Comparative FTIR Characterization of Caffeine Functional Groups in Different Varieties of Kola Nuts (Cola Acuminata and Cola Nitida)

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ABSTRACT

Transform Infrared (FTIR) spectroscopy was employed to investigate and compare the caffeine-related functional groups present in different varieties of kola nuts obtained from Oba, Anambra State, Nigeria. The kola nut varieties analyzed include White Hausa kola (*Cola nitida*), Red Hausa kola (*Cola nitida*), Four-lobed Igbo kola (*Cola acuminata*), and Common Igbo kola (*Cola acuminata*). FTIR spectra were recorded in the range of 4000–650 cm^{-1} at a resolution of 4 cm^{-1} using an ATR-FTIR technique, and the results were compared with a standard caffeine spectrum. Characteristic absorption bands corresponding to key caffeine functional groups such as C–H, C=C, C=O, C=N, and C–N were identified across all kola nut varieties, confirming the presence of caffeine. White kola and Common Igbo kola exhibited absorption bands most closely aligned with the standard caffeine spectrum, while Red kola showed minor frequency shifts. The Fourlobed Igbo kola displayed more pronounced deviations, particularly in the C–H stretching region, likely due to interactions with other phytochemical constituents. Overall, the study demonstrates that FTIR spectroscopy is an effective, rapid, and non-destructive technique for identifying and comparing caffeine functional groups in different kola nut varieties, while also revealing varietal differences influenced by their molecular environments.

Keywords:FTIR spectroscopy; Kola nut; Caffeine; Functional groups; *Cola acuminata*; *Cola nitida*; Vibrational analysis

INTRODUCTION

Kola nuts are economically, socially, and pharmacologically important plant materials widely consumed in West and Central Africa (Yahya *et al*., 2020**;** Okwuego 2023). They are derived mainly from two species, *Cola acuminata* and *Cola nitida*, which belong to the family Malvaceae. Kola nuts are traditionally chewed for their stimulant properties and are culturally significant in ceremonies, hospitality, and social interactions. Beyond their cultural relevance, kola nuts are valued for their bioactive constituents, particularly caffeine, which contributes to their physiological effects.

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring methylxanthine alkaloid widely found in beverages and plant materials such as coffee, tea, cocoa, and kola nuts (Daghbouche *et al*., 1997; Garrigues *et al*., 2000; Okwuego *et al* 2021). It acts primarily as a central nervous system stimulant and is associated with increased alertness, reduced fatigue, and enhanced cognitive performance. Due to its widespread consumption and pharmacological importance, the identification and characterization of caffeine in natural products have attracted considerable analytical interest. Several analytical techniques have been employed for the determination and characterization of caffeine, including high-performance liquid chromatography (HPLC), ultraviolet–visible (UV–Vis) spectrophotometry, gas chromatography–mass spectrometry (GC–MS), and Fourier Transform Infrared (FTIR) spectroscopy (Bouhsain *et al*., 1999; Janina *et al*., 2002; Okwuego, *et al* 2025). Among these methods, FTIR spectroscopy has gained prominence due to its rapidity, minimal sample preparation, non-destructive nature, and ability to provide direct information about molecular structure through vibrational transitions of functional groups. FTIR spectroscopy has been successfully applied to the analysis of caffeine in various matrices such as coffee, tea, and soft drinks (Daghbouche *et al.,* 1997; Bouhsain *et al*., 1999; Garrigues

