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Post-harvest quality assessment of freshly harvested and processed kola nuts [Cola nitida (Vent.) Schott & Endl.] from selected growing regions in Ghana

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Abstract: Elucidating the post-harvest quality of marketable kola nuts is essential in developing standards for grading the nuts. This was done in the present study by quantifying the phytochemicals and determining the moulds associated with kola nuts using standard laboratory methods. Mould and phytochemical assessments have both health and safety implications to marketers and consumers. Fresh and cured (3 months) kola nuts of three colour types (red, white, pink) were obtained from three different kola growing regions (Eastern, Ashanti, Ahafo) in Ghana for assessment. There were no significant differences in the amount of phytochemicals in fresh and cured nuts except for moisture (fresh = 56.21 – 59.42%; cured = 53.70–57.99%) and total polyphenol (fresh = 42.6–59.30 mg g-1; cured = 45.51–73.01 mg g-1) contents. Phytochemicals in the nuts after curing were as follows: pH 5.81–5.92, fat 0.49–0.60%, crude fibre 4.62–8.44%, total ash content 2.85–3.01%, alkaloids 0.40–0.99%, saponins 0.29–1.27%, terpenoids 0.30–1.09%, flavonoids 0.76–0.82% and tannins 38.67–45.22 mg g-1. These values are comparable to limits reported in kola nuts consumed in other countries. However, moisture, crude fibre, total polyphenols, alkaloids, saponins and terpenoids contents significantly (p < 0.05) differed across the sampled regions in Ghana. A total of 30 moulds

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PUBLIC INTEREST STATEMENT

Actors in the kola value chain who do more to maintain the quality of their produce do not enjoy any premium. This is because post-harvest quality standards have not been set for grading kola nuts. The current standards are based on mutual understanding between buyers and sellers in terms of colour, size, taste, flavour and shelf life of the nut. These features are biased by individual perception. Therefore, to assist in establishing bench mark values for quality evaluation of kola nuts, the amount of phytochemicals and the kind of moulds associated with both freshly harvested and cured kola nuts were assessed. The best ways of maintaining kola nut quality with respect to the chemical composition and reduction in mould contamination have been suggested. The information from this study will form the basis for designing a grading scheme to support the sensory (subjective) evaluation being used in the kola industry.
belonging to 14 genera were isolated from the kola nuts. These were *Absidia*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Fusoma*, *Geothricum*, *Gliocladium*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Syncephalastrum* and *Trichoderma*. Most *Aspergillus*, *Fusarium* and *Penicillium* species detected are known mycotoxin-producing moulds. The information serves as a useful basis to optimize post-harvest processes of kola nuts to maintain high quality and safety nut for consumption.

**Subjects: Biochemistry; Microbiology; Mycology; Food Chemistry**

**Keywords: Kola nuts; curing; phytochemicals; mycotoxigenic fungi; post-harvest; quality**

1. **Introduction**

*Cola nitida* (Vent) Schott. & Endl. (*Kola*) is an important commercial crop cultivated mostly in Africa (Dadzie et al., 2013). The seed of *C. nitida* fruit (pod) is referred to as kola nut and morphologically, it has three distinct colours including white, red and pink. Kola nuts are rich in essential chemical compounds including water, fat, ash, fibre, carbohydrates and proteins. Additionally, secondary metabolites, such as polyphenols, alkaloids, saponins and terpenoids, abound in kola nuts and they are produced when the plant is under stress (Pagare et al., 2015). In some West African countries including Ghana and Nigeria, the nuts are chewed mostly to suppress sleep and hunger (ADEDAYO et al., 2019; OLANIYAN et al., 2018). Moreover, it has several traditional, social and medicinal importance, such as treatment of asthma and whooping cough (ADEDAYO et al., 2019; DORATHY et al., 2014).

In terms of production capacity, Ghana produced about 25,303 tonnes of kola nuts in 2019 (FAOSTAT, 2021). Kola nuts cultivation in Ghana mainly takes place in the forest zones of Eastern, Ashanti, Western, Ahafo, Central and Volta regions where it serves as a major source of livelihood for stakeholders in the kola value chain (Dadzie et al., 2013). Kola nut is considered a neglected cash crop and its price does not attract any premium compared to crops, such as cocoa and cashew. This is attributed to the lack of quality standards for grading the nuts (AMON-ARMAH et al., 2021; OSALUSI, 2019; YAHAYA et al., 2019). There is therefore the need for research efforts geared towards formulation of quality standards for the kola market.

