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Non-invasive discrimination of roasted and unroasted cocoa bean shell of cocoa clones in Ghana and quantification of nutritional and bioactive components: a chemometric approach

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Abstract
Cocoa bean shell (CBS) remains a commonly produced by-product of cocoa bean processing. It is usually obtained from fermented and dried cocoa beans that are roasted. The study investigated the potential use of Near-infrared spectroscopy (NIRS) analysis for discriminating roasted and unroasted CBS among cocoa clones and quantifying some nutritional and bioactive components in Ghana. Five clones, comprising four important seed gardens clones used across West Africa and one criollo were evaluated. Cocoa beans from the different clones (T60/887, VENC 4, MO 20, PA 150 and T60/887 × POUND 7) were divided into two parts, with one part roasted at a temperature of 120 °C for 50 min while the other part was kept unroasted. The CBSs were milled and passed through a 425 µm pore-sized sieve to obtain the powder. A hand-held portable NIRS was used to scan the CBS powder in Ziplock bags. The nutritional and bioactive characterisation was carried out using official methods. NIRS discriminated the various clones of roasted and unroasted CBS. Carbohydrate was the predominant macronutrient, and ash content ranged from 5.25 to 8.24%. The CBS was high in potassium (2382–3144 mg/100 g) and low in sodium (25.67–51.33 mg/100 g). Total flavonoids and phenolics ranged from 8.61 to 40.71 mgQE/g and 6.34–12.25 mgGAE/g, respectively, for the roasted and unroasted CBS. To ensure better differentiation of cocoa beans from different clones using NIRS, incorporating roasting as a processing parameter is recommended.

Keywords Bioactive profile · Chemometrics · Cocoa bean shell powder · NIRS · Nutrient composition · Roasting

1 Introduction

Ghana produced 969,300, 904,700, 811,700, 1,047,000 and 822,000 tonnes of dried cocoa beans during the 2017, 2018, 2019, 2020 and 2021 cocoa growing seasons [1]. This amounted to an average quantity of about 910,940 tonnes of dried cocoa beans being produced annually [1]. Thus, Ghana remains one of the leading producers of cocoa beans worldwide [2]. Generally, the cocoa bean production chain begins with the harvesting of ripe cocoa pods that appear bright yellow during maturation [3]. The harvested ripe cocoa pods are split open using a wooden club, and the fresh, sweet pulpy cocoa beans are scooped out, collected into fresh banana leaves to form heaps and allowed to undergo spontaneous

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fermentation [3]. The fermented cocoa beans are sun-dried, packaged in jute sacks [3, 4] and sold to cocoa processing companies for further processing.

In the cocoa processing industry, dried beans are cleaned to remove certain physical hazardous materials, including stones and metallic objects. The cocoa beans are subsequently roasted, deshelled, broken down into nibs, and milled into liquor to be pressed into cocoa cake and butter, the raw materials for cocoa-based consumables [4]. The deshelling process generates cocoa bean shell (CBS), which is considered an industrial by-product. It has been reported that CBS constitutes about 12–20% of the whole dried cocoa beans [5–7]. This means that Ghana, which produces an average amount of 910,940 tonnes of cocoa beans annually, generates between 91,091 and 182,188 tonnes of CBS. Several consumer market surveys have projected increased demand for cocoa-based products, including cocoa cake and butter for confectionary, cosmeceutical and pharmaceutical applications [3, 8]. This projected increase could be attributed to factors including the increased health-conscious consumer interest in cocoa consumption, especially for the nutritional and health-promoting effects of cocoa powder, such as reducing systolic blood pressure and improving lipid profile [9, 10]. Consequent to the increased consumer demand for cocoa-based products, there will be an increased generation of large amounts of CBSs as by-products from cocoa bean processing.

The production of CBS is a nuisance to the environment and remains one of the highly underutilized food by-products globally. Recently, one of the emerging trends in the food science space has focused on increased advancement in the valorisation of cocoa processing residues, including CBS for feed and new food product development [11]. This largely has been driven by factors including the need to address United Nations Sustainable Development Goals (UN SDGs) 2, 12 and 13, which prioritise addressing zero hunger, promoting sustainable production of food and accelerating efforts to mitigate climate change challenges [12]. In regards to UN SDG 13, food by-products are major culprits as their decomposition on the environment results in the generation of greenhouse gases, which promotes climate change [12]. The use of CBS in food applications is limited in Ghana due to the paucity of data on its nutritional and bioactive composition.

In Ghana, the Cocoa Research Institute continuously select new parental clones of cocoa, leading to the development of varieties with improved cocoa bean physicochemical properties [13]. Factors that trigger these breeding programs include developing cocoa varieties with disease-resistance, better climate resilience, and improved nutritional outcomes [14]. In the case of the latter, the genetic architecture and, for that matter, the type of cocoa clone impacts the nutritional and bioactive composition of cocoa beans and their shells [15]. For example, differences in nutritional and bioactive properties were reported for CBS from different varieties [15]. Processing methods, including the use of roasting, can impact the nutritional and bioactive composition of CBS [16].

Handheld portable near-infrared spectroscopy (NIRS) for discriminating different clones of the same plant and predicting their chemical composition has gained increased interest globally [17, 18]. NIRS is a non-invasive approach to outcome assessment, providing rapid access to the results for processing and eliminating expensive reagents for sample preparation [19].

Li et al. [20] conducted an extensive review of the use of NIR to determine the quality attributes of oilseeds and oil. The authors highlighted the prospect of utilising NIR in tandem combination with chemometrics for the determination of the important macronutrients and quality parameters in oilseeds [20]. In a recent systematic review conducted using the Scopus database, the authors identified 45 studies that utilised near-infrared for the quantification of bioactive compounds in different food matrices [21]. The authors reported that NIR is a reliable device with great prospects in the analysis of bioactive compounds, including polyphenols, anthocyanins, carotenoids and ascorbic acid [21].