et al., 2000; Okwuego *et al* 2021). Previous studies have demonstrated that FTIR offers excellent sensitivity and selectivity for caffeine determination, often requiring only simple solvent extraction or solid-phase preparation. The technique enables the identification of characteristic functional groups in caffeine, including C–H, C=C, C=O, C=N, and C–N bonds, which serve as diagnostic markers for its presence (Janina *et al*., 2002**;** Okwuego 2023). In kola nuts, caffeine exists within a complex phytochemical matrix that includes polyphenols, tannins, proteins, carbohydrates, and other alkaloids (Wei *et al*., 2013; Wei *et al*., 2015**;** Okwuego 2023). These additional constituents can influence the vibrational behavior of caffeine molecules, leading to shifts in absorption bands or variations in peak intensities in FTIR spectra. Consequently, comparative FTIR analysis of different kola nut varieties can provide valuable insight into how botanical origin and species differences affect the molecular environment of caffeine. Although kola nuts are widely consumed, there is limited comparative spectroscopic information on the functional group characteristics of caffeine across different kola nut varieties. In particular, variations between *Cola acuminata* and *Cola nitida*, as well as among commonly consumed local varieties such as White Hausa kola, Red Hausa kola, Four-lobed Igbo kola, and Common Igbo kola, have not been extensively studied using FTIR spectroscopy. Therefore, this study employs Fourier Transform Infrared (FTIR) spectroscopy to identify and compare the functional groups associated with caffeine in selected varieties of kola nuts obtained from Oba, Anambra State, Nigeria, following established FTIR analytical approaches (Sim & Ting, 2012**;** Okwuego 2023). By comparing the FTIR spectra of the kola nut samples with that of standard caffeine, the study aims to confirm the presence of caffeine and evaluate spectral similarities and differences arising from varietal and phytochemical influences. The findings of this work contribute to the growing body of knowledge on the application of FTIR spectroscopy in natural product analysis and provide a scientific basis for understanding caffeine characteristics in different kola nut varieties.

A

B

C

D

Experimental

Sample Collection

Four different varieties of fresh kola nuts were used in this study, namely White Hausa kola (*Cola nitida*), Red Hausa kola (*Cola nitida*), Four-lobed Igbo kola (*Cola acuminata*), and Common Igbo kola (*Cola acuminata*), following established sampling approaches for kola nut studies (Yahya *et al.,* 2020**;** Okwuego 2023). (*Cola nitida*), Red Hausa kola (*Cola nitida*), Four-lobed Igbo kola (*Cola acuminata*), and Common Igbo kola (*Cola acuminata*). The samples were purchased from Afor Ba Market, located in Idemili South Local Government Area of Oba Town, Anambra State, Nigeria. The kola nuts were selected based on their freshness, physical

integrity, and absence of visible defects or microbial deterioration. Immediately after purchase, the samples were transported to the laboratory in clean polyethylene bags to prevent contamination. The kola nuts were washed thoroughly with distilled water to remove surface dirt and debris; after which they were air-dried at room temperature. Each variety was kept separately and clearly labeled prior to analysis to avoid crosscontamination.

Sample Preparation

The cleaned kola nut samples were cut into smaller pieces and allowed to dry further under ambient laboratory conditions to reduce moisture content, consistent with standard preparation procedures for plant-based FTIR analysis (Wei *et al*., 2015**;** Okwuego 2023). The dried samples were then ground into fine powders using a clean laboratory mortar and pestle. The powdered samples were stored in airtight sample containers and kept in a desiccator until FTIR analysis was performed.

Infrared (FTIR) Spectroscopic Analysis

Fourier Transform Infrared (FTIR) spectroscopic analysis was carried out using an Attenuated Total

Reflectance Fourier Transform Infrared (ATR-FTIR) spectrometer, in accordance with previously reported FTIR analytical methodologies (Daghbouche *et al.,* 1997; Bouhsain *et al.,* 1999; Okwuego *et al* 2021). The spectra were recorded over the mid-infrared region from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1}, as commonly adopted in caffeine FTIR studies (Garrigues *et al*., 2000; Janina *et al*., 2002; Okwuego, *et al* 2025). Prior to sample analysis, a background spectrum was collected using a clean ATR crystal to eliminate atmospheric and instrumental interferences. For each measurement, a small quantity of the powdered kola nut sample was placed directly onto the ATR crystal surface and pressed firmly to ensure good contact.

Chloroform was used to clean the ATR crystal after each scan to prevent carry-over effects. Each sample was analyzed in triplicate to ensure reproducibility and reliability of the results. The FTIR spectra were saved in Comma-Separated Values (CSV) format and processed using an automated peak detection algorithm, following the approach described by (Sim *et al* 2012; Okwuego *et al* 2021). Baseline correction and spectral normalization were performed prior to interpretation. The absorption bands in the region of 1600–1800 cm^{-1}, characteristic of caffeine carbonyl and ring vibrations, were specifically examined. Peak positions and intensities were compared with those of standard caffeine spectra for functional group identification.