In kola producing countries, quality assessment of the nut is based on traditional knowledge and mutual understanding among actors in the kola value chain (AMON-ARMAH et al., 2021; YAHAYA et al., 2019). The quality of kola nuts is based on sensory appreciation of size, flavour, taste, keeping quality and colour of the nuts, which are subjective. Quality of kola nuts, which includes the physical, chemical and safety attributes (CHERONO, 2016), requires an objective evaluation such as quantification of phytochemicals in the nuts to complement the sensory evaluation in determining the quality. Several studies from kola producing countries have reported on phytochemicals including crude fat, total ash, crude fibre, polyphenols, alkaloids and saponins in the nuts (AYEDE et al., 2014; DAH-NOUVELLESONON et al., 2015; DEWOLE et al., 2013; LWORK, 2008; LWORK et al., 2010; NGOUPOAYON et al., 2016; NWONUMA et al., 2019; NYADANU et al., 2020; ATANDA et al., 2011). However, there is limited information on the amounts of these phytochemicals in both fresh and cured nuts from a producing country to assist in establishing benchmark values.

Consumers of kola nuts mostly prefer the cured nuts to the fresh ones. This is because fresh nuts are hydrated, slimy, bitter and astringent (LWORK et al., 2010). Fresh kola nuts are subjected to curing to improve the quality of the nuts. The common method of curing kola nuts is the traditional process of keeping the nuts for months or years in either cane baskets or polypropylene sacks lined with leaves of *Musa* spp., *Mitragyna stipulosa* or polythene (AMON-ARMAH et al., 2021; LWORK et al., 2010). During curing, various biochemical changes occur in the kola nuts that lead to the development of acceptable and palatable taste of the nuts (OLANINAN
et al., 2018; Takrama et al., 2000). Curing is thus crucial in kola production as it greatly influences the quality of the nuts. Mould contamination during curing of kola nuts has been implicated in quality deterioration (Agbeniyi & Ayodele, 2010; Daouda et al., 2013; Idris et al., 2017; Atanda et al., 2011). This is attributed to the prevailing humid storage conditions in the tropics where the crop is cultivated. The most common moulds associated with kola nuts contamination during the post-harvest processing include species from the genera Aspergillus, Penicillium and Fusarium (Daouda et al., 2013; Terna et al., 2017). Mould contamination in nuts has been reported to cause health problems that are linked to the mycotoxins produced by the moulds (Atanda et al., 2013). Various levels of mycotoxins have been detected in kola nuts (Adebajo & Popoola, 2003; Dongo et al., 2007). However, in Ghana, the mycoflora associated with kola nuts is not yet known. Therefore, the aims of the present study were to i) quantify the phytochemical composition of fresh and cured kola nuts from three important kola growing regions of Ghana (Eastern, Ashanti and Ahafo) and ii) determine the kind of moulds associated with the nuts.

2. Materials and methods

2.1. Sampling location and collection
Fresh and cured kola nut (white, red and pink colours) samples were obtained from some actors of the kola value chain (farmers, processors and retailers) in Eastern (Oyoko, Afosu and Kwahu Praso), Ashanti (Agona Akrofonso, Fumso and Nyinahin) and Ahafo (Bechem, Sankore and Kenyase) regions of Ghana. The sampling locations were within the forest zones of Ghana characterised by a tropical climate. The average temperature was fairly stable within a year ranging from 25 to 27 °C. Annual average rainfall recorded in the area was around 2000 mm and was distributed between two seasons of minor and major rains (http://www.weather-atlas.com/en/ghanan). The kola nuts were cured for 12 weeks (3 months) using a traditional method that involves keeping the nuts in polypropylene sacks lined with either M. stipulosa leaves or polythene.

2.2. Experimental design
All experiments were carried out at the Cocoa Research Institute of Ghana (CRIG), Tafo—Ghana (6° 14′47.7″N, 0°21′7″W). The experimental design employed involved a 3 × 3 × 2 factorial combination with three replications. There were three kola nut types (red, white and pink), sampled from three locations (Ashanti, Ahafo and Eastern regions) with two post-harvest handling methods (fresh and cured). Thus, main and interactive effects of sampling locations, post-harvest handlings and the kola genotypes on phytochemical contents and mould contamination levels were studied.

2.3. Sample preparation
A total of 300 kola nuts (150 fresh and 150 cured nuts) were sampled from each of the three regions. From the fresh samples, 50 nuts of each colour type were separately cut into pieces, oven-dried at 40°C and pulverised to powder using laboratory mill (Cristy and Norris Ltd, Chemsford, England). Similar powders were obtained from the cured samples. The powders were passed through a sieve with pore size of 2 mm and stored in sealed plastic bags at ambient temperature until needed.

