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Effects of roasting temperature on the properties of cocoa (*Theobroma cacao* L.) bean shell wastes

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Abstract

This study aimed to evaluate the microbial, physical, and chemical properties of cocoa bean shell (CBS), a byproduct from cocoa processing, for its possible use as a food ingredient. CBS was obtained from cocoa beans that were roasted at various temperatures (110°C, 115°C, 120°C, 125°C, 130°C) for approximately 12–15 min. Quantification of microbial load, proximate composition, bioactive profile, antioxidant activity, and phenolic compounds was conducted to evaluate the quality of CBS. Results showed that total plate, yeast and mold, and coliform counts of CBS were found to be within the acceptable levels. Color measurements based on the L*, a*, and a*/b* were not significantly (p<0.05) altered between treatments. Proximate analysis revealed that crude ash and crude protein levels significantly (p<0.05) increased, while moisture and crude fiber content significantly (p<0.05) decreased at elevated temperatures. In addition, total flavonoids (TFC), total anthocyanins (TAC), and total phenolic (TPC) contents were significantly (p<0.05) reduced with increasing temperatures. Values obtained for the antioxidant activities peaked after being roasted at 115-125°C and significantly (p<0.05) decreased after being roasted at 130°C. Individual phenolics were also determined by high -performance liquid chromatography, and isolated predominant compounds isolated in CBS were identified to be catechin, epicatechin, and syringic acid. Results showed that at higher roasting temperatures, catechin and syringic acid increased, while epicatechin was reduced. Overall, this study demonstrated that extremely high roasting temperatures cause a deteriorative effect on the functional compounds present in CBS.

1. Introduction

Cocoa (*Theobroma cacao* L.), a crop of significant economic value, serves as the primary component in chocolate production. The International Cocoa Organization (ICCO) (2023) estimated that the production of cocoa (dried beans with pulp) for 2022/23 was around 4938 thousand metric tons, marking a 2.3% increase from the 4826 thousand metric tons produced in 2021/22. The diverse use of cocoa beans in industries such as manufacturing, pharmaceuticals, and cosmetics fuels the global demand for these beans. The worldwide demand for cocoa beans grows at a rate of 3% per year, while in China and India, this rate is significantly higher at 7.9% annually (Department of Agriculture, 2021). The consistently growing global demand and production of cocoa result in the rise of byproducts generated during cocoa processing. These byproducts include cocoa bean shells, pod husks, and mucilage, which represent about 70–80% of the fruit (Panak Balentić *et al*., 2018). The utilization of these byproducts as potential sources of bioactive compounds and dietary fiber is essential for producing functional food ingredients and promoting sustainable development.

Cocoa bean shell (CBS) is a processing byproduct during the manufacture of chocolate, which is recognized to contain nutritionally beneficial compounds (Manzano *et al*., 2017; Panak Balentić *et al*., 2018; Pavlović *et al*., 2019; Netania *et al*., 2022; and Soares and Oliveira, 2022). CBS is rich in polyphenolic compounds that are

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known to reduce the risk of cardiovascular, cognitive, and other chronic diseases (Martin and Ramos, 2021). Recently, the isolation and incorporation of these compounds into various products have been investigated in several studies. Reports from the literature have indicated that CBS was utilized to fortify extruded snacks, biscuits, and beverages due to its high dietary fiber content and antioxidant activity (Rojo-Poveda *et al*., 2019). The applications of polyphenolic compounds and the dietary fiber of CBS in different industries have been well explored. However, the quantity of these compounds in CBS is significantly altered by processing methods such as fermentation, drying, and roasting (Wollgast and Anklam, 2000; Razola-Díaz *et al*., 2023).

Cocoa beans undergo several processing steps before their consumption or further processing for food applications as an ingredient. Roasting in particular is essential because it promotes desirable changes such as the enhancement of the cocoa beans' characteristic chocolate flavor and aroma, dehydration, and substantial reduction of microbial load, acidity, and astringency of the bean (Peña-Correa *et al*., 2022). The roasting process also affects concentrations of certain macro- and micronutrients because of their volatile characteristic or their involvement in Maillard reactions initiated due to high-temperature processing. Furthermore, roasting produces high molecular weight compounds such as melanoidins and low molecular weight compounds with odor activity (Quiroz-Reyes and Fogliano, 2018). Several studies discussed the effect of roasting on the physicochemical characteristics and bioactive properties of cocoa beans (Peña-Correa *et al*., 2022). However, the effect of roasting on the physical and chemical composition, bioactive profile, and antioxidant properties of CBS has not yet been explored. Although roasting enhances the overall sensory characteristics of the bean, it could potentially destroy a high fraction of phenolic compounds due to high-temperature processing. Therefore, this study aimed to determine the effect of varying roasting temperatures during the processing of cocoa beans on the microbial, proximate composition, bioactive profile, and antioxidant capacity of CBS.

2. Materials and methods

2.1 Sample preparation

The preparation of cacao beans for roasting was done according to the process of Malagos-Agri Ventures Corporation (Davao, Philippines). Naturally fermented and dried cacao beans were roasted at various temperatures (110°C, 115°C, 120°C, 125°C, 130°C) for 12-15 min. Then, the CBS were manually separated from the beans, ground, placed in tubes, wrapped with aluminum foil, and stored in a freezer (-20°C) until further use.