Data Analysis

The characteristic absorption bands corresponding to major caffeine functional groups, including C–H, C=C, C=O, C=N, and C–N stretching vibrations, were identified from the FTIR spectra based on established vibrational assignments for caffeine (Janina et al., 2002; **;** Okwuego 2023 Okwuego *et al* 2021). The observed vibrational frequencies of the kola nut samples were compared with those of standard caffeine to confirm the presence of caffeine and assess variations among the different kola nut varieties. The results obtained formed the basis for comparative interpretation and discussion of the FTIR spectra presented in this study.

RESULTS AND DISCUSSION

Table 1: Characteristic Infrared Absorption Band of Varieties of kola samples and Standard

Table: FTIR Bond Assignments for Different Kola Samples

S/N	Bonds	Standard	WK	RK	CK	4-lobe Kola
1	C–H	2955.23	2925.30	2918.00	2926.42	3399.00
2	C=C	1550.03	—	1595.00	1597.30	—
3	C=O	1701.30	—	—	1723.86	—
4	C=N	1660.77	1602.00	—	—	1629.00
5	C–N	1239.95	1377.00	1369.00	1285.66	1386.20
6	C–C	950.00	—	—	—	—

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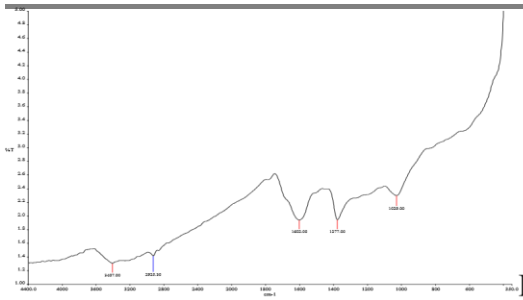