Teye et al. [19] reviewed the potential use of NIRS to determine the chemical properties of cocoa and cocoa products. The authors reported that NIRS can be used to determine the nutritional and bioactive composition of cocoa beans and its products [19]. It additionally remains a promising device for discriminating between differently treated food samples, such as those processed by roasting [22]. It has been used to detect adulterants, including Robusta coffee or chicory in Arabica roasted coffee [23]. Challenges associated with the use of NIRS have been extensively reviewed [24]. They include the reliance of NIRS prediction accuracy on reference laboratory results, difficulties associated with the initial development of models for reliance, its low sensitivity for the prediction of mineral contents due to the lack of absorbance of the minerals in the infra-red region, and in heterogenous samples, its inability to predict the spatial distribution of the quality parameters [24, 25].

That notwithstanding, there is currently a lack of data on the use of NIRS for the discrimination of CBS from cocoa clones grown in Ghana processed through roasting and quantification of their nutritional and bioactive composition. The study aimed to investigate the potential use of Near-infrared spectroscopy (NIRS) analysis for the discrimination of roasted and unroasted CBS from five cocoa clones grown in Ghana and the quantification of some nutritional and bioactive components.

2 Materials and methods

2.1 Sample acquisition

Thirty mature ripe cocoa pods of five cocoa clones were harvested from a well-spaced established clonal plots of the Cocoa Research Institute of Ghana (CRIG). The clones were T60/887, VENC 4, MO 20, PA 150 and a hybrid, T60/887 × POUND 7. Except VENC 4, these are important clones used as parents in the seed gardens across West Africa. T60/887 is a clone derived from PA 7 × NA 32 made in Trinidad and collected by Posnette in 1944 [26], VENC 4 is a clone of Criollo origin, selected in Venezuela, MO 20 is a Morona clone in the Nacional genetic group [27], PA 150 is a clone of Parinari origin, belonging to the Marañón genetic group, and T60/887 × Pound 7 is a hybrid cross between (PA 7 × NA 32) and a Nanay clone selected in the headwaters of the Amazon by Pound [26]. The pods were broken open using a wooden club. Due to the small quantities of the samples, the cocoa beans were kept in a sponge and then placed inside a box fermentation set up. The fresh pulpy cocoa beans were fermented for 6 days (except for VENC 4 which was micro-fermented in a Styrofoam box for 5 days) with intermittent turning every 2 days. The fermented cocoa beans were subsequently sun-dried on raised raffia mats to a moisture content below 12%. The collection of the cocoa bean shells used in this study complied with national guidelines. The dried cocoa beans were packaged in Ziplock bags, labelled and transported to the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

2.2 Sample preparation and treatment

About 100 g of each cocoa bean clone was weighed using an analytical balance (Ohaus PR224, USA) and divided evenly into two equal portions. Half (50 g) of each of the five samples was roasted, and the other half (50 g) was left unroasted. With the roasted samples, the cocoa beans were subjected to a temperature of 120 °C for 50 min [28] in a Memmert® Oven (SN 260, Memmert GmbH + Co.KG, Germany). The samples were placed in a desiccator to cool down. After cooling down, each of the five different dried cocoa beans was deshelled manually using a small stainless-steel knife to separate the beans from the shells and were placed separately into labelled Ziploc bags. This procedure was repeated for the unroasted samples. The 50 g of the unroasted cocoa beans resulted in an average of 8.15 g of CBS and 6.75 g for the roasted CBSs. The roasted and the unroasted shells from the cocoa beans were milled separately using a blender before passing them through a mesh with a pore size of 425 µm. This process was repeated three times to obtain three repeats per clone, resulting in 6 samples per clone (1 clone × 3 repeat × 2 treatment). In all, 30 samples of the CBS powder were packed in Ziploc bags for analytical and NIR measurement. The general processes involved in the production of the unroasted and unroasted CBS powder have been shown below (Fig. 1).

2.3 Proximate analysis

The moisture, ash, fat, fibre and protein content of the CBS powder was conducted using AOAC official methods [29]. Carbohydrate content of the CBS powder was determined by difference. This involved subtracting the percentage of moisture, ash, fat, fibre and protein content of CBS powder from 100. The energy content of the CBS powder was calculated using the Atwater factors (fat content × 9 kcal/g, protein content × 4 kcal/g and carbohydrate content × 4 kcal/g).

2.4 Mineral analysis

About 1 g of the CBS powder was weighed into a clean, empty ceramic crucible. The samples were ashed in a furnace at 500 °C for 4 h. The ashed samples were allowed to cool to room temperature and transferred into 50 ml centrifuge tubes. The crucibles were rinsed with 10 ml distilled water to enable complete transfer of the ashed samples into the centrifuge tubes. Each CBS powder sample was digested using 10 ml of aqua regia prepared by mixing hydrochloric acid and nitric acid in a 3:1 ratio. The samples were shaken on a mechanical reciprocating shaker for 5 mins to mix properly. Samples were centrifuged for 10 min at 3000 rpm and filtered using a filter paper (Whatman No. 42) into a 100 ml volumetric flask. The filtrate was diluted to the mark on the volumetric flask with distilled water [30]. The clear supernatant digest was decanted into clean reagent bottles for phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) determinations. The P content was analysed using the vanadomolybdate reagent yellow colour method with absorbance measured at 420 nm read on Spectronic 20 spectrophotometer (Libra S80PC, Biochrom Ltd., England).

Fig. 1 A flow chart showing the processes involved in the production of the unroasted and roasted cocoa bean shell powder

The K and Na contents were determined using a flame photometer. Ca and Mg contents were determined using the EDTA titration method [31].

2.5 Bioactive composition

2.5.1 Total polyphenol content determination

This was done using spectrophotometry employing Folin-Ciocalteu's method and using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) and [32]. Briefly, 20 mg of each of the 10 samples was weighed in a falcon tube using an analytical balance (Ohaus PR224, USA). A volume of 20 ml of deionized water was added to each tube and vortexed for 5 min. The samples were allowed to stand for about 10 min. A volume of 1 ml of the supernatant was pipetted using an Eppendorf pipette into a test tube. Following this, 20 µl Folin-Ciocalteu reagent and 1 ml of 20% sodium carbonate were added to the test extract. This was then incubated in the dark for 40 min, and the absorbance was measured at 760 nm. The results of phenols were expressed as Gallic acid equivalents in mg/g of extract.