2.4. Determination of chemical composition

2.4.1. Preparation of aqueous, chloroform, ethanolic and methanolic extracts of the kola nuts powder
Aqueous extract was prepared by mixing the powdered kola nut sample with sterile-distilled water at 1:10 (w/v) to a volume of 10 mL. The mixture was shaken for 24 h on an orbital shaker (Stuart, Staffordshire, UK) at room temperature and filtered (Whatman No. 1 filter paper) to obtain crude aqueous extract for phytochemical screening. A similar procedure was used to prepare the chloroform, ethanolic and methanolic (Fisher Scientific UK Ltd., Loughborough, UK) extracts.
2.4.1. Qualitative phytochemical screening
The qualitative phytochemical screening was carried out on aqueous, chloroform, ethanolic or methanolic extract of the kola nut samples to determine the presence or absence of phytochemicals using standard methods described by Sasidharan et al. (2011); for phenols) and Salman et al. (2015) (for reducing sugars). The intensity of colour change was used as an indicator to rate a photochemical qualitatively as strongly positive (++), positive (+), trace (±) or negative (-).

Test for alkaloids (Wagner’s test)

For this test, 2 mL of 1% hydrochloric acid solution (Daejung Chemicals and Metals Company Ltd., Korea) were added to 2 mL of the aqueous extract. Then, six drops of Wagner’s reagent were added to 1 mL of the resulting solution and hand-shaken for 30s. Formation of a reddish-brown precipitate showed the presence of alkaloids.

Test for anthraquinones (Borntrager’s test)

1 mL of 10% ammonia solution (Daejung Chemicals and Metals Company Ltd., Korea) was added to 2 mL of the chloroform extract. A pink-red colour in the lower (ammonical) layer indicated the presence of anthraquinones.

Test for cardiac glycosides (Keller–Kiliani Test)

For 1 mL of methanolic extract, 2 mL of chloroform and 1 mL of sulphuric acid were added. Observation of a brown ring at the interphase indicated the presence of cardiac glycosides.

Test for flavonoids (NaOH test)

1 mL dilute sodium hydroxide solution (VWR Chemicals, Germany) was added to 2 mL of methanolic extract followed by addition of dilute hydrochloric acid. Yellow colouration, which turned colourless in the dilute hydrochloric acid, indicated a positive test for flavonoids.

Test for phenols (ferric chloride test)

To 2 mL of ethanolic extract, 2 mL of 1.17 M ferric chloride solution (Park Scientific Co. Ltd., Northampton, UK) was added. Formation of a deep bluish-green solution indicated the presence of phenols.

Test for phlobatannins

A 2-mL aqueous extract of the kola sample was boiled with 2 mL of 1% hydrochloric acid solution. Formation of a red precipitate gave evidence of phlobatannins.

Test for saponins (frothing test)

To 0.5 mL of aqueous extract, 5 mL of SDW was added and shaken vigorously for a stable, persistent froth. This indicated the presence of saponins.

Test for steroids (Liebermann-Burchard test)

About 1–2 drops of concentrated sulphuric acid and 2–3 mL of acetic anhydride were added to 1 mL of methanolic extract of sample. A colour change to dark green indicated the presence of steroids.

Test for reducing sugars (Benedict’s test)
To 1 mL of aqueous extract, 5 mL of Benedict’s solution was added and boiled for 2 min. This
was allowed to cool and formation of a red precipitate indicated the presence of reducing sugars.

**Test for tannins (Braemer’s test)**

Alcoholic ferric chloride solution (10%) was added at 3 mL to 3 mL of the ethanolic extract.
A positive test showed a deep bluish-green colouration.

**Test for terpenoids (Salkowski Test)**

For this test, 5 mL of methanolic extract was mixed with 2 mL of chloroform and 3 mL of
concentrated sulphuric acid. A reddish-brown colouration at the interface indicated a positive
result for the presence of terpenoids.

**Test for volatile oil**

Dilute sodium hydroxide solution was added at 0.1 mL to 2 mL of aqueous extract. The solution
was swirled while 2–3 drops of dilute hydrochloric acid were added. Formation of a white pre-
cipitate indicated the presence of volatile oil.

### 2.4.2. Quantitative analysis

The pH, moisture, crude fibre, fat and total ash contents in fresh and cured kola nut samples were
determined according to Association of Analytical Chemists AOAC (2005). Total polyphenol content
was determined as described by a modified protocol of Lowor et al. (2010). Briefly, 30 mL of
acidified methanol was added to 0.2 g of fat-free sample and shaken on an orbital shaker for 2 h at
maximum speed. One mL of appropriately diluted (with sterile) filtrate was reacted with 5 mL
Folin-Ciocalteu reagent (VWR Chemicals, Germany) at 1:10 (v/v) for 8 min and then 4 mL sodium
carbonate solution (75 g L⁻¹; Acros Organics, New Jersey, USA) was added to the reaction mixture.
The absorbance readings were taken at 760 nm on a Cecil CE 7400 UV-visible spectrophotometer
(Cecil Instruments, Cambridge, England) after incubation at 30°C and 0°C for 1 h each. The total
phenolic content was determined from a catechin (Sigma Aldrich, St. Louis, USA) calibration curve
prepared and analyzed concurrently with the samples.