Fig.A.Whitekola

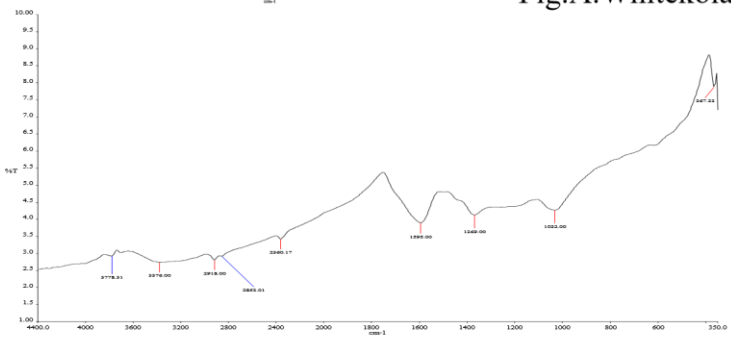

Fig.B. Red kola

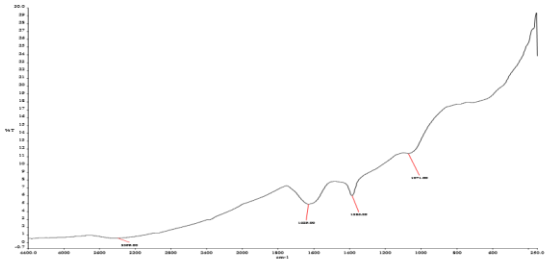

Fig.C. 4-lobe kola

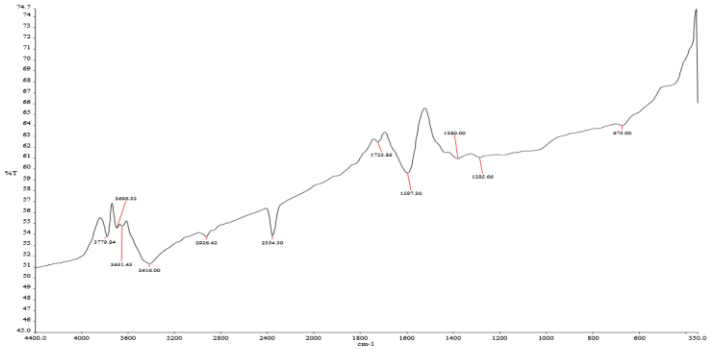

Fig.D.Common kola

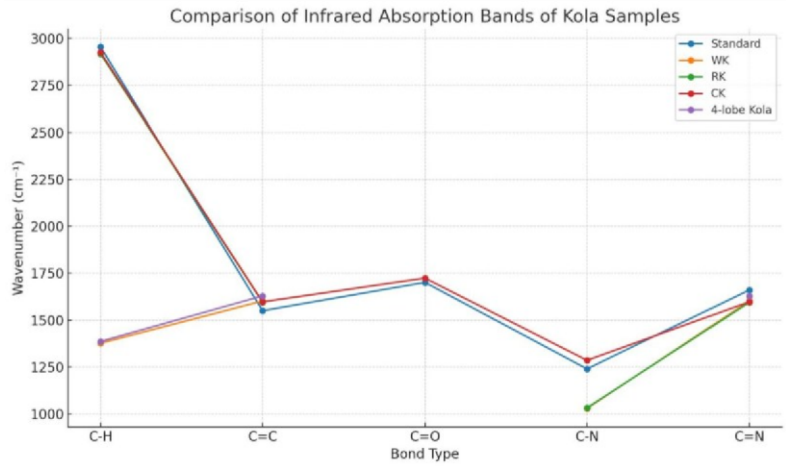

Fig.2. A Graph of Comparison of Infrared Absorption Bands of Different Varieties of Kola nuts.

FTIR Spectral Characteristics of Standard Caffeine

The Fourier Transform Infrared (FTIR) spectrum of standard caffeine was recorded in the mid-infrared region (4000–650 cm^{-1}) and used as a reference for functional group identification in the kola nut samples

(Daghbouche *et al.,* 1997; Garrigues *et al*., 2000; Okwuego *et al* 2021)**.** The spectrum exhibited characteristic absorption bands corresponding to the major functional groups present in the caffeine molecule. A strong absorption band observed at approximately 2955 cm^{-1} was assigned to C–H stretching vibrations of the methyl ($–CH_3$) groups. The aromatic C=C stretching vibration appeared around 1550 cm^{-1}, while the carbonyl (C=O) stretching vibration of the lactam groups was observed at approximately 1701 cm^{-1}. Additional bands at about

1661 cm^{-1} and 1240 cm^{-1} were attributed to C=N and C–N stretching vibrations, respectively. These absorption bands are consistent with previously reported FTIR spectra of caffeine and served as diagnostic markers for comparative analysis **(**Bouhsain *et al.,* 1999; Janina *et al*., 2002; Okwuego, *et al* 2025; Ochie *et al* 2025)**.**

FTIR Analysis of Kola Nut Varieties

The FTIR spectra of the four kola nut varieties, White Hausa kola (*Cola nitida*), Red Hausa kola (*Cola nitida*), Four-lobed Igbo kola (*Cola acuminata*), and Common Igbo kola (*Cola acuminata*) were analyzed and compared with the standard caffeine spectrum, following comparative FTIR approaches reported in the literature **(**Wei *et al*., 2015; Ochie *et al* 2025**)**. The characteristic absorption bands observed for each sample are summarized in Table 1.

C–H Stretching Vibrations

The C–H stretching vibration, characteristic of methyl groups in caffeine, was observed at 2925.30 cm^{-1} for White kola, 2918.00 cm^{-1} for Red kola, and 2926.42 cm^{-1} for Common kola. These values are in close agreement with the standard caffeine peak at 2955.23 cm^{-1}, indicating the presence of methylated xanthine structures in these samples **(**Janina *et al.,* 2002**;** Okwuego 2023**;** Okwuego, *et al* 2025**)**. In contrast, the Fourlobed kola exhibited a broad absorption band at 3399.00 cm^{-1}, which deviates significantly from the standard. This shift is likely due to overlapping O–H or N–H stretching vibrations arising from other phytochemicals present in this kola variety.