2.5.2 Total flavonoids content determination

The total flavonoids content was determined using the aluminum chloride test. An amount of 20 mg of each of the 10 samples was weighed in a falcon tube using an analytical balance (PR224, Ohaus Corporation, USA). A volume of 20 ml of 80% ethanol was added to each tube and vortexed for 5 min. The samples were allowed to stand still for about 10 min. A volume of 1 ml of the supernatant was pipetted using an Eppendorf pipette into a test tube. After this, 1 ml of 2% aluminum chloride was added and incubated in the dark for 40 min. The absorbance was measured at 420 nm. The calibration curve was constructed using quercetin standard solutions, and results were expressed as mg of quercetin equivalent per gram [33].

2.6 NIR scanning of cocoa bean shell powder and statistical analysis for analytical measurements

All 30 samples were placed in low-density polyethylene (LDPE) Ziplock bags and scanned. The scanning was carried out using a handheld DLP NIRScan Nano instrument (Texas Instruments, Dallas, TX, USA), which had a wavelength of 900–1700 nm and operated using a 3 nm spectral resolution. For each sample, six spectral measurements were taken consecutively, yielding 180 spectra in total. The whole spectrum capture procedure was carried out at room temperature.

The spectra obtained from the NIR scanning process were first preprocessed with a Savitzky-Golay smoothing filter to minimise the noise additive effect of the collected spectra [34]. Subsequently, principal component analysis (PCA) was carried out to detect, visualise, and eliminate outliers from all the samples. Additionally, it was used to reduce dimension while maintaining the relevant information [35].

Models were developed using linear discriminant analysis (LDA) to enable the classification of the different clones for both roasted and unroasted bean treatments. The predictive value of each LDA model was evaluated following the division of the data into two categories (training and validation) [36]. The first and second replicates constituted two-thirds of the data, and their spectra were used in the training set. The third replication spectra were used to build the validation set. During the data splitting phase, the calibration and validation sets' replicates were switched out three times [36].

Analyses were done in triplicates. Data analysis was done using Standard Package for Social Scientists (SPSS) 24.0 software. Data was checked for normality using the Sharpiro-Wilk test, where $p > 0.05$ refers to implied normality. The test for significant differences between the means of the proximate, mineral and bioactive composition of the cocoa clones was determined using the Analysis of Variance (ANOVA). Tukey's test was used to compare the mean values, and significance differences were established at $p < 0.05$. The statistical metrics used to assess the effectiveness of the LDA models included average recognition accuracy for calibration and average prediction accuracy for cross-validation.

3 Results

3.1 Proximate analysis of five clones of roasted and unroasted cocoa bean shells

The proximate analysis was carried out for protein, ash, moisture, fat, fibre and total carbohydrate contents. The CBSs were high in carbohydrate, with roasted MO 20 having the highest value of 52.58% and lower in unroasted VENC 4 with a value of 38.31%. There was an increase in carbohydrate in all the clones after the roasting process. Roasting decreased the protein content in the cocoa clones except VENC 4. There was, however, no notable change in the protein content of MO 20 after roasting. Protein was the second highest macronutrient, ranging from 15.32 to 23.42%, with roasted VENC 4 recording the maximum value. Both unroasted and roasted MO 20 recorded the minimum protein value as 15.32%. The fat content decreased with roasting in all the clones except T60/887 and PA 150. Unroasted VENC 4 contained the maximum fat value of 12.34%. The minimum fat content of 8.23% was found in roasted MO 20. The fibre contents of the CBSs were in the range of 6.57%-11.48%, with roasted VENC 4 recording the maximum and T60/887 recording the minimum. The fibre content increased in VENC 4 and MO 20 after roasting and decreased in the other clones. Unroasted PA 150 had the highest moisture content of 11.96%, and roasted VENC 4 had the lowest moisture content of 6.57%. There was a decrease in moisture content in all the five clones after roasting. The maximum ash value of 8.42% was recorded in roasted VENC 4. Roasted T60/887 had the minimum ash content of 5.25%. The ash content increased after roasting in the clones except for T60/887 and T60/887 × POUND 7. Roasted PA 150 recorded the maximum energy value of 374.46 kcal/100 g, while roasted VENC 4 had the minimum (341.06 kcal/100 g) (Table 1).

3.2 Mineral composition of the unroasted and roasted cocoa bean shells from five cocoa clones grown in Ghana

The CBS was high in potassium, ranging from 2382 to 3114 mg/100 g. There was an increase in the potassium content after roasting the clones, with the exception of MO 20 and PA 150. Roasted VENC 4 had the maximum potassium content, and roasted PA 150 had the minimum. Phosphorus was the second most predominant mineral, ranging from 483.67 to 1698 mg/100 g. Roasting decreased the potassium content in the clones except VENC 4 and T60/887 × POUND 7. The sample with the maximum phosphorus content was roasted VENC 4 with 1698 mg/100 g, and unroasted T60/887 had the minimum phosphorus content of 483.67 mg/100 g. Roasted T60/887 × POUND 7 had the maximum calcium content

Table 1 Proximate composition of unroasted and roasted cocoa bean shell from five cocoa clones grown in Ghana

