. Ether layer was discarded while aqueous layer was retained and 60 ml n- butanol extract was wash twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation; the sample was dried in an oven at 40°C to a constant weight. The saponin content was calculated as percentage of the initial weight of sample taken.

Caffeine determination

Caffeine content was determined according to Irgolic *et al.* (1982) methods. Two samples of grated kola nuts were put into a round bottom flask and 300 ml of distilled water was added to each. The mouth of the flask was covered with condenser and then connected to a closed jar of water and each of the flasks was placed on electric heater. As soon as the content begins to boil the close tap was open to drain the water and was allowed to stand for one hour. As the content was boiling, the refluxing system was turned on and the reflux was sieved out. The residue was discarded and the filtrate was retained and placed in ice block for 15 mins; thereafter, 100 ml of the filtrate was placed in a 250 ml separatory funnel and 120 ml chloroform was added gradually. The corked separatory funnel was shaken until the chloroform, water interface was established and after 50 mins clear solution was formed into which caffeine dissolved in chloroform. It was later put into 50 ml beaker and chloroform evaporated over a water bath. The weight of the resultant yellowish white caffeine crystals was taken on mettler P-165 electric balance.

Enzyme determinations

Cellulase activity

The enzyme extract was prepared by grinding 1 g of nut with 1/10 M dibasic sodium phosphate (K_2HPO_4) in a mortar maintained at 5°C with crushed ice (Norkrans, 1979). The ensuing suspension was centrifuge at 18,000 g for 30 mins at 2°C using the M.SE ultra-high speed centrifuge. To 1 ml of the supernatant was added 1 ml of 1% carboxymethyl cellulose in 0.05 M phosphate buffer (pH 5.0) and the mixture allowed to stand for 1 h at 30°C (Singh and Kunene, 1980). The enzyme action was stopped with 3, 5 - dinitrosalicylic acid (DNSA) reagent and the amount of reducing sugar formed determined by taking the absorbance at 540 nm against a blank containing 1 ml of boiled enzyme extract which was similarly treated.

Total amylase (α and β activity)

Enzyme extract was prepared by grinding 1 g of the nuts with sodium acetate buffer (pH 5.0) in a mortar maintained at 5°C with crushed ice and the extract centrifuge at 18,000 g for 30 mins at 2°C. One millimeter of the supernatant was added to 1 ml of 1% soluble starch in 1/10 M sodium acetate buffer and the mixture was incubated at 27°C for 1 h. The enzyme action was stopped with DNSA reagent and the quantity of reducing sugar formed was determined by taking the absorbance at 540 nm against a blank containing 1 ml of boiled enzyme extract treated similarly (Swain and Dekker, 1966).

Proteinase activity

Enzyme extract was prepared in a manner similar to total amylase activity. Except that 0.05 M sodium phosphate buffer (pH 6.0) was used as the extracting buffer. Proteinase activity in the enzyme was determined using the Lowry Folin – Ciocalteu method of Mcdonald *et al*. (1965).

Lipase activity

Enzyme extract was obtained in the same way for total amylase activity. Lipase activity in the enzyme extract was determined using the method of Young *et al.* (1977).

Ascorbic acid content

The determination of ascorbic acid was carried out using the 2, 6-dicloroindophenol method described by (Association Official Analytical Chemists, 1984).

Ten gram of fresh kola nuts was weighed into mortar and 48 ml metaphosphoric acid was added. The mixture was stirred for about 20 mins and rapidly filtered using a suction pump and Buchner funnel. 10 ml of the filtrate was titrated to the end point (change from blue to a permanent pink colour) with the standardized 2, 6-dichlorophenol-indophenol solution. The titration was repeated in triplicates and blank determination was also carried out following the above procedure but using 10 ml of mataphosphoric acetic acid instead of the filtrate (AOAC, 1984)

The phenolic content was analyzed using Folin-Ciocalteu reagent following a modified procedure of Singleton and Rossi (1965). One ml of the appropriately diluted sample was mixed with 5 ml of Folin-Ciocalteu reagent (1:10, v/v, diluted with distilled water). The reaction was neutralized by adding 4 ml of 75g l^{-1} sodium carbonate. Samples was held for 2 h at 25 ± 2°C and the absorbance of the resulting blue colour was measured at 760 nm against a reagent blank on a Cecil CE 7400 UV-visible Spectrophotometer (Cecil Instruments, Cambridge, England).