2.2 Microbiological analysis

Aerobic plate count was determined using plate count agar and incubated at 35°C for 48 hs (Maturin and Peeler, 2001). The coliform count was enumerated using the violet, red bile agar incubated at 35°C for 24 hs (Feng *et al*., 2020). Yeast and mold count was determined using potato dextrose agar acidified with 10% tartaric acid. The plates were incubated at 25°C for 5 days. The diluent used for all the analyses was 0.1% peptone water (Tournas *et al*., 2001).

2.3 Proximate analysis

Proximate composition was determined according to the procedures of the AOAC INTERNATIONAL (2000). Specifically, moisture was determined by oven drying (AOAC Official Method 934.01), ash by muffle furnace incineration (AOAC Official Method 923.03), crude fat by Soxhlet extraction (AOAC Official Method 920.39), crude protein by the Micro-Kjeldahl method (AOAC Official Method 960.52), and crude fiber by acid -alkali digestion (AOAC Official Method 962.09). Nitrogen-free extract (NFE) was calculated by difference.

2.4 Water activity

CBS samples were placed in a round container for water activity determination using the Novasina Labswift^{TM} portable water activity analyzer.

2.5 Color

Color evaluation was measured using the Chromameter (Konica Minolta, Japan). The color was expressed in CIELAB color values (L*, a*, b*) where the L-value represents the lightness to darkness, the a-value represents the greenness to redness, and the b-value represents the blueness to yellowness of CBS samples. ΔE reflects the color change of a sample from the reference. It was calculated based on the equation:

$$\Delta E^* = \sqrt{((L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2)}$$

The values with subscript 2 are the color values obtained for the target sample, while those with subscript 1 are color values obtained for the reference sample.

2.6 Bioactive compounds

2.6.1 Sample extraction

Sample extraction was conducted according to Larrauri *et al* (1997) with minor modifications. Two grams of ground and sieved sample were mixed with 60 mL of a methanol:water:acetic acid solution (50:50:1 v/v/v) and placed in a shaker (Benchmark Scientific, Inc., U.S.A.) for 1 h at 3000 rpm at room temperature. Then,

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it was centrifuged at 3000 rpm for 15 min. The supernatant was filtered using Whatman® No. 1 filter paper and stored at -20°C until use. The quantification of bioactive compounds (phenolics, monomeric anthocyanin, and flavonoid content) and antioxidant properties (ABTS, DPPH, and FRAP) was carried out using a UV-vis spectrophotometer (Shimadzu Corp., Japan).

2.6.2 Total phenolic content

The total phenolic content (TPC) was measured by Folin-Ciocalteu's method following ISO 14502-1 with minor modifications. Approximately 0.15 mL of diluted CBS extract was added with 0.75 mL of 10% Folin-Ciocalteu reagent and 0.60 mL of 4% Na_2CO_3. The mixture was then allowed to stand at room temperature for 1 h in a dark room. After incubation, absorbance was recorded at 765 nm. Gallic acid in methanol was used as a standard with a curve plot ranging from 0–150 mg/L (R^2 = 0.9947). Results were expressed as milligrams gallic acid equivalents per gram dry weight (mg GAE/g DW).

2.6.3 Total monomeric anthocyanin content

The total monomeric anthocyanin content (TAC) was analyzed using the pH differential method based on Lee *et al.* (2005) with minor modifications. TAC was quantified by diluting CBS extracts in buffers of potassium chloride (pH = 1.0) and sodium acetate (pH = 4.5). The absorbance of the extract was read at 520 nm and 700 nm against a blank (distilled water). Results were expressed as milligrams of cyanidin-3-glucosidase equivalents per gram dry weight (mg C3GE/g DW) and calculated using the following equation:

$$TAC, \left(mg\frac{C3GE}{g\ DW}\right) = \frac{\Delta A \times MW \times df \times 10^3 \times 100}{\varepsilon \times l \times 1}$$

Where: $\Delta A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$; MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol), df = dilution factor, ε = extinction coefficient of cyanidin 3-glucoside (26,900 L × mol^{-1} × cm^1), l is the cell path length (1 cm), and 10^3 is the factor conversion from g to mg.

2.6.4 Total flavonoid content

The total flavonoid content was measured using the flavonoid-aluminum chloride complexation method according to Fattahi *et al.* (2014) with minor modifications. Approximately 0.05 mL of diluted CBS extract was added with 2 mL of distilled water and 0.150 mL of 5% $NaNO_2$. After 5 min, 0.150 mL of 10% $AlCl_3$ was added, left to stand again for 6 min, and then 1 mL 1M NaOH and 1.65 mL distilled water. After thorough mixing, the sample absorbance was read at 510 nm. Catechin in absolute methanol was used as a standard with a curve plot ranging from 0–400 mg/L (R^2 = 0.9902). Results were expressed as milligrams of catechin equivalents per gram dry weight (mg CE/g DW).

2.7 Antioxidant properties

2.7.1 DPPH scavenging activity assay

The DPPH scavenging activity (DPPH) was conducted according to Pisoschi and Negulescu (2012) with minor modifications. CBS extract was added to the DPPH solution (1:1 v/v) and was left to stand in a dark room for 30 min at room temperature. The absorbance of the extracts was read at 517 nm. Trolox in methanol was used as a standard with a curve plot ranging from 0-12 mg/L (R^2 = 0.9947). Results were expressed as milligrams of Trolox equivalents per gram dry weight (mg TE/g DW).