Parameter	Samples (Mean ± SD)									
	Unroasted T60/887	Unroasted VENC 4	Unroasted T60/887 × POUND 7	Unroasted M0 20	Unroasted PA 150	Roasted T60/887	Roasted VENC 4	Roasted T60/887 × POUND 7	Roasted MO 20	Roasted PA 150
Moisture (%)	11.20 ± 0.41^{bc}	7.77 ± 0.92^{a}	10.84 ± 0.18^{bc}	10.37 ± 0.16^{bc}	11.96 ± 0.84^{c}	9.84 ± 1.23^{b}	6.51 ± 0.62^{a}	7.84 ± 0.28^{a}	7.52 ± 0.21^{a}	6.94 ± 0.47^{a}
Ash (%)	5.49 ± 0.36^{ab}	8.07 ± 0.54^{d}	6.52 ± 0.32^{bc}	5.85 ± 0.02^{abc}	6.01 ± 0.62^{abc}	5.25 ± 0.22^{a}	8.24 ± 0.16^{d}	5.91 ± 0.43^{abc}	6.60 ± 0.36^{c}	6.16 ± 0.25^{abc}
Crude fat (%)	9.18 ± 0.92^{abc}	12.34 ± 0.35^{f}	9.94 ± 0.54^{bcde}	9.18 ± 0.16^{abc}	10.60 ± 0.37^{cde}	11.09 ± 0.77^{def}	8.94 ± 0.15^{ab}	9.69 ± 0.5^{abcd}	8.23 ± 0.65^{a}	11.48 ± 0.38^{ef}
Crude fibre (%)	8.60 ± 0.14^{c}	10.39 ± 0.24^{e}	9.72 ± 0.04^{d}	9.34 ± 0.02^{d}	9.51 ± 0.01^{d}	6.57 ± 0.02^{a}	11.16 ± 0.12^{f}	6.8 ± 0.16^{a}	9.75 ± 0.61^{de}	7.64 ± 0.19^{b}
Protein (%)	17.36 ± 1.10^{bcd}	23.13 ± 0.46^{e}	18.75 ± 0.12^{d}	15.32 ± 0.44^{a}	17.43 ± 0.32^{bcd}	15.83 ± 1.21^{ab}	23.42 ± 0.22^{e}	17.95 ± 1.15^{cd}	15.32 ± 0.44^{a}	16.34 ± 0.25^{abc}
Carbohydrate (%)	48.17 ± 1.83^{c}	38.31 ± 0.41^{a}	44.22 ± 0.87^{b}	49.94 ± 0.12^{cd}	44.50 ± 0.67^{b}	51.41 ± 0.83^{d}	41.73 ± 0.75^{b}	51.82 ± 1.51^{d}	52.58 ± 1.52^{d}	51.44 ± 0.80^{d}
Energy (kcal/100 g)	344.70 ± 5.06^{a}	356.79 ± 4.33^{b}	341.38 ± 1.29	343.66 ± 0.20^{a}	343.06 ± 4.92^{a}	368.79 ± 6.62^{c}	341.06 ± 2.68^{a}	366.29 ± 4.35^{bc}	345.65 ± 2.05^{a}	374.46 ± 1.20^{c}

Mean values with different superscripts in the same row are significantly different ($p < 0.05$)

of 1481.67 mg/100 g, and roasted T60/887 had the minimum of 661.33 mg/100 g. The amount of calcium and magnesium decreased after roasting in the CBSs except for T60/887 × POUND 7 and PA 150. The maximum magnesium content was recorded in unroasted MO 20 with a value of 551 mg/100 g. Unroasted PA 150 had the least magnesium content of 233.33 mg/100 g. Sodium was the lowest mineral in the CBS, ranging from 25.67 to 51.33 mg/100 g. Unroasted MO 20 recorded the maximum sodium content, and unroasted VENC 4 recorded the minimum. The sodium content in all the clones increased after roasting except MO 20 (Table 2).

3.3 Bioactive composition of CBS from five cocoa clones grown in Ghana

The CBSs were high in flavonoid, ranging from 8.45 to 40.71 mgQE/g. Flavonoid was found to be high in unroasted VENC 4 with a value of 40.71 mgQE/g. Unroasted T60/887 × POUND 7 had the minimum amount of flavonoid of 8.45 mgQE/g. Roasting increased the flavonoid contents in the CBSs except VENC 4 and PA 150. Roasted PA 150 had the maximum phenolic content of 12.25 mgGAE/g, while roasted VENC 4 had the lowest of 6.34 mgGAE/g. Total phenolic contents in the CBSs decreased after roasting except T60/887 × POUND 7 and PA 150 (Table 3).

4 Discrimination of cocoa clones

4.1 Principal component analysis results

Figure 2 shows the plot of the Principal Component Analysis (PCA) scores, visualising the variations in roasted and unroasted beans irrespective of clone differences (A) and the variations in clones regardless of treatment (B). From Fig. 2, roasted and unroasted beans could be differentiated in the plot, but their clones could not be differentiated. This implied that temperature made a difference in the chemical composition of the beans, but PCA analysis did not show enough separation in the clones, where chemometric analysis was required.

4.1.1 Linear discriminant analysis (LDA) results

Figure 3 shows the linear discriminant analysis (LDA) plot for the discrimination of unroasted (A) and roasted (B) cocoa bean clones. Apart from clone PA 150 and T60/887, all the cocoa bean clones could be clearly classified in the plots when they were not roasted (Fig. 3A). From the confusion matrix (Table 4), there was an overall average clone class prediction of 95.32% when beans were not roasted. There was a misclassification of PA 150 (11%) as T60/887. Although slight overlapping seems to be visually observed between clone PA 150 and VENC 4 when beans were roasted (Fig. 3B), there was 100% average clone class prediction.

5 Discussion

Moisture content is a parameter widely recognized in food industries as it indicates the shelf life of foods [37]. However, moisture content of ≤ 7 can halt enzymatic and microbial activity of cocoa beans and makes them safe for storage [38, 39]. The moisture content values in the unroasted samples ranged from 7.77% to 11.96% (Table 1). The roasted samples showed lower moisture contents, which ranged between 6.51- and 9.84%. There was a significant difference in moisture content between roasted T60/887 and unroasted PA 150. The moisture content values of the unroasted and roasted samples were in agreement with previous research, which showed that the moisture content of unroasted CBS reduced from 13.13% to 4.32% after roasting [40]. Additionally, it was consistent with the study done by Rojo-Poveda et al. [15], which reported a moisture content of 3.6 to 13.13%. The moisture content value of the roasted CBS was similar to the moisture values (6.41% to 9.23%) reported by Djali et al. [41].The study showed that the moisture content of the unroasted bean shell was higher than that of the roasted bean shell.