The average data were analyzed using ANOVA. The treatment means were compared using a Duncan Multiple Range Test at the 5% probability.

Results and Discussion

Quantitative phytochemical content (g/100g) of leaves of botanicals used for storing *C. nitida* nut

The leaf of *T. grandis* had highest mean values in alkaloid (4.76 g/100g), flavonoid (0.41 g/100g) and theobromine (0.004 g/100g); the leaf of *A. indica* recorded the highest mean values in saponin (4.85 g/100g), tannin (0.37 g/100g), polyphenol (0.28 g/100g), anthraquinone (0.58 g/100g) and kolatin (0.006 g/100g) while *M. paradisiaca* recorded the lowest mean values for all the phytochemical analyzed (Table 1 and 2).

Table 1. phytochemical content (g/100g) of the botanicals used in storing kola nuts.

Botanicals	Alkaloid	Saponin	Tannin	Polyphenol	Anthraquinone
T. grandis	4.76[a]	3.83[b]	0.35[b]	0.24[b]	0.32[b]
A. indica	4.40[b]	4.85[a]	0.37[a]	0.28[a]	0.58[a]
A. cordifolia	4.12[c]	2.34[c]	0.29[c]	0.23[c]	0.14[c]
M. paradisiaca	3.75[d]	1.48[d]	0.27[d]	0.22[d]	0.11[d]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 2. phytochemical content (g/100g) of the botanicals used in storing kola nuts.

Botanicals	Flavonoid	Caffeine	Kolatin	Theobromine
T. grandis	0.41[a]	0.016[b]	0.003[b]	0.004[a]
A. indica	0.35[b]	0.110[a]	0.006[a]	0.003[b]
A. cordifolia	0.34[c]	0.012[c]	0.002[c]	0.002[c]
M. paradisiaca	0.25[d]	0.010[c]	0.002[b]	0.001[d]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 3 revealed that a common trend of increase was obtained from the lowest nut weight to the largest nut weight of fresh *C. nitida* with the preponderance of anthraquinone. Red *C. nitida* nut above 21 g recorded the highest mean values in tannin (4.22 g/100 g), flavonoid (3.03 g/100 g), caffeine (4.83 g/100 g) theobromine (2.28 g/100 g), kolatin (4.78 g/100 g) and polyphenol (2.76 g/100 g). Pink *C. nitida* nut recorded the highest mean values in saponin (4.01 g/100 g), alkaloid (1.87 g/100 g) and anthraquinone (5.67 g/100 g) and the result was significant. (Table 3). The leaves of *T. grandis* had the highest mean values for tannin (3.49 g/100 g), saponin (3.45 g/100 g), flavonoid (2.46 g/100 g), anthraquinone (5.19 g/100 g), caffeine (4.29 g/100 g) and polyphenol (1.65 g/100 g) and the least was obtained for phostocin in red *C. nitida* nut above 21 g. (Table 5 and 6). Similar result was observed for pink *C. nitida* nut except for anthraquinone and polyphenol. *A. indica* recorded the highest mean values in flavonoid (2.23 g/100 g), theobromine (5.07 g/100 g), kolatin (3.86 g/100 g) and polyphenol (1.44 g/100 g) for white nut above 21 g and the lowest mean value was obtained for phostocin (Table 8).. Decreasing quantities for all the phytochemical content assayed using botanicals as preservative were observed for cured *C. nitida* nut of different colours as the number of weeks after curing increases with the preponderance of anthraquinone and the least mean value was obtained for polyphenol and was significantly *different* (Table 7, 8, 9 and 10). The implication is that stored *C. nitida* nuts do not tend to colour the teeth when consumed compared to the fresh nuts because of the reduction in polyphenol responsible for teeth browning. Fresh kola nuts are highly hydrated, have very bright colours and are astringently bitter. They contain a wide array of complex secondary plant metabolites and polyphenolics known to be mainly responsible for the astringency and bitter taste in the nuts. Several curing and preservation methods have been tried to remove the astringency, bitterness and high moisture content to make the nuts palatable and acceptable (Osei Bonsu *et al.*, 1977; Takrama *et al.*, 2000). 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).