2.7.2 Ferric reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) was carried out according to Tomasina *et al.* (2012) with modifications. Approximately 0.15 mL diluted CBS extract was added to 1.35 mL FRAP reagent diluted in 50% ethanol. The absorbance of the extracts was read at 620 nm. Trolox in ethanol was used as a standard with a curve plot ranging from 0-40 mg/L (R^2 = 0.9923). Results were expressed as milligrams of Trolox equivalents per gram dry weight (mg TE/g DW).

2.7.3 ABTS scavenging activity

ABTS scavenging activity (ABTS) was carried out according to the method used by Zubia *et al.* (2023) with modifications. Approximately 0.15 mL diluted CBS extract was added to 1.35 mL ABTS diluted ABTS solution and placed in a dark room for 15 min at room temperature. The absorbance of the extracts was read at 734 nm. Trolox in methanol was used as a standard with a curve plot ranging from 0-40 mg/L (R^2 = 0.9981). Results were expressed as milligrams of Trolox equivalents per gram dry weight (mg TE/g DW).

2.8 Quantification of phenolic compounds by High performance liquid chromatography

Extracts were filtered through a 25 µm syringe filter and diluted to a 1:8 (sample extract:distilled water) ratio. Sample extracts were run on a Shimadzu Prominence (Shimadzu Corp., Japan) with an LC-20 AD pump with DGU-20A5R degasser, Sil-20AHT UFLC autosampler, SPD-M20A diode array detector, and Lab Solution software. Filtered solvents were placed in 1.5 mL HPLC vials in the autosampler. An approximate volume of 20 µL was injected into the reverse-phase HPLC system.

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Separation was obtained using an Inertsil ODS-3 (250 mm × 4.5 mm × 5 µm) reverse phase column protected with an Inertsil ODS-3 (4.0 mm × 10 mm × 5 µm) guard column. The column was kept at 30°C using a CTO-10ASVP column oven and maintained at a flow rate of 0.8 mL/min. Solvents used were A [2% Acetonitrile (ACN):98% distilled water acidified with acetic acid (HOAc)] and B (98% ACN:2% distilled water acidified with HOAc) using a gradient elution system consisting of time 0 min, 10% B; 3 min, 10% B; 6 min 30% B; 8 min, 30%B; 12 min, 50% B; 17 min, 50% B; 25 min, 90% B; 35 min, 90% B; and 45 min, 10% B (Flandez *et al*., 2023). Detection was done at 275 nm.

2.9 Statistical analysis

Data are presented as the mean ± standard deviation (n=3). Values obtained were evaluated for statistical difference using one-way analysis of variance (ANOVA), and Tukey's honest significant difference (HSD) was done as the post-hoc analysis. The significant difference was evaluated at a 95% confidence level using Statsoft STATISTICA 10 and Minitab Statistical Software 19 (Minitab LLC, USA). Principal component analysis (PCA) was performed to create a graphical representation of the correlation of the bioactive profile, antioxidant capacity, and quantified phenolic compounds of CBS with each other. The correlation matrix, scree plot, and PCA biplot were generated using R Studio version 4.3.2.

3. Results and discussion

3.1 Microbiological analysis

Table 1 presents the microbiological analysis results of various CBS samples collected from cocoa beans roasted at different temperatures (110°C, 115°C, 120°C, 125°C, 130°C). The highest total plate count (TC) and yeast and mold count (YMC) were observed at a roasting temperature of 110°C. As the roasting temperature increased, a decreasing trend in both YMC and TC was noted. This aligns with the findings of Gutiérrez (2017), who reported a significant reduction in microbial load due to high temperature exposure and dehydration of CBS. Additionally, no coliforms were detected in CBS at any roasting temperature, which can be attributed to the slightly acidic nature of the CBS samples (Oracz *et al*., 2019; Pavlović *et al*., 2019; Bobiles *et al*., 2022). These results conform to the standards for cocoa powder outlined in the "Guidelines on the Microbiological Requirements and Assessment of Certain Prepackaged Processed Food Products" set by the Philippine Food and Drug Administration (2022). Moreover, Table 1 shows that the microbial load of CBS obtained from cocoa beans roasted at 120°C and higher was within acceptable limits according to the standards set by the Philippine Food and Drug Administration (2022). Roasting at higher temperatures effectively reduces the microbial load of CBS. However, it may also impart an undesirable scorched or burnt flavor. Therefore, it is crucial to control the roasting temperature of cocoa beans to preserve their organoleptic properties.

3.2 Proximate composition

Table 2 presents the proximate composition and physicochemical properties of CBS from cocoa beans processed at different roasting temperatures. Moisture content generally decreased as roasting temperatures increased, except at 115°C and 120°C. Higher roasting temperatures trigger moisture evaporation, as evidenced by the observed trend. The values obtained in this study ranged from 4.04-6.27%, consistent with the results (3.6-7.8%) reported by Serra Bonvehí and Ventura Coll (1999). Additionally, the moisture content values were within the standard limits for cocoa powder (below 7.50%) to ensure stability during storage (Codex Alimentarius Commission, 2022).