Ash content, also referred to as mineral content, is the remains of inorganic residue after the complete combustion of organic components in food samples [42]. The ash content values for the unroasted samples ranged from 5.49 to 8.07%, while those for the roasted samples were from 5.25 to 8.24%. VENC 4 recorded the maximum ash content in both cases, while T60/887 recorded the minimum. There was no significant difference in ash content between the roasted and unroasted VENC 4. However, they were significantly different from the other samples. Agus et al. [40] reported that

Table 2 Mineral composition of cocoa bean shell from five cocoa clones grown in Ghana

Parameter	Samples (Mean ± SD)									
	Unroasted T60/887	Unroasted VENC 4	Unroasted T60/887 × POUND 7	Unroasted M0 20	Unroasted PA 150	Roasted T60/887	Roasted VENC 4	Roasted T60/887 × POUND 7	Roasted MO 20	Roasted PA 150
P (mg/100 g)	807.33 ± 18.04^{b}	1522.33 ± 28.75^{f}	993.33 ± 33.71^{c}	1320.33 ± 30.37^{e}	1010 ± 22.91^{c}	483.67 ± 3.06^{a}	1698 ± 71.84^{g}	1130.33 ± 45.01^{d}	1213.33 ± 2.08^{d}	881.67 ± 28.10^{b}
K (mg/100 g)	2448.33 ± 22.50^{a}	2783 ± 34.70^{bc}	2790.33 ± 23.46^{bc}	3107 ± 20.30^{d}	2410.33 ± 34.59^{a}	2711.67 ± 94.88^{b}	3114 ± 23.00^{d}	2914.67 ± 28.68c	3084.67 ± 34.15^{d}	2382 ± 134.73^{a}
Ca (mg/100 g)	774.66 ± 6.11^{c}	895 ± 9.45^{d}	977.33 ± 12.22^{e}	1284.67 ± 28.15^{g}	673.33 ± 14.05^{a}	661.33 ± 12.22^{a}	732 ± 4.00^{b}	1418.67 ± 12.22^{h}	955.67 ± 9.02^{e}	1094.67 ± 10.07^{f}
Mg (mg/100 g)	335 ± 33.96^{c}	312.33 ± 6.11^{bc}	336 ± 20^{c}	551 ± 32.36^{f}	233.33 ± 9.61^{a}	240.67 ± 7.09^{a}	272 ± 3.61^{ab}	424.67 ± 10.69^{d}	486.33 ± 8.08^{e}	325.67 ± 3.51^{c}
Na (mg/100 g)	28 ± 0^{ab}	25.67 ± 0.58^{a}	30.33 ± 0.58^{bc}	51.33 ± 0.58^{f}	27 ± 0^{a}	34.33 ± 0.58^{d}	28.33 ± 0.58^{ab}	32.67 ± 0.58^{cd}	41.33 ± 0.58^{e}	33.33 ± 2.89^{d}

Mean values with different superscripts in the same row are significantly different ($p < 0.05$)

Table 3 Bioactive composition of cocoa bean shell from five cocoa clones grown in Ghana

Parameter	Samples (Mean ± SD)									
	Unroasted T60/887	Unroasted VENC 4	Unroasted T60/887 × POUND 7	Unroasted M0 20	Unroasted PA 150	Roasted T60/887	Roasted VENC 4	Roasted T60/887 × POUND 7	Roasted MO 20	Roasted PA 150
Total phenolics (mg/g)	6.47 ± 0.04^{a}	7.75 ± 0.02^{c}	8.22 ± 0.11^{d}	9.99 ± 0.13^{f}	10.08 ± 0.03^{f}	6.42 ± 0.03^{a}	6.34 ± 0.08^{a}	8.56 ± 0.03^{e}	7.36 ± 0.07^{b}	12.25 ± 0.11^{g}
Flavonoids (mg/g)	8.61 ± 0.03^{a}	40.71 ± 0.26^{g}	8.45 ± 0.23^{a}	12.72 ± 0.37^{c}	27.11 ± 0.21^{f}	15.58 ± 0.10^{e}	9.74 ± 0.40^{b}	14.23 ± 0.26^{d}	14.59 ± 0.06^{d}	15.00 ± 0.32^{de}

Mean values with different superscripts in the same row are significantly different ($p < 0.05$)

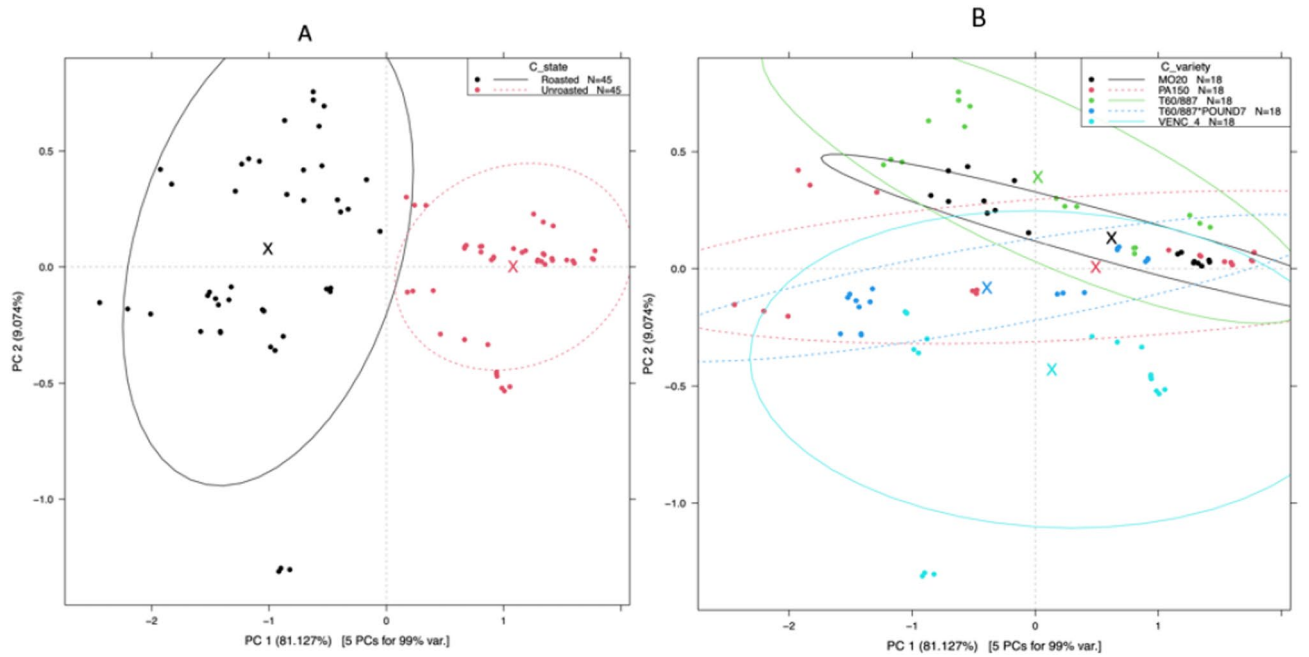

Fig. 2 Principal Component Analysis (PCA) scores plot visualizing the variations in roasted beans and unroasted beans irrespective of clone differences (**A**) and also the variations in clone irrespective of treatment (**B**)