Cured kola nuts that are not slimy, not astringently bitter in taste but palatable and crispy are normally considered to have the desirable characteristics of good quality (Quarcoo, 1973). 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 found 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; Kumaran and Karunakaran, 2007). 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, its stimulant effects and its consumption in high amounts may become toxic (Graham, 1978). 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 2.28 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). In addition, phenols are widely used in the manufacture of resins, plastics, insecticides, explosives, dyes and detergents as a raw material for the production of drugs such as aspirin (Michael, 2008). Therefore, *C. nitida* can then be of economic importance in these aforementioned areas.

The result in Table 11 showed a common trend of increase from the lowest nut weight to the largest nut weight with the preponderance of total amylase activity (0.148 mg/glucose/min/g protein), followed by catalase (0.145 mg/$NaBO_3.4H_2O$/min/g protein) and the lowest mean value was obtained for proteinase (0.121 mg/tyrosine/min/g protein). Similar result was observed for different colours where red nut above 21 g recorded the highest mean values for all the enzymes assayed except for glucose-6-phosphatase (0.137 mg/inorganic phosphate/min/g protein) in white *C.nitida* nut >21 g and pink nut recorded the lowest mean values.

Table 3. Effect of different weights and varying colours on phytochemical content (g/100 g) of fresh C. nitida nut

Nut weight before curing	Tannin	Saponin	Alkaloid	Flavonoid	Anthraquinone
1-10	2.75[c]	2.66[c]	1.44[c]	2.04[c]	3.51[c]
11-20	3.97[b]	3.68[b]	1.84[b]	2.32[b]	5.50[b]
21-40	4.42[a]	4.25[a]	2.02[a]	3.03[a]	5.42[a]
Coloured nut before curing					
Pink (>21 g)	3.23[c]	4.01[a]	1.87[a]	2.58[b]	5.67[a]
Red (>21 g)	4.22[a]	3.61[b]	1.68[b]	3.01[a]	5.17[b]
White (>21 g)	3.69[b]	2.96[c]	1.79[a]	1.79[c]	4.43[c]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 4. Effect of different weights and varying colours on phytochemical content (g/100 g) of fresh C. nitida nut

Nut weight before curing	Caffeine	Theobromine	Kolatin	Polyphenol
1-10	1.65[c]	1.65[c]	3.44[c]	1.56[c]
11-20	4.50[b]	1.97[b]	4.27[b]	2.28[b]
21-40	5.42[a]	2.28[a]	4.78[a]	2.76[c]
Coloured nut before curing				
Pink (>21 g)	4.46[b]	2.13[b]	4.55[b]	1.45[c]
Red (>21 g)	4.83[a]	2.29[a]	4.69[a]	3.61[a]
White (>21 g)	3.97[c]	1.47[c]	3.25[c]	1.55[b]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 5: Effect of different weights and varying colours on phytochemical content (g/100 g) of fresh C. nitida nut

Treatments	Tannin	Saponin	Alkaloid	Flavonoid	Anthraquinone
Cured red nut (>21 g)					
T. grandis	3.49[a]	3.45[a]	1.66[c]	2.46[a]	5.19[a]
A. indica	3.38[b]	3.23[ab]	1.69[ab]	2.36[b]	4.93[b]
A cordifolia	3.38[b]	3.26[ab]	1.70[a]	2.31[b]	4.95[b]
M. paradisiaca	3.35[b]	3.20[ab]	1.67[bc]	2.34[b]	4.94[b]
Phostocin	3.33[b]	3.11[b]	1.63[d]	2.31[b]	4.87[b]
Cured pink nut (>21 g)					
T. grandis	3.49[a]	3.40[a]	1.70[ab]	2.40[a]	5.46[b]
A. indica	3.38[b]	3.30[ab]	1.73[a]	2.31[b]	5.72[a]
A cordifolia	3.37[b]	3.30[ab]	1.70[ab]	2.30[b]	5.45[b]
M. paradisiaca	3.84[b]	3.25[ab]	1.66[b]	2.26[b]	4.86[c]
Phostocin	3.32[b]	3.14[b]	1.62[c]	2.24[b]	4.85[c]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 6. Effect of different weights and varying colours on phytochemical content (g/100 g) of fresh C. nitida nut