Alkaloids, saponins and flavonoids contents were determined by the gravimetric method
described by Harborne (1973), Obadoni and Ochuko (2002), and Harborne (1998) respectively.
Terpenoids content was determined by a modified protocol described by Indumathi et al.
(2014) where 1 g sample was soaked in 25 mL of absolute ethanol for 24 h. The solution
was filtered and terpenoids in the filtrate were extracted with 20 mL of petroleum ether (Fisher
Scientific UK Ltd., Loughborough, UK). The ether extract was separated in pre-weighed glass
vials (∑i) and allowed to dry completely (∑j). The yield (%) of total terpenoids content was
calculated using the formula \(\% = \frac{\text{mass of \ ether extract}}{\text{mass of \ sample}} \times 100\). Tannins were assayed using the procedure of Katoch
(2011). Five mL of vanillin/hydrochloride reagent (2 g vanillin in 4% hydrochloric acid in
methanol (v/v)) was added to 1 mL of polyphenol extract (0.1 g of sample in 5 mL methanol).
The mixture was incubated for 20 minutes and absorbance was read at 500 nm. The standard
used was catechin. The analyses were carried out in triplicates.

### 2.5. Isolation and identification of moulds

In this study, the agar plating technique was used to determine the moulds on fresh and cured
kola nuts. A total of 100 fresh kola nuts from each colour type (white, red and pink) were
randomly selected from the regional samples. The fresh nuts were surface sterilized in 5%
commercial sodium hypochlorite solution (Berrak Manufacturing Co. Ltd., Tema, Ghana) for
2 min, rinsed twice in sterile distilled water and blot-dry on tissue paper. Each sterilized nut
was aseptically divided into 12 pieces (1 cm × 1 cm approx.) and four pieces placed at
equidistance on chloramphenicol (100 mg L⁻¹) potato dextrose agar (Oxoid PDA) plates. The
procedure was repeated for cured kola nuts and the entire 1,800 plates (900 each from fresh nuts and 900 from cured nuts) were arranged in completely randomised design in a dark incubator at 28°C for 7 days. Moulds growing out of the nuts were sub-cultured onto PDA and frequencies of their occurrence recorded. The fungal isolates were identified using their morphological characteristics. Macroscopic and microscopic characters including colony and hyphal morphology, fruiting bodies and spore characteristics of the isolates were compared with published literature (Barnett & Hunter, 1972; Klich, 2002; Mathur & Kongsdal, 2003; Nelson et al., 1983; Samson et al., 1995).

2.6. Statistical analysis
The experiment was a completely randomised design and data obtained in percentages (except moisture content) were arcsine transformed (Sin−1(x/100)) prior to the analyses. The data was subjected to 3-way analysis of variance using GenStat 11.1 (2008). The differences in the means were separated using Duncan multiple-range test at p < 0.05 level.

3. Results and discussion

3.1. Qualitative phytochemical analysis
The present study revealed that the fresh and cured kola nuts contained alkaloids, cardiac glycosides, flavonoids, phenols, saponins, reducing sugars, tannins, terpenoids, and volatile oil (Table 1). Akpakpan et al. (2020) also assessed phytochemicals in fresh kola nuts and reported chemical constituents similar to those detected in the current study. Qualitatively, the content of phytochemicals in the fresh nuts was similar to the cured ones.

3.2. Quantitative determination of phytochemical constituents
The amounts of phytochemicals in the fresh or cured kola nuts of three different kinds (red, white and pink colours) sampled from Eastern, Ashanti and Ahafo regions did not show any significant (p > 0.05) interaction. Sampling location significantly (p < 0.05) influenced moisture content, crude fibre, total polyphenols, alkaloids, saponins and terpenoids in the fresh and cured kola nuts (Table 2). This could be attributed to differences in curing methods at the locations. Some of the kola actors cured their kola nuts in baskets, polypropylene bags or buried them in pits in the ground. In most cases, they lined the storage bag, pit or basket with either leaves or polythene (Amon-Armah et al., 2021). These practices possibly affected some of the chemical constituents in the nuts during curing.