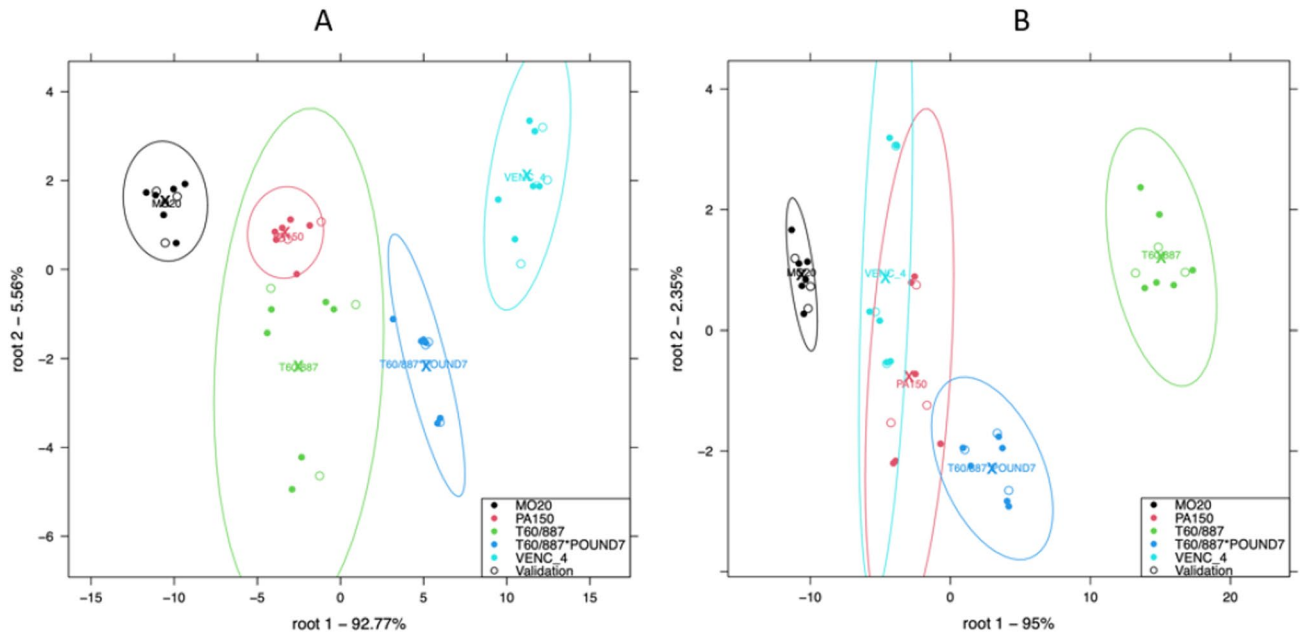

Fig. 3 LDA scores plot for the discrimination of non-roasted (**A**) and roasted (**B**) for five cocoa bean clones

roasted CBS showed higher ash content than unroasted CBS. The results from this study corroborate this, although the differences were not significant ($p > 0.05$). However, clones T60/887 and T60/887 × POUND 7 showed otherwise. Similarly, the ash content reported by Djali et al. [41] was higher in the roasted CBS (6.48–8.21%) compared to the unroasted (3.74–4.74%). The levels of ash content observed for the samples in the present study were similar to those reported in previous studies [15, 43]. A key difference observed in this study was the ash content of the unroasted samples, which differed from findings from previous research [41]. This could be attributed to varietal and geographical differences.

Regarding the fat composition, the unroasted VENC 4 recorded the maximum value (12.34%), whereas roasted MO 20 had the minimum (8.23%). There were significant differences among roasted MO 20, unroasted T60/887 × POUND 7,

Table 4 Confusion matrix showing the LDA classification accuracies for five cocoa bean clones

Average prediction of unroasted bean clones:95.32%		MO 20	PA 150	T60/887 × POUND 7	T60/887	VENC 4
	MO 20	100	0	0	0	0
	PA 150	0	89	0	12.41	0
	T60/887 × POUND 7	0	0	100	0	0
	T60/887	0	11	0	87.59	0
	VENC 4	0	0	0	0	100
Average prediction of roasted bean clones:100%		MO 20	PA 150	T60/887 × POUND 7	T60/887	VENC 4
	MO 20	100	0	0	0	0
	PA 150	0	100	0	0	0
	T60/887 × POUND 7	0	0	100	0	0
	T60/887	0	0	0	100	0
	VENC 4	0	0	0	0	100

unroasted and roasted PA 150, roasted T60/887 and unroasted VENC 4. A fat content of 15.1% was reported in a study by Delgado-Ospina et al. [44] for CBS, which is higher than the lipid content in this study. Both Agus et al. [40] and Djali et al. [41] reported a decrease in fat content in CBS after roasting, which was in close agreement with all the clones except T60/887 and PA 150. Also, the fat content of unroasted CBS was reported to have increased after roasting from 4.09% to 5.8%, which is lower than the fat content in both the unroasted and roasted CBS in this study [45]. It has been reported, however, that genotype, soil, climate, and harvest conditions can all have an impact on the makeup of cocoa beans [46].

Crude fibre indicates the level of the edible part of plants which has some resistance to digestion and absorption in the intestine [43]. The maximum amount of fibre for both the unroasted and roasted samples was observed in VENC 4, while the minimum was in T60/887. The fibre content of the roasted samples were within the ranges of 6.75% to 11.16%, and that of the unroasted ranged from 8.60% to 10.39%. There was no significant difference among unroasted MO 20, unroasted PA 150, unroasted T60/887 × POUND 7 and roasted MO 20. Roasted VENC 4, roasted PA 150 and unroasted T60/887 were significantly different from one another and the rest of the samples. It is reported that the roasting process affects the quantity of dietary fibre in CBS [47]. Agus et al. [40] reported increased fibre content in CBS after the roasting process. This increment in the fibre content of CBS was due to Maillard reaction compounds, polysaccharides, polyphenolic and protein interactions during roasting [48]. The fibre content reported by Agus et al. [36] for both the unroasted (13.86%) and roasted (16.06%) CBS were higher than the results from this study. Roasting decreased the fibre contents in all the samples except MO 20 and VENC 4.