The crude protein content of CBS showed a general increasing trend with elevated cocoa bean roasting temperatures, except between 115°C and 120°C. This aligns with the findings of Djali *et al*. (2023), who reported that roasted CBS samples have 50% higher crude protein compared to their unroasted counterparts. The increase in crude protein content can be explained by the study conducted by Pätzold and Brückner (2006), which observed the racemization mechanism and

Table 1. Microbiological quality of cocoa bean shell waste from cocoa beans processed under different roasting temperatures.

Parameters	Standard		CBS processing temperature, °C				
	m	M	110	115	120	125	130
Total plate count, CFU/g	104	106	7.53×10^6	1.59×10^6	2.63×10^5	$<2.5\times10^4$	3.67×10^5
Coliform count, CFU/g	<10	102	<10	<10	<10	<10	<10
Yeast and mold count, CFU/g	102	104	1.6×10^2	$<1.5\times10^2$	$<1.0\times10^2$	<1.0×10	<1.0×10

Values are presented as mean from 3 replicates. M – Level which when exceeded in one or more samples would cause the lot to be rejected as this indicates potential health hazard or imminent spoilage; m – acceptable level of microorganisms determined by a specific method; the values are generally based on levels that are achievable under GMP ("Guidelines on the Microbiological Requirements and Assessment of Certain Prepackaged Processed Food Products" |Philippine Food and Drug Administration, 2022).

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Table 2. Proximate composition and physicochemical profile of cocoa bean shell from cocoa beans processed under different roasting temperatures.

Parameters	CBS processing temperature, °C				
	110	115	120	125	130
Moisture, g/100 g	6.27±0.04^{a}	5.77±0.02^{b}	5.67±0.12^{b}	4.88±0.04^{c}	4.04±0.21^{d}
Ash, g/100 g	4.57±0.16^{c}	6.53±0.46^{b}	7.24±0.14^{b}	7.20±0.50^{b}	8.67±0.26^{a}
Crude protein, g/100 g	14.4±0.26^{c}	16.93±0.88^{b}	16.11±0.34^{b}	18.72±0.6^{a}	19.97±0.09^{a}
Crude fiber, g/100 g	15.69±0.28^{a}	15.59±0.37^{ab}	14.42±0.74^{b}	12.54±0.29^{c}	11.88±0.49^{c}
Crude fat, g/100 g	47.00±0.35^{a}	28.44±0.76^{b}	25.49±0.07^{c}	26.00±0.02^{c}	28.32±0.82^{b}
Carbohydrate, g/100 g	12.12±0.20^{c}	26.74±1.67^{b}	31.06±0.69^{a}	30.66±0.93^{a}	27.10±0.73^{b}
Aw	0.46±0.00^{b}	0.42±0.00^{c}	0.50±0.00^{a}	0.41±0.00^{cd}	0.40±0.01^{d}
Color analysis					
L*	47.77±0.55	48.64±1.68	47.04±0.94	46.72±2.76	44.90±0.73
a*	7.58±0.90	13.52±5.87	10.80±0.14	10.98±1.21	9.79±0.19
b*	8.10±0.34^{b}	11.72±0.36^{ab}	12.10±0.39^{ab}	13.94±3.35^{a}	10.09±0.19^{ab}
b*/a*	1.07	0.87	1.12	1.27	1.03
a*/b*	0.94	1.15	0.89	0.79	0.97
ΔE*	5.19	3.18	0	1.88	3.1

Values are presented as mean ± SD (n=3) and evaluated using one-way ANOVA. Significant differences among the means were determined using Tukey's HSD test using STATISTICA 10 software. Values within the same row with different superscripts are significantly different from each other at $p<0.05$.

identified a small percentage of protein called D-amino acids that increase upon roasting. D-amino acids are products generated from the Amadori rearrangement, representing stable intermediates formed during the Maillard reaction.

The crude fiber content showed significant differences ($p<0.05$) across the treatments at various roasting temperatures. The highest crude fiber content was observed at roasting temperatures of 110°C and 115°C, followed by CBS from cocoa beans roasted at 120°C. The lowest values were recorded for CBS from cocoa beans roasted at 125°C and 130°C. These results demonstrated a clear decreasing trend in crude fiber content, as indicated by the distinct groupings. This trend can be due to the redistribution of insoluble fibers present in the CBS and the possible solubilization of these non-starch polysaccharides at higher roasting degrees, transforming them into their soluble counterparts (Panak Balentić *et al*., 2018). This result aligns with Fakhlaei *et al*. (2020), who determined the effect of roasting conditions up to 150°C and found a significant reduction in crude fiber between unroasted cocoa beans (64.35%) and roasted cocoa beans up to 130°C (32.01%). Furthermore, the results obtained in this study are closely related to those reported by Agus *et al*. (2018) (16.06%) for cocoa shells at all roasting temperatures.

The highest fat content was observed at the lowest roasting temperature applied (110°C). This could be due to the incomplete separation of the nibs and the CBS. Interestingly, higher roasting temperatures facilitate the separation process of the nibs from the shells, a natural characteristic of the material. Additionally, this study recorded higher CBS fat content at all roasting temperatures compared to the fat content (1.5-8.49%) reported by Rojo-Poveda *et al*. (2020). According to Oracz and Nebesny (2019), fat loss in cocoa beans can be attributed to the generation of lipid-derived and lipid-oxidation byproducts, resulting in approximately 3.8% fat loss. However, it is also important to consider the migration of fat from the nib to the shell during the roasting process (Fakhlaei *et al*., 2020). High roasting temperatures can simultaneously melt the fats in the nibs and create porous gaps in the membranes of cocoa bean shells, providing pathways for fat migration, which may lead to increased fat content in the shells (Peña-Correa *et al*., 2022).