| Constituent     | Fresh | Cured |
|-----------------|-------|-------|
| Alkaloids       | +     | +     |
| Anthraquinones  | -     | -     |
| Cardiac glycosides | ++  | ++    |
| Flavonoids      | +     | +     |
| Phenols         | +     | ±     |
| Phlobatannins   | -     | -     |
| Saponins        | +     | +     |
| Steroids        | -     | -     |
| Reducing sugars | +     | ++    |
| Tannins         | +     | ++    |
| Terpenoids      | +     | +     |
| Volatile oil    | +     | +     |

Strong positive = ++, Positive = +, Trace = ±, Negative = -
Moisture content of kola nut is an index of water activity, stability or susceptibility of the nuts to microbial contamination (Wijaya et al., 2015). The fresh nuts had significantly ($p < 0.05$) higher moisture content than the cured ones. Moisture content of the fresh nuts was between 56.21 and 59.42% while that of the 12-week cured-nuts ranged from 53.70 to 57.99% (Table 2). Olaniyan et al. (2018) reported moisture content of 57.03% for fresh kola nuts and 50.95% for 12-week cured nuts. In Ghana, Lowor et al. (2010) reported moisture content of 52.45–56.84% for the fresh kola nuts but lower values (42.49–44.16%) for the nuts after 9-week curing in cane basket. The rapid loss of moisture could be attributed to the drying up of kola nuts during curing. Curing in polypropylene sacks lined with leaves of Musa spp. or M. stipulosa allows gradual drying of the kola nuts as compared to rapid drying where the nuts are kept in cane baskets or left in the open. Rapid drying of fresh kola nuts results in undesirable characteristics, such as wrinkling and poor nut colour (Takrama et al., 2000). Therefore, gradual drying of fresh kola nuts through curing in polypropylene sacks should be practiced. Reduction in the high moisture content during curing makes the nuts crispy, improves the taste and prevents mould contamination. These are important quality standards for grading kola nuts. It was estimated that pH of the kola nuts was slightly acidic and ranged from 5.81 to 5.92 for fresh kola nuts and 5.81–5.92 for cured nuts. There were no significant differences between pH values obtained for the fresh and the cured nuts (Table 2). Nyadanu et al. (2020) assessed the pH of 25 different kola genotypes in Ghana and reported pH values (5.65–6.88) within the acidic range. The pH values recorded in this study compares well with that reported by Nyadanu et al. (2020).

The fat content in kola nuts constitutes the amount of extractable lipids. Fat is an important source of energy but when consumed in excess can lead to weight gain with several deleterious effects. The content of fat in the kola nuts ranged from 0.51 to 0.53% in the fresh and 0.49 to 0.60% in the cured nuts (Table 2). These values were higher than 0.20% obtained in kola nut from Benin (Dah-Nouvlessounon et al., 2015). Consequently, kola nut can be reported as a low-fat food and the low-fat is a desired attribute important in selecting high-quality kola nuts for consumers. In the case of crude fibre, the content ranged from 4.26 to 6.89% for fresh nuts and 4.62 to 8.44% for the cured. There were no significant differences in the crude fibre content in the fresh and the cured kola nuts implying that it was well preserved during curing of the nuts. The crude fibre content gives an indication of the amount of non-digestible constituents, such as cellulose, lignin and pectin in the kola nuts (Dhingra et al., 2012). It is an important attribute of the nut because it helps consumers to reduce appetite, blood sugar levels, risk of cardiovascular diseases, relieves constipation and aids in digestion (Dhingra et al., 2012). The ash content is the amount of inorganic residue from oxidation of the kola nuts at high temperature. It reflects the mineral content of kola nut (Park, 1996). Total ash contents obtained were 2.74–2.88% and 2.85–3.01% for the fresh and cured nuts, respectively. Values of the mineral content are desirable in the nutrition of consumers because levels in fresh foods are usually less than 6% (Anon, 2022). As observed in other chemical constituents of kola nut, the curing process did not affect the minerals in the nuts. A similar result has been reported by Olaniyan et al. (2018) for fresh nuts and those cured for 12 weeks.