Protein is an essential macromolecule in cocoa beans known to play a role in the development of flavour through the Maillard reaction during roasting [49]. It was observed that a roasted sample of a clone was not significantly different from its unroasted sample. The protein content of the CBS is decreased during roasting processes [47]. There was a reduction in the protein content of the CBSs after roasting, except VENC 4. This reduction in the protein content of CBS is as a result of the Maillard reaction, which is triggered by the heat from roasting and the interaction of the carbonyl group of the reducing sugar with the free amino acid from protein [40]. The results showed lower protein content in CBS (roasted and unroasted) than the study by Agus et al. [40], which stated the protein content of 27.43% for unroasted and 25.07% for roasted CBS. Fakhlaei et al. [45] reported 18% for unroasted and 17.17% (at 120 °C) for roasted CBS, which is in the range of the protein content reported in this study. Similarly, the protein content of the roasted CBS was in close agreement with the study by Djali et al. [41] which reported the protein content of roasted CBS in the range of 15.7% to 18.79%. Again, Delgado-Ospina et al. [44] observed a protein value of 15.2% for CBS, which is within the range of our study. There was no significant difference observed in the crude protein content before and after roasting of the cocoa bean in this study.

Carbohydrate is an essential macronutrient in cocoa beans since it is used in fermentation and roasting processes to produce flavour processor and cocoa flavor [50]. The value for carbohydrate the CBS ranged between 38.31% and 52.58% for unroasted VENC 4 and roasted MO 20 respectively. Unroasted VENC 4 was significantly different from the rest of the samples. It was observed that roasting significantly affected the carbohydrate content in the CBS. The carbohydrate content of CBS (roasted and unroasted) in this study was in close agreement with the study by Agus et al. [40], which reported 44.63% and 55.85% for unroasted and roasted CBS, respectively. The carbohydrate content reported in this study is within the range reported by Djali et al. [41]. A carbohydrate content of (14.7%) for CBS was reported by

Delgado-Ospina et al. [44], which is lower compared to the study. Also, Rojo-Poveda et al. [15] reported that carbohydrates comprise 7.85–70.25% of the CBS dry weight. Similarly, Sánchez et al. [47] reported the carbohydrate content of CBS to range from 13.2 to 70.3%. The carbohydrate content of all the samples increased after the roasting process. It has been shown that roasted CBS has more carbohydrate content than unroasted CBS due to the movement of sugars in the CBS toward the outer shell as it roasts [40, 47].

CBS can be utilized in animal feed due to its high energy source [51]. In this study, roasted PA 150 had the maximum energy value (374.46 kcal/100 g), while roasted VENC 4 had the minimum (341.06 kcal/100 g). Unroasted T60/887 × POUND 7 differed from the rest of the samples. Compared to findings by Rojo-Poveda et al. [15] and Adamafio [52] who reported energy content of 122 and 121.81 kcal/100 g, respectively, energy content from the current study were higher.

Table 2 shows the mineral constituent of the roasted and unroasted CBS. CBS is known to have a considerable amount of ash content, which serves as an indicator of the minerals present [42]. VENC 4 had the highest phosphorus content among the unroasted samples, while T60/887 had the lowest. There were significant differences among the unroasted samples except for T60/887 × POUND 7 and PA 150, which were not significantly different. The phosphorus content of the roasted samples ranged between 483.67 mg/g (T60/887) and 1698 mg/100 g (VENC 4). All the roasted samples significantly differed in phosphorus content except for T60/887 × POUND 7 and MO 20. CBS is mainly abundant in minerals such as potassium, calcium, magnesium and phosphorus [15]. The phosphorus content of unroasted T60/887, T60/887 × POUND 7 and roasted T60/887 and PA 150 aligns with the phosphorus content in both the roasted and unroasted CBS than those reported by Soares & Oliveira [43], which ranged between 580 and 1000 mg/100 g. A similar value (0.58–1.00 g/100 g, which is equivalent to 580–1000 mg/100 g) was reported by Rojo-Poveda et al. [15]. However, mineral content in CBS may vary depending on its geographical origin since the absorption of minerals by plants highly depends on the availability of minerals in the soil and, therefore, depends on the type and quality of the soil [53, 54].

Potassium content ranged from 2382 mg/100 g to 3114 mg/100 g, with roasted VENC 4 having the maximum and minimum amounts in roasted PA 150. Roasted VENC 4 was significantly different from all the samples except for unroasted MO 20 and roasted MO 20. Roasted and unroasted PA 150 were significantly different. Also, roasted T60/887 × POUND 7 and unroasted T60/887 × POUND 7 were significantly different. Soares & Oliveira [43] reported lower potassium content of CBS, which ranged between 1250 and 1820 mg/100 g compared to this study.

Calcium content ranged from 673.33 mg/100 g to 1284.67 mg/100 g for unroasted samples, and 661.33 mg/100 g to 1418.67 mg/100 g for roasted samples, exceeding the reported range of 230–440 mg/100 g by Rojo-Poveda et al. [15].

The magnesium content of the unroasted samples was observed to be high in MO 20 and low in PA 150. For the roasted samples, MO 20 had the highest value of magnesium, while T60/887 had the lowest. The magnesium content for roasted and unroasted samples ranged between 233.33 mg/100 g and 551 mg/100 g. Among the unroasted samples, PA 150 and MO 20 were significantly different from each other and the other samples. There was a significant difference among roasted T60/887 × POUND 7, roasted MO 20 and unroasted MO 20. The unroasted samples were significantly different from their roasted counterparts in magnesium content except VENC 4 (Table 2). The magnesium content (480-1290 mg/100 g) reported by Soares and Oliveira [43] was consistent with the magnesium content of this study.

MO 20 recorded the maximum sodium content in the unroasted and roasted CBSs (51.33 and 41.33 mg/100 g respectively). VENC 4 also recorded the minimum in both unroasted and roasted samples (25.67 and 28.33 mg/100 g respectively). Unroasted and roasted MO 20 were significantly different from each other and from the rest of the samples. Soares and Oliveira [43] reported sodium content of 16–192 mg/100 g, which is consistent with findings from this study since values for sodium content for the roasted and unroasted CBS fall within this range.