C=C Aromatic Stretching Vibrations

The aromatic C=C stretching vibration observed at 1550.03 cm^{-1} in the standard caffeine spectrum was also detected in Red kola (1595.00 cm^{-1}) and Common kola (1597.30 cm^{-1}). The presence of this band confirms the aromatic purine ring system characteristic of caffeine **(**Garrigues et al., 2000; Okwuego *et al* 2021; Ochie *et al* 2025**)**. The slight upward shift in wave numbers may be attributed to intermolecular interactions between caffeine molecules and other constituents within the kola nut matrix.

Carbonyl (C=O) Stretching Vibrations

Caffeine contains two carbonyl functional groups that produce a strong absorption band at approximately

1701.30 cm^{-1} in the standard spectrum **(**Bouhsain *et al*., 1999; Chinweuba *et al* 2024; Ochie *et al* 2025; Okwuego, *et al* 2025**)**. Among the kola nut samples, a corresponding peak was clearly observed in Common kola at 1723.86 cm^{-1}. The shift towards a higher wave number may be due to hydrogen bonding or conjugation effects with surrounding phytochemicals.

C=N Stretching Vibrations

The C=N stretching vibration, characteristic of the heterocyclic purine ring of caffeine, was observed at 1660.77 cm^{-1} in the standard caffeine spectrum (Janina *et al*., 2002; Ochie *et al* 2025**;** Okwuego 2023). Corresponding bands were detected in White kola at 1602.00 cm^{-1} and in Four-lobed kola at 1629.00 cm^{-1}, further confirming the presence of nitrogen-containing aromatic systems.

C–N Stretching Vibrations

The C–N stretching vibration of alkylated amine groups in caffeine appeared at 1239.95 cm^{-1} in the standard spectrum **(**Daghbouche *et al*., 1997**;** Okonkwo *et al* (2025**;** Okwuego 2023**)**. All kola nut samples exhibited absorption bands within this region, confirming the presence of $N–CH_3$ groups across all kola varieties.

C–C Skeletal Vibrations

The C–C skeletal vibration observed at 950.00 cm^{-1} in the standard caffeine spectrum was not clearly detected in the FTIR spectra of the kola nut samples. This absence may be due to masking by stronger absorption bands from other phytochemical constituents or reduced sensitivity in complex biological matrices *(*Wei *et al., 2013***;** Odilora *et al* 2025; Okwuego, *et al* 2025**)**.

Comparative Evaluation of Kola Nut Varieties

Overall, the FTIR spectral comparison revealed that White kola and Common kola exhibited the closest agreement with the standard caffeine spectrum, similar to trends reported for caffeine-containing plant matrices (Wei *et al*., 2013; Wei *et al*., 2015**;** Okwuego 2023**)**. Red kola also showed good correspondence with minor shifts, while the Four-lobed kola exhibited the most pronounced deviations.

Implications of FTIR Findings

The results of this study highlight the effectiveness of FTIR spectroscopy as a rapid and non-destructive tool for identifying caffeine functional groups in natural products, as demonstrated in previous analytical studies (Sim & Ting, 2012; Garrigues *et al*., 2000**;** Okwuego *et al* 2021; Okwuego, *et al* 2025**)**.

CONCLUSION

FTIR spectroscopy was successfully applied to the comparative identification of caffeine functional groups in different kola nut varieties. Characteristic absorption bands corresponding to C–H, C=C, C=O, C=N, and C–N vibrations were observed in all samples, confirming the presence of caffeine. White Hausa kola (*Cola nitida*) and Common Igbo kola (*Cola acuminata*) showed the closest agreement with the standard caffeine spectrum, whereas Red Hausa kola exhibited minor spectral shifts. The Four-lobed Igbo kola displayed more pronounced deviations, particularly in the C–H stretching region, attributable to interactions with other phytochemical components. These results demonstrate the suitability of FTIR as a rapid and non-destructive technique for caffeine characterization and for assessing varietal differences in kola nuts.

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