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Research Article

Cacao Bean Shell as a Source of Protein-Rich Functional Extracts: A Comparative Study with Spent Coffee Grounds

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Abstract

Food-processing by-products can serve as alternative sources of proteinaceous and bioactive compounds when their recoverable fractions are effectively utilized. In this study, cacao bean shell (CBS) and spent coffee grounds (SCG) were compared under identical extraction conditions to evaluate their suitability for recovering protein-rich functional fractions. CBS showed approximately 3.6-fold higher crude extract recovery than SCG (0.18 vs 0.05 g/g) and a 1.6-fold higher protein-equivalent content (530 vs 330 mg BSA eq./g extract). Antioxidant capacity was also higher in CBS, with ABTS and DPPH values of 178 and 128 μmol TE/g extract, respectively, compared with 78 and 58 μmol TE/g extract for SCG. CBS additionally showed higher co-recovery of several mineral elements, particularly Mg, Ca, Cu, and Fe. The CBS extract exhibited a clear dose-dependent increase in ABTS and DPPH radical-scavenging activity over the 10–100 mg range, while lactose was not detected under the analytical conditions used. FT-IR analysis further showed extraction-associated changes in the CBS matrix consistent with the removal of proteinaceous, phenolic, and other soluble constituents. Overall, CBS showed more favorable recoverability and functional characteristics than SCG under the conditions tested, supporting its use as a feedstock for protein-rich multifunctional extracts and the valorization of cacao-processing residues.

Keywords: Cacao Bean Shell, Spent Coffee Grounds, Protein-Equivalent Content, Antioxidant Activity, Food By-Product Valorization.

1. Introduction

Food loss and waste across the global food supply chain have become a major challenge in efforts to develop more sustainable food systems. Approximately 1.3 billion tonnes of edible food are lost or wasted annually, generating an estimated 3.3 billion tonnes of greenhouse gas emissions and accounting for approximately 8–10% of global greenhouse gas emissions (Roy *et al.,* 2023). Against this background, the valorization of solid by-products generated during food processing has received increasing attention as a means of recovering residual proteins, phenolic compounds, minerals, and other functional components for further use (Galanakis, 2012; Capanoglu *et al.,* 2022). Such approaches extend beyond waste reduction by redirecting constituents already present in processed biomass into new material streams, thereby improving resource utilization within a circular economy framework.

Cacao bean shell (CBS) is a major food-processing by-product generated by separation of the outer shell from the bean during the processing of *Theobroma cacao* L. CBS accounts for approximately 10–17% of the total cacao bean mass, and its annual generation has been estimated to exceed 0.48 million tons based on global cacao production (Okiyama *et al.,* 2017). CBS is characterized by a high dietary fiber content of approximately 39–66% and contains 10–27% protein, together with polyphenolic compounds such as epicatechin, catechin, and procyanidins and methylxanthines including theobromine and caffeine (Okiyama *et al.,* 2017; Rebollo-Hernanz *et al.,* 2019). Extracts derived from CBS have shown antioxidant activity in several assays, including ABTS, DPPH, and ORAC (Rebollo-Hernanz *et al.,* 2019; Lolato *et al.,* 2026), and its use as a functional food ingredient and dietary fiber source has been investigated as part of circular utilization strategies for cacao-

processing residues (Okiyama *et al.,* 2017). Because the shell is physically separated from the cacao bean before or during roasting, substantial amounts of proteinaceous and phenolic constituents can remain in the residual matrix, making CBS a potential feedstock for further recovery.

Spent coffee grounds (SCG) are the solid residues remaining after coffee beverage preparation and are generated on a scale of several million tons annually, considering global coffee production of more than 10 million tons per year (Mussatto *et al.,* 2011; Ballesteros *et al.,* 2014). SCG consist largely of polysaccharides such as cellulose and hemicellulose and contain approximately 10–17% protein and 10–15% lipids, together with residual chlorogenic acids, caffeine, and mineral elements including magnesium, potassium, and phosphorus (Ballesteros *et al.,* 2014). Bioactive compounds recovered from SCG have been associated with antioxidants and anti-inflammatory activities and with potential modulation of the gut microbiota, leading to continued interest in SCG as a feedstock for functional food ingredients and biorefinery applications (Zuorro and Lavecchia, 2012). SCG, however, differ fundamentally from CBS in their processing history. Coffee beans undergo roasting, during which Maillard reactions and other heat-induced transformations occur, followed by hot-water extraction during beverage preparation. Consequently, SCG represent a residual matrix from which a substantial fraction of water-soluble proteins and low-molecular-weight phenolic compounds has already been removed. These differences in processing history can influence both the composition and recoverability of residual components when the two by-products are subjected to the same extraction conditions.