The amount of alkaloids, saponins and terpenoids obtained in the fresh and cured kola nuts were not significantly different (Table 2). Alkaloids are nitrogen-containing compounds, which have a strong bitter taste and possess antioxidant, anti-inflammatory and anticarcinogenic properties (Badyal et al., 2020; Kurek, 2019). Caffeine is reported as the main alkaloid (1.5%) in kola nut and it confers stimulant properties to the nuts (Lowor et al., 2010). The alkaloids in fresh nuts ranged from 0.29 to 1.09% and the cured nuts ranged from 0.40 to 0.99%. The content was higher than 0.22% obtained in kola nuts from Nigeria (Dewole et al., 2013) but lower than 2.06% obtained in nuts from Benin (Dah-Nouvlessounon et al., 2015). The saponin content of the fresh nuts ranged from 0.27 to 1.13% and the cured nuts was 0.29 to 1.27%. It confers bitter taste but with health promoting effects like anticancer, antimicrobial, cholesterol-lowering and antimutagenic properties (Güçlü-Üstünadağ & Mazza, 2007). Terpenoids are aromatic and volatile organic compounds that contribute to the flavour and aroma of kola nuts. The terpenoids also have health benefits
| Phytochemical constituent | Eastern Region | Ashanti Region | Ahafo Region |
|---------------------------|----------------|----------------|--------------|
|                           | Fresh          | Cured          | Fresh        | Cured          | Fresh          | Cured          |
| Moisture Content (%)      | 59.42±0.86     | 57.99±0.62     | 56.67±1.12   | 54.87±0.91     | 56.21±1.46     | 53.70±0.81     |
| pH                        | 5.81±0.07      | 5.92±0.04      | 5.92±0.01    | 5.85±0.04      | 5.86±0.01      | 5.86±0.01      |
| Fat (%)                   | 0.52±0.02      | 0.60±0.02      | 0.51±0.02    | 0.53±0.02      | 0.53±0.03      | 0.49±0.04      |
| Crude fibre (%)           | 5.35±0.34      | 5.40±0.67      | 4.26±0.68    | 4.62±1.53      | 6.89±0.97      | 8.44±1.09      |
| Total ash (%)             | 2.74±0.10      | 2.85±0.23      | 2.88±0.14    | 2.92±0.22      | 2.80±0.19      | 3.01±0.19      |
| Alkaloids (%)             | 1.09±0.26      | 0.88±0.25      | 0.51±0.20    | 0.40±0.14      | 0.29±0.05      | 0.99±0.63      |
| Saponins (%)              | 0.27±0.04      | 0.37±0.20      | 0.40±0.11    | 0.29±0.05      | 1.13±0.30      | 1.27±0.25      |
| Terpenoids (%)            | 0.32±0.05      | 0.30±0.02      | 0.68±0.43    | 1.09±0.47      | 1.10±0.55      | 0.94±0.40      |
| Total polyphenol content (mg g⁻¹) | 42.60±6.50 | 45.51±1.27 | 45.67±1.68 | 65.40±3.81 | 59.29±8.50 | 73.01±6.99 |
| Flavonoids (%)            | 1.03±0.21      | 0.82±0.25      | 1.00±0.27    | 0.76±0.19      | 1.00±0.20      | 0.81±0.24      |
| Tannins (mg g⁻¹)          | 54.61±19.34    | 39.78±12.99    | 41.17±6.70   | 45.22±10.16    | 34.01±6.87     | 38.67±3.99     |

Values in each row followed by the same letters are not significantly different at p<0.05. The values after ± are standard error of means.
The range of terpenoids in fresh and cured nuts was 0.32% to 1.10% and 0.30 to 1.09%, respectively. Phytochemical screening of kola nuts in Côte d’Ivoire (Ayebe et al., 2014), Cameroon (Ngoupayo et al., 2016) and Nigeria (Adedayo et al., 2019; Dorothy et al., 2014) have all indicated the presence of terpenoids in kola nuts. However, the levels measured in the current study were comparatively lower possibly reducing the flavour and the aroma precursors of the nut.

Polyphenols constitute one of the major and diverse groups of plant secondary metabolites. They all have at least an aromatic ring with a hydroxyl group attached (Rasouli et al., 2017). Polyphenols contribute to the colour, astringency, and bitter taste of kola nuts (Badyal et al., 2020; Bele et al., 2010). The total polyphenol content in the nuts ranged from 42.60 to 59.29 mg g⁻¹ in fresh nuts and from 45.51 to 73.01 mg g⁻¹ in cured nuts (Table 2). In all instances, polyphenol content in the cured kola nuts was higher when compared to the fresh nuts. Olanijan et al. (2018) also observed an increase in the polyphenol content of kola nuts after 12 weeks of curing. The increase in polyphenol content may be attributed to formation of new compounds that have antioxidant properties, such as melanoids (end products of Maillard reaction). In the test, melanoids can react with the Folin-Ciocalteu reagent to increase the total polyphenol content (Pérez-Martínez et al., 2010). This is because the reagent is non-specific for antioxidants present in a reaction. In addition, the melanoids are brown pigments and may contribute to the loss of bright colours of the fresh kola nuts observed after curing (Figure 1). Tannins are high-molecular-weight polyphenols with astringent and bitter-tasting properties (Bele et al., 2010). The tannin content in the nuts ranged from 34.01 to 54.61 mg g⁻¹ for fresh nuts and 38.67 to 45.22 mg g⁻¹ for cured nuts (Table 2). Flavonoids are low-molecular-weight polyphenols responsible for pigmentation (red, pink, white) in the kola nuts (Chen et al., 2007; Panche et al., 2016). The range of flavonoids in fresh and cured kola nuts were from 1.00 to 1.03% and 0.76 to 0.82%, respectively. The flavonoid and tannin contents determined in the fresh kola nuts were also maintained after curing (Table 2). Thus, maintaining the market value of the nuts.