The ash content of CBS exhibited a significant ($p<0.05$) increasing trend with cocoa bean roasting temperatures. This is consistent with the results obtained by Agus *et al*. (2018), where roasted samples showed higher ash content compared to their unroasted counterpart. The same was observed in a study by Djali *et al*. (2023), where they observed that unfermented and unroasted samples have lower ash contents. The increase in crude ash content of CBS can be attributed to the evaporation of moisture, leading to a reduction in the overall mass of the CBS and the reduction of its organic components, retaining the inorganic minerals (Putri *et al*., 2023). Roasting can also reduce the antinutrient compounds present in CBS during roasting, further

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increasing the concentration of retained inorganic compounds in the CBS, which is reflected by the higher ash content at elevated temperatures (Djali *et al*., 2023).

The highest carbohydrate content was recorded in the CBS processed at 120°C. Increasing the temperature showed a significant (p<0.05) rise in carbohydrates, peaking at 120°C and 125°C. This is consistent with the findings of Agus *et al*. (2018) and Djali *et al*. (2023), who reported higher carbohydrate levels in fermented and roasted samples compared to plain cocoa beans. However, the carbohydrate content of CBS decreased after roasting at 130°C. This decrease can be attributed to the production of Maillard reaction products, which alter the properties of sugars present in CBS (Oracz and Nebesny, 2019). Additionally, the roasting process can degrade carbohydrates and produce volatile byproducts, further reducing the carbohydrate content in CBS (Putri *et al*., 2023).

The water activity of CBS samples ranged from 0.40 –0.50. The highest water activity was observed at 120°C (0.50), followed by 110°C (0.46). The lowest water activity was recorded at 130°C (0.40), which was not significantly different (p<0.05) from 125°C (0.41). This aligns with the findings of García-Alamilla *et al*. (2017), who demonstrated that roasting temperature and time significantly affect water activity and moisture content. Exposure of CBS samples to higher roasting temperatures dehydrates the free water in their matrix, resulting in lower moisture and water activity. The water activity values obtained at all temperatures are within the standard for cocoa powder (less than 0.60) (ADM Cocoa, 2009; Codex Alimentarius Commission, 2022).

3.3 Color

Color is an important physical aspect in determining the quality of CBS during processing. Polyphenolic compounds and anthocyanins formed during the fermentation process contribute to the characteristic brown color of the CBS (Krysiak *et al*., 2013). During roasting, the main factors affecting the degree of change in the browning of cocoa beans are temperature and duration of heating (Krysiak, 2006; Krysiak *et al*., 2013).

The measurement of color was done using the CIE L*a*b* method, where L* values indicate the lightness or darkness, a* values indicate the greenness to redness, and b* values indicate the blueness to yellowness of the sample (Zyzelewicz *et al*., 2014). The L* and a* values of the CBS samples showed no significant (p<0.05) difference as the roasting temperature increased. Meanwhile, the highest b* value was recorded for CBS samples from cocoa beans roasted at 125°C, which is significantly (p<0.05) different from that roasted at 110°C and 130°C. The roasting temperatures used in this study provide beneficial changes in the CBS samples since they improve the color by providing a deeper brown color upon increased roasting temperature. However, Nebesny and Rutkowski (1998) found that increasing the roasting temperature above 135°C will initiate burning that leads to the destruction of organoleptic properties such as flavor, aroma, and color.

The ΔE values of each sample were calculated based on the 120°C reference since it is the current roasting temperature applied to the cocoa beans processed by the industry. The ΔE values are categorized as not perceptible by human eyes (<1), perceptible through close observation (1-2), perceptible at a glance (2-10), colors are more similar than opposite (11-49), and colors are exactly opposite (100). In this study, the ΔE values are all greater than 1, which means there are perceivable changes in color from the standard reference. On the other hand, a*/b* and b*/a* ratios obtained in this study were not significantly (p<0.05) different as the roasting temperature increased. A high ratio of a*/b* indicates a measure of the deepness of orange to red color in samples, whereas the ratio of b*/a* indicates a weaker brown-orange color. Overall, the roasting process had no notable effect on the a*/b* and b*/a* ratios, suggesting no significant changes in color depth at temperatures between 110-130°C.

3.4 Bioactives profile and antioxidant properties

Phenolic compounds found in CBS are reported to have several health benefits and mainly contribute to its antioxidant properties. The quantity of phenolics isolated from CBS can be affected by several factors, such as variety (Borja Fajardo *et al*., 2022), fermentation process (Taranto *et al*., 2017), roasting or drying (Valadez-Carmona *et al*., 2017), and other geographical factors (Rojo-Poveda *et al*., 2021). Table 3 shows the bioactive profile and antioxidant activities of CBS as affected by cocoa bean roasting temperature.