Despite the substantial interest in both materials, direct comparisons of CBS and SCG under identical extraction conditions remain limited. Most previous studies have examined the two by-products independently or have applied different extraction procedures, making direct assessment of feedstock-dependent recoverability difficult. Total protein content alone is insufficient to determine the practical value of a food-processing by-product as an alternative protein source. In plant-derived matrices, proteins can interact with cell-wall polysaccharides, lignin, and phenolic compounds through covalent and non-covalent associations, and their solubility and extractability can therefore vary considerably with processing history (Sari *et al.,* 2015).

Evaluating crude extract recovery together with the protein-equivalent content of the recovered fraction under the same extraction protocol can provide a more direct comparison of the amount of proteinaceous material that is practically recoverable from each feedstock. Co-extraction of antioxidant-active compounds also does not necessarily need to be regarded solely as an impurity when the intended product is not a highly purified protein isolate. When a recovered fraction contains both protein-equivalent material and measurable antioxidant activity, it may be more appropriately considered a multifunctional extract in which multiple residual components of the original biomass are retained within a single valorization stream (Galanakis, 2012).

In this study, CBS and SCG were processed under identical extraction conditions to compare crude extract recovery and protein-equivalent content of the resulting fractions. The functional and compositional characteristics of the recovered extracts were evaluated through ABTS and DPPH radical-scavenging assays and analysis of selected mineral elements, including Cu, Fe, Mo, Mg, and Ca. Because the CBS extract showed higher recovery and antioxidant activity in the direct comparison, its dose-dependent radical-scavenging response was examined further. Lactose content and FT-IR spectral profiles before and after extraction were also assessed to provide additional compositional information on the recovered CBS fraction and the corresponding raw material. The study therefore aimed to compare the practical extractability of two representative food-processing by-products and to evaluate CBS as a feedstock for recovering a protein-rich functional fraction.

2. Materials and Methods

2.1. Raw Materials and Reagents

Cacao bean shell (CBS) was obtained from a domestic chocolate manufacturer in Korea, and spent coffee grounds (SCG) were collected from a coffee shop located in Seoul, Republic of Korea. Prior to extraction, CBS was dried at 60 °C. SCG was dried under the same conditions and subsequently used for extraction. Skim milk powder, used as a lactose-containing reference material, was purchased from Sigma-Aldrich, Korea. Bovine serum albumin (BSA) used as the protein calibration standard, Bradford reagent, ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid], potassium persulfate, and reagents used for lactose analysis were purchased from Sigma-Aldrich (Republic of Korea). All other chemicals and reagents were of analytical grade or higher.

2.2. Extraction Procedure

Crude extracts from CBS and SCG were prepared using identical extraction conditions to allow direct comparison between the two feedstocks. Each dried material was suspended in 1 M NaOH and extracted at room temperature with continuous agitation at 150 rpm for 2 h. After extraction, the suspension was centrifuged to separate the insoluble solids from the supernatant. The recovered supernatant was subsequently freeze-dried to obtain the crude extract. Crude extract recovery was calculated as the dry mass of the recovered extract divided by the dry mass of the initial raw material and expressed as g extract/g raw material. All extractions were performed in triplicate.

2.3. Determination of Protein-Equivalent Content

The protein-equivalent content of the extracts was determined using a BSA-based colorimetric assay. A calibration curve was prepared using bovine serum albumin as the standard, and absorbance was measured using a UV–Vis spectrophotometer (Genesys 10-S, Thermo Fisher Scientific). The protein-equivalent content was calculated from the BSA calibration curve and expressed as mg BSA equivalent (BSA eq.)/g extract. Because the assay response was quantified relative to BSA rather than by direct determination of total protein, the resulting values were interpreted as protein-equivalent content rather than absolute protein concentration.