Botanicals	Caffeine	Theobromine	Kolatin	Polyphenol
Cured red nuts				
T. grandis	4.29[a]	1.84[b]	3.93[ab]	1.65[a]
A. indica	4.24[ab]	1.89[a]	3.98[a]	1.63[a]
A. cordifolia	4.16[b]	1.82[b]	3.98[a]	1.60[a]
M. paradisiaca	4.17[b]	1.83[b]	3.84[b]	1.
Phostocin	4.16[b]	1.83[b]	3.84[b]	1.62[a]
Cured pink nut (>21 g)				
T. grandis	3.99[a]	1.97[b]	3.99[ab]	1.44[b]
A. indica	3.86[ab]	2.09[a]	4.03[a]	1.52[a]
A cordifolia	3.71[b]	1.86[b]	3.78[b]	1.46[b]
M. paradisiaca	2.99[c]	1.81[b]	3.81[b]	1.47[b]
Phostocin	2.99[c]	1.72[c]	3.80[b]	1.44[b]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 7. Effect of botanicals as preservative on phytochemical content (g/100 g) of cured C. *nitida* nut of different colours and time above 21 g

Treatments	Tannin	Saponin	Alkaloid	Flavonoid	Anthraquinone
Cured white nut (>21 g)					
T. grandis	3.39[a]	3.44[a]	1.67[a]	2.11[b]	4.99[b]
A. indica	3.30[b]	3.20[b]	1.60[ab]	2.23[a]	5.07[a]
A cordifolia	3.38[b]	3.19[b]	1.57[b]	2.18[ab]	5.02[ab]
M. paradisiaca	3.42[a]	3.23[b]	1.56[b]	2.10[b]	4.86[b]
Phostocin	3.26[b]	3.20[b]	1.57[b]	2.01[c]	4.84[b]
Red (>21 g)					
3	3.74[a]	3.59[a]	1.85[a]	2.48[a]	5.16[a]
6	3.70[a]	3.52[a]	1.83[a]	2.47ab	5.12[a]

9	3.60[b]	3.35[a]	1.75[b]	2.40[b]	5.04[a]
12	2.50[c]	2.54[b]	1.26[c]	2.07[c]	4.62[b]
Pink (>21 g)					
3	3.33[a]	3.21[a]	2.22[a]	2.47[a]	5.88[a]
6	3.30[b]	3.09[b]	1.98[a]	2.47[a]	5.32[ab]
9	3.10[c]	2.60[c]	1.68[b]	2.40[b]	5.04[b]
12	2.70[d]	2.51[c]	1.46[b]	2.10[c]	4.81[b]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 8. Effect of botanicals as preservative on phytochemical content (g/100 g) of cured C. *nitida* nut of different colours and time above 21 g

Treatments	Caffeine	Theobromine	Kolatin	Polyphenol
Cured white nut (>21 g)				
T. grandis	3.86[a]	1.51[b]	2.99[b]	1.39[b]
A. indica	3.85[a]	1.61[a]	3.86[a]	1.44[a]
A cordifolia	3.79[ab]	1.50[b]	3.86[a]	1.40[b]
M. paradisiaca	3.40[b]	1.49[b]	2.91[b]	1.36[b]
Phostocin	3.40[b]	1.48[b]	2.86[b]	1.25[c]
Red (>21 g)				
3	4.43[a]	1.95[a]	4.04[a]	1.75[a]
6	4.32[b]	1.92[a]	4.01[ab]	1.70[ab]
9	4.18[c]	1.85[b]	3.90[b]	1.66[b]
12	3.89[d]	1.66[c]	3.71[c]	1.38[c]
Pink (>21 g)				
3	4.00[a]	1.95[a]	4.01[a]	1.50[a]
6	3.98[a]	1.93[a]	4.01[a]	1.46[a]
9	3.77[b]	1.84[b]	3.90[b]	1.36[b]
12	3.46[c]	1.70[c]	3.73[c]	1.30[b]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 9. Effect of colours and time on phytochemical content (g/100 g) of cured C. *nitida* nut above 21 g