3.4.1 Bioactives profile

The highest total phenolic content (TPC) was obtained for samples processed under 120°C (168.73 mg GAE/g DW), which is significantly (p<0.05) different from samples processed under 110°C and 130°C. It can also be observed that CBS samples from cocoa beans processed under 115°C, 120°C, and 125°C showed no significant (p<0.05) difference. The slight increase in TPC from CBS roasted at 115-125°C from 110°C agrees with the finding reported by Oracz and Nebesny (2016) for the cultivar *Trinitario* from Papua New Guinea, where unroasted samples displayed the lowest TPC. This can be attributed to the degradation of cellular structure

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Table 3. Bioactive compound and antioxidant properties of cocoa bean shell waste from cocoa beans processed at different roasting temperatures.

Parameters	CBS processing temperature, °C				
	110	115	120	125	130
TPC, mg GAE/g FW	141.24±0.37^{c}	168.13±2.33^{a}	168.73±0.95^{a}	164.22±2.90^{a}	155.71±1.16^{b}
TAC, mg cyanidin-3-glucoside eq/ g FW	0.0214±0.0007^{a}	0.0095±0.0007^{b}	0.0010±0.0000^{c}	Below detectable limit	Below detectable limit
TFC, mg catechin eq/g FW	21.41±0.82^{a}	17.85±1.25^{ab}	15.64±0.93^{b}	11.29±0.95^{c}	12.40±0.82^{c}
DPPH, mg TE/g FW	5.65±0.09^{ab}	5.85±0.09^{a}	5.58±0.07^{b}	5.70±0.06^{ab}	5.71±0.12^{ab}
FRAP, mg TE/g FW	34.83±0.34^{b}	37.09±1.25^{ab}	35.20±3.04^{ab}	39.19±1.43^{a}	34.05±0.42^{b}
ABTS, mg TE/g FW	41.26±0.13^{b}	45.57±1.42^{a}	41.99±1.23^{b}	45.54±0.27^{a}	35.06±0.34^{c}

Values are presented as mean ± SD (n=3) and evaluated using one-way ANOVA and significant differences among the means were determined using Tukey's HSD test using Statsoft STATISTICA 10 software. Values within the same row with different superscripts are significantly different at $p<0.05$.

*Bioactive Compounds: TPC – Total Phenolics Content; TAC – Total Anthocyanin Content; TFC – Total Flavonoids Content

*Antioxidant Activity: FRAP – Ferric Reducing Antioxidant Power; DPPH and ABTS radical scavenging assay TE – Trolox Equivalent; GAE – Gallic Acid Equivalent; FW – Fresh Weight.

during heat treatment that results in the release of bound phenolic compounds (Oracz *et al*., 2015; Quiroz-Reyes and Fogliano, 2018; Wu *et al*., 2022).

The total phenolic content (TPC) of cocoa bean shells (CBS) peaked at 120°C, followed by a decreasing trend from 120 to 130°C. Roasting generally decreases the TPC of samples analyzed, and the extent of loss depends on the temperature applied (Payne *et al*., 2010). This decrease can be attributed to the Maillard reaction and oxidation of phenolic compounds, including their interactions to form high molecular weight compounds, which significantly reduce their content and directly affect antioxidant capacity (Bordiga *et al*., 2015).

A significant decrease in total monomeric anthocyanin (TAC) is observed as the cocoa bean processing temperature increases. This decrease becomes particularly noticeable at roasting temperatures of 125°C and 130°C, where TAC levels in CBS samples are almost negligible. This suggests that higher roasting temperatures lead to the degradation of TAC in CBS samples. This trend can be attributed to the conversion of anthocyanin into brown pigments, a result of the Maillard reaction combined with protein degradation, oxidation, and polyphenol polymerization, as reported by Bordiga *et al*. (2015). Furthermore, the low levels of anthocyanin observed in this study can be attributed to the fermentation process, which involves the formation of enzymes such as polyphenol oxidase and peroxidase that break down anthocyanin into smaller compounds (Quiroz-Reyes and Fogliano, 2018; Li *et al*., 2022; Djali *et al*., 2023). Bordiga *et al*. (2015) also noted that anthocyanins are particularly scarce in cocoa samples and further degrade during fermentation and drying.

A significant ($p<0.05$) decreasing trend in total flavonoid content (TFC) of CBS was observed as roasting temperature increased. This aligns with the findings of Zzaman and Al-din Sifat (2023), who reported that roasting significantly decreased TFC in CBS. This decreasing trend can be attributed to the oxidation or degradation of flavanols during roasting (Zzaman *et al*., 2017). In their study, Zzaman and Al-din Sifat (2023) measured the TFC of cocoa beans categorized as raw, roasted using superheated steam, and roasted using the conventional method. The highest TFC was found in raw cocoa beans (8.33 mg/100 g), followed by beans roasted with superheated steam (7.14 mg/100 g) and those roasted conventionally (5.14 mg/100 g). Additionally, Oracz *et al*. (2015) have observed that the individual and total flavan-3-ols content of five cocoa types from Brazil were significantly decreased at roasting temperatures 110–150°C, including epicatechin, which is the predominant flavonoid in cocoa beans.

3.4.2 Antioxidant activity

The highest DPPH values were observed for CBS from cocoa beans processed at 115°C, 125°C, and 130°C. Generally, as the processing temperature increases, DPPH values are expected to decrease. However, the observed increase in DPPH values with higher roasting temperatures may be due to the formation of reducing substances during thermal degradation, which degrade at higher temperatures and more intense roasting conditions (Summa *et al*., 2006). This result is in agreement with the findings of Tomaino *et al*. (2005), where roasting up to 120°C initially increased the DPPH values of spice essential oils.