2.4. ABTS Radical-Scavenging Capacity

ABTS radical-scavenging capacity was determined using a modified method based on Re *et al.,* (1999). The ABTS radical cation (ABTS•+) was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to react in the dark for 24 h. Before analysis, the ABTS•+ solution was diluted with phosphate-buffered saline (PBS, pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 10 µL of sample was mixed with 990 µL of the diluted ABTS•+ solution. The mixture was incubated in the dark for 6 min, after which absorbance was measured at 734 nm using a Microplate reader. Antioxidant capacity was calculated using a Trolox calibration curve and expressed as µmol Trolox equivalent (TE)/g extract.

2.5. DPPH Radical-Scavenging Capacity

DPPH radical-scavenging capacity was determined using a modified method based on Brand-Williams *et al.,* (1995). A 0.2 mM DPPH solution was prepared in methanol. A 50 µL aliquot of sample was mixed with 950 µL of the DPPH solution and incubated in the dark for 30 min. Absorbance was then measured at 517 nm. Antioxidant capacity was calculated from a Trolox calibration curve and expressed as µmol TE/g extract. For the dose-dependent assay, CBS extract was tested at five dosages (10, 20, 40, 80, and 100 mg) using the same ABTS and DPPH assay procedures. Radical-scavenging activity was expressed as a percentage (%).

2.6. Analysis of Mineral Elements

The concentrations of Cu, Fe, Mo, Mg, and Ca in the recovered extracts were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES; Agilent 5110, Agilent). Prior to analysis, the samples were filtered using 0.22 µm syringe filter. Calibration curves for individual elements were prepared using certified standard solutions purchased from Sigma-Aldrich. Element concentrations were calculated from the corresponding calibration curves and expressed as mg/L.

2.7. Determination of Lactose Content

Lactose content in the CBS extract was determined by high-performance liquid chromatography (HPLC; Shimadzu, LC-20 HPLC systems). Skim milk powder was analyzed under the same chromatographic conditions as a lactose-containing reference material to confirm the analytical response of the method.

2.8. FT-IR Analyses

Fourier transform infrared (FT-IR) spectroscopy was used to examine changes in the chemical profile of CBS before and after extraction. Raw CBS and the residual solid fraction obtained after extraction were analyzed using a Cary 360 FT-IR spectrometer (Agilent, USA) equipped with an attenuated total reflectance (ATR) accessory.

3. Results and Discussion

3.1. Comparison of Crude Extract Recovery and Protein-Equivalent Content

Under identical extraction conditions, crude extract recovery was approximately 0.18 g/g for cacao bean shell (CBS) and 0.05 g/g for spent coffee grounds (SCG), corresponding to an approximately 3.6-fold higher recovery from CBS (Figure 1A). This difference indicates that a larger fraction of the CBS biomass was recovered as soluble extract under the extraction conditions used in this study. CBS is generated through physical separation

of the outer shell during cacao processing and may therefore retain a relatively large fraction of the soluble constituents originally present in the biomass(Mussatto *et al.,* 2011; Okiyama *et al.,* 2017). SCG, in contrast, represents a residue that has already undergone roasting and hot-water extraction, during which part of the water-soluble fraction is transferred to the coffee beverage. This processing history provides a plausible explanation for the lower crude extract recovery observed for SCG.

The protein-equivalent content of the recovered extracts showed the same trend. The CBS extract contained approximately 530 mg BSA eq./g extract, compared with approximately 330 mg BSA eq./g extract for SCG, representing an approximately 1.6-fold difference (Figure 1B). Thus, CBS not only yielded more extract per unit of raw material but also produced an extract with a higher protein-equivalent content. Because protein content was determined using a colorimetric assay calibrated against bovine serum albumin (BSA), these values should be interpreted as BSA-equivalent responses rather than absolute protein concentrations (Bradford, 1976; Mattoo and Edelman, 1987). Phenolic compounds and other co-extracted constituents present in plant-derived samples may also influence colorimetric protein measurements. The term protein-equivalent content is therefore used throughout this study to reflect the analytical basis of the measurement.

The concurrent increase in both crude extract recovery and protein-equivalent content is an important feature of the CBS results. Combining these two parameters gives an estimated protein-equivalent recovery of approximately 95 mg/g raw material for CBS (0.18 × 530) and approximately 17 mg/g for SCG (0.05 × 330), corresponding to an approximately 5.6-fold difference. This calculation is based on the BSA-equivalent assay and approximate values obtained from Figure 1 and is therefore best regarded as a supporting estimate rather than an independent quantitative endpoint. Nevertheless, it illustrates that the difference between the two feedstocks becomes more pronounced when both the amount of recovered extract and its protein-equivalent content are considered together.