Botanicals	Tannin	Saponin	Alkaloid	Flavonoid	Anthraquinone
White (>21 g)					
3	2.98[a]	3.00[a]	2.21[a]	2.30[a]	5.10[a]
6	2.81[a]	2.78[a]	2.20[a]	2.29[a]	5.08[a]
9	2.68[b]	2.41[b]	2.18[a]	2.20[b]	5.00[a]
12	2.01[c]	2.38[b]	2.00[b]	2.16[b]	4.86[b]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 10. Effect of colours and time on phytochemical content (g/100 g) of cured C. *nitida* nut above 21 g

Botanicals	Caffeine	Theobromine	Kolatin	Polyphenol
White (>21 g)				
3	3.86[a]	1.80[a]	3.72[a]	1.49[a]
6	3.81[a]	1.78[a]	3.70[a]	1.40[a]
9	2.80[b]	1.68[b]	3.51[b]	1.38[b]
12	2.71[b]	1.46[b]	3.01[c]	1.21[c]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Table 11. Effect of different weights and varying colours on enzyme activities of fresh C. *nitida* nut

Treatments	Catalase Activity	Cellulase Activity	Polyphenol oxidase Activity	Total amylase Activity	Proteinase Activity	Glucose-6-phoshatase activity	Lipase activity
Nut weight before curing							
1-10	0.136[c]	0.124[c]	0.130[c]	0.137[c]	0.113[c]	0.118[c]	0.119[c]
11-20	0.140[b]	0.128[b]	0.132[b]	0.145[b]	0.117[b]	0.119[b]	0.121[b]
21-40	0.145[a]	0.143[a]	0.140[a]	0.148[a]	0.121[a]	0.123[a]	0.134[a]
Coloured nut before curing							
Pink(>21 g)	0.130[b]	0.121[a]	0.126[b]	0.131[b]	0.115[b]	0.116[a]	0.116[b]
Red (>21 g)	0.146[a]	0.147[a]	0.139[a]	0.149[a]	0.118[a]	0.121[a]	0.122[a]
White (>21 g)	0.145[b]	0.128[a]	0.139[a]	0.149[a]	0.118[a]	0.137[a]	0.122[a]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Note unit of these enzymes are:

Total amylase: mg/glucose/min/g protein

Polyphenol oxidase: mg/quinine/min/g protein

Glucose-6- phosphatase: mg/inorganic phosphate/min/g protein

Catalase: mg/$NaBO_3.4H_2O$/min/g protein

Lipase: ml 0.02M NAOH/min/g protein

Proteinase: mg/tyrosine/min/g protein

Cellulase: mg/glucose/min/g protein

Table 12. Effect of botanicals as preservative on enzyme activities of cured C. *nitida* nut above 21 g by weight.

Treatments	Catalase Activity	Cellulase Activity	Polyphenol oxidase Activity	Total amylase Activity	Proteinase Activity	Glucose-6-phoshatase activity	Lipase activity
Cured red nut (>21 g)							
T. grandis	0.154[a]	0.125[a]	0.127[b]	0.141[a]	0.121[a]	0.120[b]	0.113[b]
A. indica	0.135[b]	0.123[b]	0.133[a]	0.136[a]	0.117[c]	0.125[a]	0.118[a]
A cordifolia	0.135[b]	0.123[b]	0.128[b]	0.138[a]	0.115[c]	0.118[b]	0.110[b]
M. paradisiaca	0.136[b]	0.124[b]	0.126[b]	0.140[a]	0.119[b]	0.124[b]	0.117[b]
Phostocin	0.134[b]	0.120[b]	0.127[b]	0.137[a]	0.114[d]	0.118[b]	0.104[c]
Cured pink nut (>21 g)							
T. grandis	0.140[a]	0.126[a]	0.130[b]	0.140[a]	0.119[a]	0.119[b]	0.111[b]
A. indica	0.135[b]	0.123[b]	0.137[a]	0.138[b]	0.117[b]	0.122[a]	0.118[a]
A cordifolia	0.135[b]	0.124[b]	0.134[b]	0.137[b]	0.114[b]	0.120[b]	0.109[b]
M. paradisiaca	0.134[b]	0.122[b]	0.131[b]	0.136[b]	0.115[b]	0.117[c]	0.114[b]
Phostocin	0.134[b]	0.122[b]	0.132[b]	0.136[b]	0.114[b]	0.118[c]	0.101[c]