Similar to the DPPH assay, the highest ABTS values were obtained for CBS from cocoa beans roasted at 115°C and 125°C, with no significant difference between these values ($p<0.05$). The increase in ABTS values up to 115°C, followed by a decrease at higher temperatures,

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may be due to the combined contributions of melanoidins, low molecular weight-reducing substances, and polyphenolic compounds (Jung *et al*., 2021; Wu *et al*., 2022).

The highest FRAP value was recorded for CBS samples from cocoa beans roasted at 125°C, which are significantly different (p<0.05) from samples processed at 110°C and 130°C. Similar to DPPH, Tomaino *et al* (2005) also reported an increase in FRAP at roasting temperatures up to 120°C, and a reduction when roasted at 180°C for spice essential oils. This pattern is also seen in other studies, such as the work by Delgado-Ospina *et al*. (2020) on cocoa beans, where moderate (120°C) roasting increased FRAP values compared to very dark roasted or unroasted samples.

3.5 Quantification of phenolic compounds by High-performance liquid chromatography

Table 4 presents the effect of increasing cocoa bean roasting temperatures on the catechin, epicatechin, and syringic acid content of CBS. Catechin content of CBS increased with elevated roasting temperatures up to 130°C. This can be due to the thermal processing-induced epimerization of cis-configured catechin into its trans-configured form (Quiroz-Reyes and Fogliano, 2018; Goya *et al*., 2022). However, Goya *et al*. (2022) reported that with exposure at 150°C for 20 min, trans-configured catechin reaches a plateau and begins to degrade. This suggests that while heat can initially increase catechin levels, excessive or prolonged heat can lead to its degradation.

The level of epicatechin in CBS decreases as cocoa bean roasting temperatures increase. This decrease can be attributed to the epimerization of cis-configured epicatechin into trans-configured catechin during thermal processing. Catechin and epicatechin are stereoisomers, and the epimerization process explains the effect of roasting based on the decrease in epicatechin and the corresponding increase in catechin (Quiroz-Reyes and Fogliano, 2018; Delgado-Ospina *et al*., 2020; Goya *et al*., 2022). The study by Delgado-Ospina *et al.* (2020) and Kongor *et al.* (2016) further supports this, indicating that cis-configured catechin, which includes epicatechin, consistently decreases as temperature (>120°C) rises.

Table 4. Catechin, Epicatechin, and Syringic acid of CBS from cocoa beans at different roasting temperatures.

Roasting Temperature, °C	Catechin mg/g FW	Epicatechin mg/g FW	Syringic acid mg/g FW
110	8.11±0.08^{d}	0.8851±0.01^{a}	0.1091±0.00^{b}
115	10.23±0.03^{c}	0.6905±0.00^{b}	0.1235±0.00^{ab}
120	10.77±0.02^{b}	0.5970±0.02^{c}	0.1303±0.00^{ab}
125	10.75±0.03^{b}	0.4955±0.02^{d}	0.1207±0.00^{ab}
130	11.05±0.02^{a}	0.4733±0.01^{d}	0.1409±0.00^{a}

Data are presented as mean ± SD (n=3) and evaluated using one-way ANOVA. Significant differences among the means were determined using Tukey's HSD test using Minitab 19 statistical software. Values within the same column with different superscripts are significantly different from each other at p<0.05.

The amount of syringic acid in CBS increased as the roasting temperature rose to 130°C. This increase is likely due to the breakdown of larger phenolic compounds, which transform into smaller compounds like syringic acid (Mattioli *et al*., 2020). Roasting can also degrade tannins and other phenolic precursors, further contributing to syringic acid accumulation. This finding is consistent with the study by Wu *et al*. (2022), which showed a significant increase (p<0.05) in syringic acid under light (<210°C) to medium (250°C) roasting conditions for coffee beans. These results suggest that roasting temperature plays a key role in enhancing syringic acid content in both CBS and coffee beans. However, the optimal roasting conditions may vary depending on the type of bean and the desired outcome.

3.6 Principal component analysis

Principal component analysis (PCA) was applied to identify the primary characteristics of bioactive compounds in CBS samples by converting correlated variables into distinct principal components for easier interpretation. Figure 1 displays the PCA correlation matrix, scree plot, and biplot, offering a comprehensive visualization of how the bioactive compounds and antioxidant capacities of CBS responded to elevated roasting temperatures.

The CBS at different roasting temperatures were mainly separated by two principal components. The first and second components explained 56.2% and 27.5% of the overall variation, respectively, as shown in Figure 1B. This indicates that the roasting temperatures of cocoa beans have a large influence on the bioactive and antioxidant properties of CBS. From the PCA results (Figure 1C), the first principal component discriminated samples with high TFC, TAC, and epicatechin from samples with high syringic acid, TPC, and catechin. Meanwhile, the second principal component is largely dependent on DPPH, ABTS, and FRAP.