The highest phenolic content for the unroasted samples was 12.25 mgGAE/g in PA 150, whereas T60/887 recorded the lowest value for phenolic content. For the roasted samples, the phenolic content ranged from 6.34 mg GAE/g to 12.25 mg GAE/g, with VENC 4 having the minimum and PA 150 recording the maximum for the phenolic value. There was no significant difference among roasted VENC 4, roasted and unroasted T60/887. Also, no significant difference was found between unroasted MO 20 and unroasted PA 150. The rest of the samples were significantly different from one another. Barbosa-Pereira et al. [55] reported total phenolic content ranging between 5.8 and 7.5 mg GAE/g in CBS, which is consistent with this study. Similarly, total phenolic content of 7 mg GAE/mg and 10.8 mgGAE/g were reported by Manzano et al. [56] and Delgado-Ospina et al. [55] respectively, which were in the range of the total phenolic content of this study [44, 56]. However, the total phenolic content in the CBS from the present study is lower than 17.2 mg GAE/g [57]. Agus et al. [40] reported decreased total phenolic content from 9.056 µg GAE/ml to 5.947 µg GAE/ml after roasting. This was consistent with all the clones except PA 150 and T60/887 × POUND 7 which increased in total phenolic content after roasting. However, increasing roasting temperature resulted in increased total phenolic and total flavonoid contents of

pumpkin seeds [58]. CBSs from different kinds of cocoa beans show different types and quantities of phenolic compounds depending on their origin and genotype [47].

Flavonoids are important groups of phenolic compounds that have gained research interest because of their bioactive properties, which include anti-inflammatory, anti-oxidant, and cardio-protective effects [59]. Table 4 shows that roasted VENC 4 had the minimum flavonoid content, whereas roasted T60/887 had the maximum flavonoid content. The value ranged between 9.74 mg QE/g and 15.58 mg QE/g. For the unroasted samples, T60/887 × POUND 7 had the minimum flavonoid content of 8.45 mg/g and 40.71 mgQE/g for VENC 4 being the maximum. Significant differences were observed among unroasted PA 150, unroasted MO 20, unroasted VENC 4 and roasted T60/887 × POUND 7. The total flavonoid content in CBS is higher than 1.8–3.6 mg QE/ g [55]. Also, Sánchez et al. [47] reported a range of 1.6–43.9 mg QE/g total flavonoid. However, the flavonoid content falls within this range. Similarly, Delgado-Ospina et al. [44] reported total phenolic content ranging between 1.65 and 40.72 mg QE/g, which aligns with the total flavonoid content in this study.

Linear discriminant analysis (LDA) is a supervised classification approach that utilizes information from a training dataset to accurately assign unknown samples to their appropriate categories [60]. The differentiation stems from the unique biochemical and physical traits displayed by individual groups of CBSs, leading to a clearly evident pattern of separation. This offers additional insights into the detectable absorption bands and their associated chemical constituents [61].

By selecting suitable projection axes in LDA, similar samples are positioned close to each other in the projections, while samples from different classes are maximally separated. Consequently, the classification of each sample is based on its proximity to the projection points of other samples [62]. Specifically, PA 150 and T60/887 exhibited a closer relationship compared to the other clones, as PA 150 fell within the cluster associated with T60/887. In contrast, VENC 4 and MO 20 were notably distant from each other on the graph, indicating minimal to no similarities between them. NIR pre-treatment is usually performed using the Savitzky-Golay filter to eliminate undesirable noise issues and overlapping spectra, thereby increasing the reliability of the results [63].

Figure 3 presents the outcomes of Linear Discriminant Analysis (LDA) applied to the five roasted CBS clones. Following the roasting process, an interesting pattern emerged. VENC 4 and PA 150 displayed substantial similarities, as indicated by their close clustering in the analysis. Additionally, there was an overlap between the characteristics of T60/887 × POUND 7 and PA 150. In contrast, T60/887 stood apart significantly from the rest of the CBS clones. Notably, roasting had a limited impact on MO 20, as its characteristics remained largely unaffected, as shown in Fig. 3.

The result from the NIR showed that similar samples were positioned close to each other on the graph, implying they are similar in their biochemical and physical traits (Fig. 3). The unroasted PA 150 and T60/887 CBSs were notably close to each other on the graph. Comparing this to the nutritional and bioactive analysis shows a minimal significant difference between the unroasted PA 150 and unroasted T60/887. However, after roasting the samples, it was observed that all the samples were distinct. Roasting, therefore, affected the biochemical and physical composition of the CBS. PA 150 and T60/887 were notably distant from each other, indicating minimal to no similarities between them after the roasting process. These changes were also observed in the nutritional and bioactive analysis. The nutritional and bioactive analysis showed a significant difference between the roasted PA 150 and the roasted T60/887. These findings are consistent with those reported in other studies where NIR was used to accurately predict the presence of bioactive compounds, including polyphenols, anthocyanins, carotenoids and ascorbic acid [21]. For example, NIR was used to accurately predict the presence of total polyphenols in cocoa beans produced from pods stored for different days with the beans fermented for different periods [64]. Similarly, the presence of bioactives from cocoa bean husk, including theobromine and total phenols, was accurately predicted [65].

6 Conclusions

Roasting of the five clones of CBS resulted in varied nutritional and bioactive composition. Roasted VENC 4 had the highest ash, protein, fibre and low moisture content compared to the other clones. Roasted PA 150 recorded the highest energy and phenolic content. The highest flavonoid content was observed in unroasted VENC 4. The CBS was rich in potassium and phosphorus and low in sodium. Roasted VENC 4 was high in potassium and low in sodium. CBS can, therefore, be recommended as an ingredient in functional foods due to its rich bioactive content. Moreover, it can have beneficial applications in food products for people living with high blood pressure, given its potassium and low magnesium content. Also, the use of NIRS shows noteworthy efficacy in distinguishing between different clones of CBSs and

detecting differences between roasted and unroasted CBSs. To ensure better differentiation of cocoa beans from different clones using near infrared (NIR) spectroscopy, it is recommended to incorporate a roasting process.

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Declarations

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