The lower recoverability of SCG may be related to the sequence of thermal and aqueous processing applied during coffee production (Mussatto *et al.,* 2011; Sari *et al.,* 2015). Roasting can promote protein denaturation and Maillard reactions between proteins and carbohydrate-derived compounds, potentially decreasing protein solubility or incorporating proteinaceous material into less extractable complexes. Subsequent beverage extraction removes additional water-soluble components from the roasted coffee matrix. As a result, the proteinaceous fraction remaining in SCG may contain a greater proportion of components associated with the cell-wall matrix or altered by thermal processing. CBS does not undergo an equivalent beverage-extraction step, which may allow a larger fraction of soluble proteins, low-molecular-weight peptides, and other extractable constituents to remain available for recovery.

The present comparison was performed using a single extraction protocol, and the observed difference should therefore be interpreted within the conditions tested rather than as an intrinsic difference that applies to all extraction systems (Sari *et al.*, 2015). More intensive extraction procedures, including alkaline, enzyme-assisted, or high-temperature treatments, can alter the recovery characteristics of SCG. Under the conditions applied here, however, CBS consistently showed higher crude extract recovery and protein-equivalent content than SCG.

Overall, the results in Figure 1 identify CBS as the more favorable feedstock for recovering a protein-containing fraction under the extraction conditions used in this study. Subsequent analyses therefore compared the antioxidant properties and mineral composition of the extracts obtained from both feedstocks (Sections 3.2 and 3.3), followed by further characterization of the CBS extract, which showed the higher overall recoverability (Sections 3.4 and 3.5).

3.2. Comparison of Antioxidant Activity: ABTS and DPPH Radical-Scavenging Capacity

The antioxidant properties of the recovered fractions were evaluated by comparing the ABTS and DPPH radical-scavenging capacities of cacao bean shell (CBS) and spent coffee grounds (SCG) extracts (Figure 2). The ABTS antioxidant capacity of the CBS extract was approximately 178 μmol TE/g extract, whereas that of the SCG extract was approximately 78 μmol TE/g extract, corresponding to an approximately 2.3-fold difference (Figure 2A). On an equal extract-mass basis, the CBS fraction therefore exhibited a substantially greater capacity to scavenge ABTS radicals than the SCG fraction. A similar pattern was observed in the DPPH assay (Figure 2B). The CBS extract showed an antioxidant capacity of approximately 128 μmol TE/g extract, compared with approximately 58 μmol TE/g extract for SCG, representing an approximately 2.2-fold difference. ABTS and DPPH assays differ in their reaction characteristics and in their responses to individual antioxidant compounds (Brand-Williams *et al.,* 1995; Re *et al.,* 1999). The ABTS assay can respond to both

hydrophilic and lipophilic antioxidants and involves electron- and hydrogen-transfer reactions, whereas the DPPH assay is more closely associated with the ability of compounds to donate hydrogen or electrons to the DPPH radical. The consistent ranking of CBS > SCG in both assays therefore indicates that the higher antioxidant capacity of the CBS extract was not restricted to a single assay system.

The magnitude of the difference in antioxidant capacity was also greater than that observed for protein-equivalent content. CBS showed approximately 2.2-2.3-fold higher ABTS and DPPH values than SCG, whereas the difference in protein-equivalent content was approximately 1.6-fold (Figure 1B). This pattern indicates that the antioxidant activity of the CBS extract cannot be attributed solely to its higher protein-equivalent content. CBS is known to contain polyphenolic constituents such as epicatechin, catechin, and procyanidins, together with methylxanthines including theobromine and caffeine (Okiyama *et al.,* 2017; Rebollo-Hernanz *et al.,* 2019; Lolato *et al.,* 2026). Co-extraction of these compounds with the proteinaceous fraction could therefore contribute to the higher radical-scavenging capacity observed for CBS.