Means followed by the same letters on the same columns are not significantly different according to Duncan Multiple Range (DMRT) Test at 5% level of probability

Note unit of these enzymes are:

Catalase: mg/$NaBO_3$. $4H_2O$/min/g protein
Total amylase: mg/glucose/min/g protein
Lipase: ml 0.02M NAOH/min/g protein
Polyphenol oxidase: mg/quinine/min/g protein
Proteinase: mg/tyrosine/min/g protein
Glucose-6- phosphatase: mg/inorganic phosphate/min/g protein
Cellulase: mg/glucose/min/g protein

Table 13. Effect of botanicals as preservative on enzyme activities of cured C. *nitida* nut above 21g.

Treatments	Catalase Activity	Cellulase Activity	Polyphenol oxidase Activity	Total amylase Activity	Proteinase Activity	Glucose-6-phoshatase activity	Lipase activity
Cured white nut (>21 g)							
T. grandis	0.134[a]	0.125[a]	0.128[c]	0.141[a]	0.120[a]	0.118[b]	0.104[c]
A. indica	0.131[b]	0-.123[b]	0.136[a]	0.136[b]	0.116[b]	0.122[a]	0.114[a]
A cordifolia	0.133[b]	0.123[b]	0.132[b]	0.134[b]	0.114[b]	0.122[a]	0.110[b]
M. paradisiaca	0.132[b]	0.122[b]	0.131[b]	0.135[b]	0.116[b]	0.118[b]	0.108[b]
Phostocin	0.129[b]	0.122[b]	0.127[c]	0.133[b]	0.113[b]	0.116[b]	0.100[c]
Red (>21 g)							
3	0.148[a]	0.126[a]	0.133[a]	0.143[a]	0.120[a]	0.125[a]	0.119[a]
6	0.140[b]	0.124[b]	0.130[b]	0.142[a]	0.119[b]	0.122[b]	0.117[a]
9	0.137[b]	0.122[c]	0.126[c]	0.141[a]	0.116[c]	0.120[c]	0.111[b]
12	0.132[c]	0.119[d]	0.123[d]	0.134[a]	0.113[d]	0.116[d]	0.103[d]

Means followed by the same letters on the same columns are not significant different according to Duncan Multiple Range Test at 5% probability level.

Note unit of these enzymes are:

Catalase: mg/$NaBO_3$. $4H_2O$/min/g protein
Total amylase: mg/glucose/min/g protein
Lipase: ml 0.02M NAOH/min/g protein
Polyphenol oxidase: mg/quinine/min/g protein
Proteinase: mg/tyrosine/min/g protein
Glucose-6- phosphatase: mg/inorganic phosphate/min/g protein
Cellulase: mg/glucose/min/g protein

Table 14. Effect of colours and time on enzymes activity of cured *C. nitida* above 21 g.