3.3. Mould incidence on kola nut samples
A total of 30 moulds belonging to 14 taxonomic genera were isolated from fresh and cured kola nuts (Table 3). The genera Absidia, Aspergillus, Colletotrichum, Fusarium, Fusoma, Geothricum, Gliocladium, Mucor, Neurospora, Penicillium, Rhizoctonia, Rhizopus, Syncephalastrum and Trichoderma were consistently associated with the kola nuts. There were no differences in occurrence of the different fungi on the three kola nut types (red, white and pink). Among the fresh kola

Figure 1. Samples of the red, white and pink types of kola nuts showing bright colours at harvest (A) and dull colours after 12-week curing (B).
Table 3. Moulds associated with freshly harvested and cured kola nuts from three major production regions in Ghana

| Mould species      | Eastern region | Ashanti region | Ahafo region |
|--------------------|----------------|----------------|--------------|
|                    | Fresh | Cured | Fresh | Cured | Fresh | Cured |
| Absidia ramosa     | 0.0   | 0.0   | 0.0   | 0.0   | 0.5   | 3.3   |
| Aspergillus flavus | 9.9   | 9.0   | 16.3  | 25.7  | 26.7  | 12.0  |
| Aspergillus fumigatus | 9.7  | 6.5   | 1.2   | 1.8   | 7.5   | 8.4   |
| Aspergillus Niger  | 31.2  | 18.2  | 37.1  | 53.4  | 18.7  | 25.3  |
| Aspergillus tamarii| 7.5   | 44.2  | 4.2   | 4.6   | 45.8  | 36.9  |
| Aspergillus terreus| 1.2   | 0.9   | 0.2   | 3.0   | 1.2   | 0.7   |
| Aspergillus versicolor | 0.0  | 0.0   | 0.0   | 0.0   | 0.7   | 1.7   |
| Aspergillus wentii | 0.0   | 0.0   | 0.0   | 0.0   | 0.7   | 1.7   |
| Calletotrichum sp. | 13.0  | 11.5  | 24.3  | 15.4  | 2.5   | 0.0   |
| Fusarium oxysporum | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   |
| Fusarium sp.       | 0.0   | 0.0   | 0.8   | 0.0   | 0.0   | 0.0   |
| Fusarium sporatrichioides | 0.0 | 12.4 | 0.0 | 0.0 | 0.3 | 0.5 |
| Fusoma rubrascosa  | 0.0   | 0.0   | 0.0   | 0.0   | 0.0   | 1.2   |
| Geotrichum sp.     | 0.0   | 0.0   | 1.1   | 0.0   | 0.0   | 0.0   |
| Gloxodium sp.      | 0.0   | 0.0   | 0.3   | 0.0   | 0.0   | 0.0   |
| Mucor plumbeus     | 0.0   | 0.0   | 3.3   | 1.1   | 0.2   | 0.0   |
| Mucor racemosus    | 0.2   | 0.0   | 2.8   | 1.4   | 0.0   | 0.0   |
| Neurospora crassa  | 1.4   | 0.4   | 1.7   | 1.0   | 0.8   | 0.3   |
| Penicillium citrinum | 0.0  | 0.4   | 0.0   | 0.0   | 0.0   | 0.0   |
| Penicillium expansum | 25.8 | 25.8  | 3.8   | 4.6   | 17.2  | 7.3   |
| Penicillium hirsutum | 0.0  | 0.0   | 0.0   | 0.2   | 0.0   | 0.0   |
| Penicillium viridicatum | 0.1 | 0.0   | 0.6   | 0.0   | 0.0   | 0.0   |
| Rhizoctonia solani | 17.7  | 13.8  | 0.0   | 0.0   | 18.3  | 5.1   |