Correlation analysis was done to determine the relationships and behavior of the bioactive and antioxidant properties found in CBS. Table 5 shows the degree of correlation of these properties with each other. TFC and epicatechin had the highest correlation (r=0.98, p<0.05). The high correlation between TFC and epicatechin can be attributed to the abundance of

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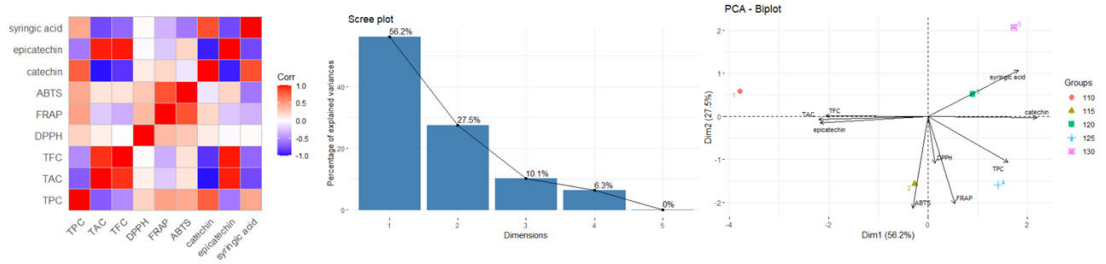

Figure 1A Figure 1B Figure 1C

Figure 1. Principal component analysis (PCA). A: graphical representation of the correlation matrix, B: scree plot, and C: PCA biplot of the relationships between CBS from cocoa beans at different roasting temperatures and their bioactive and antioxidant properties generated using R studio version 4.3.2.

flavonoids in cocoa, which consist of (-) epicatechin and (+) catechin (Pavlović *et al*., 2019; Demir *et al*., 2023). Moreover, a high correlation between TAC and TFC (r=0.93, $p<0.05$) is anticipated, given that anthocyanins are a class of water-soluble flavonoids (Mattioli *et al*., 2020), which means the degradation of TAC at higher temperatures reduces the amount of TFC measured. Furthermore, ABTS and FRAP assays also displayed a positive correlation (r=0.83, $p<0.05$), which suggests that the compounds obtained in CBS reacted correspondingly to these assays due to the similarity in their mechanisms (Thaipong *et al*., 2006).

The PCA biplot (Figure 1C) also revealed the correlation of the bioactive compounds and antioxidant capacities to each sample analyzed. For example, the CBS sample from cocoa bean roasted at 115°C has the highest positive correlation with ABTS, DPPH, and FRAP, while the CBS sample from cocoa bean roasted at 110°C has the strongest positive correlation with TFC, TAC, and epicatechin, and a negative correlation with catechin. The PCA biplot (Figure 1C) illustrates the relationships between bioactive compounds and antioxidant capacities across the analyzed samples. For instance, the CBS sample roasted at 115°C exhibited a strong positive correlation with antioxidant activity, as measured by ABTS, DPPH, and FRAP. In contrast, the CBS sample roasted at 110°C showed a strong positive correlation with total flavonoid content (TFC), total antioxidant capacity (TAC), and epicatechin, while displaying a negative correlation with catechin.

These findings indicate that roasting temperature significantly influences the bioactive and antioxidant properties of CBS. Higher roasting temperatures lead to increased levels of syringic acid and catechin, while lower temperatures are associated with elevated levels of epicatechin. The PCA results suggest that optimizing roasting conditions could help achieve the ideal temperature for maximizing the retention of bioactive compounds and antioxidants in CBS. Therefore, based on the results of this study, the recommended cocoa bean roasting temperature to achieve desirable CBS properties is between 115–120°C.

4. Conclusion

This study demonstrated that cocoa bean shell (CBS), a byproduct of chocolate production, possesses significant potential as a source of bioactive compounds with high antioxidant capacity. Data gathered from this study showed that cocoa bean roasting temperatures had a significant effect on the microbial quality, proximate composition, bioactive profile, and antioxidant properties of CBS. Roasting between 115°C and 125°C was identified as optimal for maximizing antioxidant activity and preserving key phenolic compounds while ensuring microbial safety. Higher roasting temperatures led to a

Table 5. Correlation matrix between the bioactive profile and antioxidant activity of CBS as affected by different roasting temperatures.

	TPC	TAC	TFC	DPPH	FRAP	ABTS	Catechin	Epicatechin	Syringic acid
TPC	1								
TAC	-0.71	1							
TFC	-0.50	0.93	1						
DPPH	0.23	0.081	0.0070	1					
FRAP	0.48	-0.23	-0.35	0.36	1				
ABTS	0.44	0.14	0.15	0.29	0.83	1			
Catechin	0.78	-0.97	-0.87	0.10	0.21	-0.11	1		
Epicatechin	-0.58	0.97	0.98	-0.020	-0.24	0.22	-0.94	1	
Syringic acid	0.45	-0.79	-0.66	0.020	-0.33	-0.59	0.84	-0.79	1

Values presented are normalized. Values from 0 to 1 show positive correlation while values from 0 to -1 show negative correlation between parameters.

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decrease in moisture, crude fiber, and bioactive compounds, particularly flavonoids and anthocyanins, which were more susceptible to degradation. Meanwhile, roasting increased the content of catechin and syringic acid, contributing to the overall antioxidant potential of CBS. However, excessive heat (above 125°C) resulted in the deterioration of phenolic compounds and antioxidant capacity. These findings suggest that optimizing roasting conditions is critical in harnessing the full potential of CBS as a functional food ingredient. Future research should explore alternative processing methods or pre-roasting treatments to further enhance the bioactive properties of CBS while minimizing nutrient loss.

Conflict of interest

The authors declare no conflict of interest.

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