Treatments	Catalase Activity	Cellulase Activity	Polyphenol oxidase Activity	Total amylase Activity	Proteinase Activity	Glucose-6-phoshatase activity	Lipase activity
Pink (>21 g)							
3	0.147[a]	0.127[a]	0.134[a]	0.144[a]	0.123[a]	0.124[a]	0.117[a]
6	0.140[b]	0.125[b]	0.131[b]	0.142[a]	0.120[b]	0.121[b]	0.113[b]
9	0.139[b]	0.123[c]	0.127[c]	0.141[a]	0.117[c]	0.119[c]	0.111[c]
12	0.132[c]	0.120[d]	0.124[d]	0.135[a]	0.114[d]	0.115[d]	0.108[d]
White (>21 g)							
3	0.135[a]	0.126[a]	0.128[a]	0.140[a]	0.118[a]	0.122[a]	0.114[a]
6	0.133[b]	0.124[b]	0,124[b]	0.139[a]	0.114[b]	0.120[b]	0.111[b]
9	0.131[c]	0.121[c]	0.122[c]	0.137[b]	0.112[c]	0.118[c]	0.108[c]
12	0.129[d]	0.119[d]	0.120[d]	0.133[b]	0.109[d]	0.116[d]	0.101[d]

Means followed by the same letters on the same coloums are not significantly different according to Duncan Multiple Range (DMRT) Test at 5% level of probability

Note unit of these enzymes are:

Catalase: mg/$NaBO_3$. $4H_2O$/min/g protein
Total amylase: mg/glucose/min/g protein
Lipase: ml 0.02M NAOH/min/g protein
Polyphenol oxidase: mg/quinine/min/g protein
Proteinase: mg/tyrosine/min/g protein
Glucose-6- phosphatase: mg/inorganic phosphate/min/g protein
Cellulase: mg/glucose/min/g protein

Red *C.nitida* nut above 21 g preserved with the leaves of *T.grandis* recorded the highest mean values for catalase (0.154 mg/$NaBO_3.4H_2O$/min/g protein), cellulase (0.125 mg/glucose/min/g protein), total amylase (0.141 mg/glucose/min/g protein) and proteinase (0.121 mg/tyrosine/min/g protein). *A. indica* had the highest values for glucose-6-phosphatase (0.125 mg/inorganic phosphate/min/g protein), lipase (0.118 ml 0.02M NAOH/min/g protein) and polyphenol oxidase (0.133 mg/quinine/min/g protein) while the lowest mean values were obtained for phostocin. Similar result was obtained for pink and white *C. nitida* nut and the result was significant (Table 12 and 13). Decrease in the activity of enzymes were observed for pink, red and white *C. nitida* nut as the number of weeks after curing increases (Table 13 and 14). Purseglove (1991) reported values of polyphenol in the range of 0.8% to 1.3 % for C. garcina kola and 2.5 % to 3.0 % for the varieties of *C. nitida*. Work carried out by Ducksworth and Coleman (1970) showed the white cultivars of most crop products such as bitter kola and kola nuts lack carotene, a polyphenol, which is responsible for the pigmentation, noticed in *Cola nitida* especially the red cultivar. The relatively considerable values of polyphenols in this study may therefore explain the incidence of enzymatic browning in Nigeria kola nuts. The levels of polyphenol also vary from variety to variety with the highest value occurring in the red cultivar of *Cola nitida* and the lowest obtained in pink. An interesting occurrence takes place when kola nuts are half eaten or handled in a manner that exposes their tissues; brown particles begin to appear with time, this is what is responsible for staining of consumers teeth. This principle is referred to as "browning". Browning is attributed to the oxidation of phenolic compound present in the plant product. This is catalyzed by polyphenol oxidase. This enzyme utilizes molecular oxygen in producing quinines and melanin, which on interaction with other constituents yield brown pigments (Mayer and Havel, 1979). Polyphenols make animo-acids unavailable by binding strongly to them (Elias and Bressani, 1979).

Conclusion and Recommendation

❖Large *C. nitida* nut recorded the highest mean values in phytochemical and enzymes activities that were determined.

❖Red *C. nitida* nuts above 21g by weight recorded the highest mean values in all the enzymes activities

❖Preservative botanical is recommended to farmers as it gave higher amount of nutrients compared to phostocin which the farmers are presently using.

❖Large *C. nitida* nut above 21 g by weight should be cured for about 12 weeks using botanicals as as this reduces the polyphenol content which is responsible for teeth browning.

❖*C. nitida* nut should be stored above 12 weeks to reduce the caffeine content thereby making it safe for human consumption.

❖The presence of phytochemicals (secondary plant metabolites) justifies their therapeutic functions.

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