(Continued)
| Mould species       | Eastern region | Ashanti region | Ahafo region |
|---------------------|----------------|----------------|--------------|
|                     | Fresh | Cured | Fresh | Cured | Fresh | Cured |
| Rhizoctonia sp.     | 37.2  | 35.7  | 22.9  | 26.0  | 0.0   | 0.0   |
| Rhizopus oryzae     | 4.0   | 0.0   | 0.0   | 1.0   | 0.0   | 0.0   |
| Rhizopus sp.        | 0.0   | 0.0   | 61.8  | 56.2  | 0.0   | 0.0   |
| Rhizopus stolonifer | 2.8   | 0.7   | 0.0   | 26.4  | 47.5  | 65.8  |
| Syncephalastrum     | 0.0   | 0.0   | 0.0   | 0.0   | 1.3   | 1.2   |
| racemosum            |       |       |       |       |       |       |
| Trichoderma harzianum| 2.0  | 2.2   | 0.0   | 1.8   | 0.6   | 3.7   |
| Trichoderma sp.     | 0.0   | 0.0   | 0.1   | 0.2   | 0.0   | 0.0   |
| Average mould count | 9.6   | 10.7  | 8.7   | 10.7  | 10.9  | 9.6   |

Values are average count of different moulds isolated from 900 kola nuts (100 each of red, white and pink kola nuts from 3 locations per region).
nuts, samples from Ahafo region were the most contaminated with moulds. This could be attributed to the fact that the kola nuts were obtained from pods that were not harvested but allowed to drop before they were picked (Amon-Armah et al., 2022). Damages from the drop, insects and rodents usually increase the chance of such pods becoming contaminated. However, for the cured nuts, samples from Eastern region were the most contaminated (Table 3). The results indicated a general increase in the occurrence of the moulds after curing. This may be attributed to the prevailing environmental conditions during curing which was humid and therefore maintained the nuts moisture content at levels above 50% (51 to 58%) favouring moulds growth. Although slow drying of the nuts during curing is desirable in maintaining the integrity of the nuts, losses due to mouldiness will be difficult to control completely and hence compromise on the quality of the cured nuts.

Moulds of different taxonomic genera were detected on the kola nuts from Ahafo (12 genera), Eastern (11 genera) and Ashanti (9 genera) regions. This may be as a result of poor post-harvest handling of the nuts. The method of curing, storage of the kola nuts and the levels of cleanliness of the storage environment can predispose the nuts to higher mould contamination. Prior to curing, sorting of damaged (weevilled, broken) nuts is recommended (Lowor, 2008) and the basis of sorting is that damaged nuts often contain high levels of mould that can contaminate the lots during curing. Consequently, appropriate sanitary measures aimed at preventing moulds contamination for safe storage of kola nuts is crucial and highly recommended. It is suggested that kola nuts should be packaged in bags that are porous and placed on treated pallets to allow air space between the floor and the bottom of the bags. This will prevent build-up of heat and moisture favourable for fungal growth. The storage room should also be disinfected with appropriate pesticide.

Aspergillus was the most common genera detected from the three regions, and seven species were identified (Table 3). This was followed by Penicillium (four species) and Rhizopus (three species) genera. Aspergillus Niger, A. flavus, Fusarium sp., Mucor sp. Penicillium sp. and Colletotrichum spp. have been implicated in deterioration of the nutritive quality of stored kola nuts (Agbeniyi & Ayodele, 2010; Idris et al., 2017). Aspergillus, Penicillium and Fusarium species are also well-known producers of major mycotoxins (Carlson & Ensley, 2003). Earlier investigators from Nigeria (Alebajo & Popoola, 2003) and Côte d’Ivoire (Douada et al., 2013) detected mycotoxins such as aflatoxin, Ochratoxin A and Zearalenone in stored kola nuts. In this study, 14 of the moulds consistently found on kola nuts belong to the mycotoxin-producing genera. Further studies are therefore required to determine the presence and levels (if any) of mycotoxins in kola nuts from Ghana.

4. Conclusion
The present study showed that fresh and cured kola nuts grown from three major kola producing regions of Ghana have important chemical compounds, such as polyphenols, fat, water, as well as secondary metabolites (alkaloids, saponins, terpenoids, flavonoids and tannins). Generally, the kola nuts were slightly acidic, low in fat but have high crude fibre content. Total polyphenol content increased as the moisture content decreased in the cured kola samples when compared to the fresh nuts. The amount of phytochemicals did not differ among the different kinds (red, pink and white) of kola nuts examined. The phytochemical quality was maintained after curing. However, the nuts were contaminated with moulds affecting the safety quality of the kola nuts. Nuts should be stored inside porous bags inside well-ventilated rooms instead of burying them in underground pits lined with polythene sheets. There were 14 genera of moulds identified on the kola nuts from the three regions of Ghana and some of them are known mycotoxins producing genera. Information from this study will form the basis for optimization of the post-harvest handling processes of kola nuts to improve on the quality and safety of kola nuts. This will also support the sensory (physical) evaluation currently undertaken by actors in the kola value chain.
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