The lower antioxidant capacity of the SCG extract may also be related to its previous processing history. Coffee roasting induces extensive thermal reactions, including degradation and transformation of chlorogenic acids and the formation of Maillard reaction products (Farah *et al.,* 2005; Mussatto *et al.,* 2011). Hot-water extraction during beverage preparation subsequently removes a substantial proportion of the remaining soluble low-molecular-weight compounds. SCG therefore represents a residual matrix that has already undergone both thermal treatment and aqueous extraction before the extraction procedure applied in this study. In contrast, CBS retains a different compositional profile because it is not subjected to an equivalent beverage-extraction step. These differences provide a plausible basis for the lower antioxidant activity observed in the SCG fraction under the same extraction conditions. The present measurements represent the overall antioxidant capacity of each crude extract and do not distinguish the contribution of individual proteinaceous, phenolic, or other co-extracted constituents. Accordingly, the CBS fraction should not be described as an antioxidant protein based on these data. The terms protein-rich extract with antioxidant activity or protein-containing antioxidant-active fraction more accurately reflects the measured characteristics of the extract. The antioxidant results therefore extend the difference observed in extract recovery and protein-equivalent content in Figure 1. CBS yielded a larger amount of extract, a higher protein-equivalent content, and approximately twofold higher antioxidant capacity than SCG under the conditions tested. The concurrent recovery of proteinaceous material and antioxidant-active constituents supports the interpretation of CBS as a feedstock for obtaining a multifunctional fraction rather than solely as a source of isolated protein.

3.3. Co-Recovery of Mineral Elements in the Extracts

The mineral composition of the recovered fractions was evaluated by measuring Cu, Fe, Mo, Mg, and Ca in the cacao bean shell (CBS) and spent coffee grounds (SCG) extracts (Figure 3). The largest difference between the two extracts was observed for Mg (Rojo-Poveda *et al.,* 2020). Its concentration was approximately 50 mg/L in the CBS extract, whereas only about 1–2 mg/L was detected in the SCG extract. This was the most pronounced difference among the five elements analyzed and indicates that a substantial fraction of Mg present in the CBS matrix was transferred into the soluble fraction during extraction.

Ca also showed a clear difference between the two extracts, with concentrations of approximately 8 mg/L in CBS and 2 mg/L in SCG (Rojo-Poveda *et al.,* 2020). Cu and Fe followed the same general pattern. Their concentrations in the CBS extract were approximately 3.5 and 5 mg/L, respectively, compared with approximately 1.5 and 0.5 mg/L in the SCG extract. Mo was present at relatively low concentrations in both samples, generally below 1 mg/L, and the difference between CBS and SCG was less pronounced than for the other elements.

These results show that the CBS extract contained not only protein-equivalent and antioxidant-active constituents but also several mineral components. The recovered fraction should therefore be regarded as a multicomponent extract rather than as a purified organic fraction. From the perspective of high-purity protein isolation, the presence of inorganic constituents would represent material requiring further separation. In the context of a multifunctional extract, however, their co-recovery reflects the broader compositional complexity of the original biomass.

The particularly high Mg concentration in the CBS extract is consistent with the mineral profile generally reported for cacao-derived materials, in which Mg and Ca are among the more abundant inorganic constituents. The relatively high Mg and Ca concentrations observed here are therefore likely to reflect, at least in part, the mineral composition of the original CBS matrix. By contrast, SCG had already undergone hot-water

extraction during coffee preparation. Because a portion of water-soluble inorganic constituents can be transferred into the beverage during this step, the residual SCG matrix may contain a smaller readily extractable mineral fraction under the conditions used in the present study (Campos-Vega *et al.,* 2015).

The mineral data should not be interpreted as evidence that the CBS extract functions as a mineral supplement or provides a defined nutritional contribution. The present analysis did not assess serving size, bioavailability, or dietary intake. Rather, the results demonstrate that selected inorganic constituents were co-extracted together with the organic fraction during the recovery process.

Overall, the CBS extract contained higher concentrations of Mg, Ca, Cu, and Fe than the SCG extract, with the largest difference observed for Mg (Figure 3). Together with the higher crude extract recovery in Figure 1 and greater antioxidant capacity in Figure 2, this mineral profile further characterizes CBS as a multicomponent feedstock from which proteinaceous, antioxidant-active, and inorganic constituents can be recovered within the same extraction process.

3.4. Dose-Dependent Antioxidant Response of the CBS Extract

Because cacao bean shell (CBS) showed higher crude extract recovery, protein-equivalent content, antioxidant capacity, and mineral co-recovery than spent coffee grounds (SCG) in the preceding comparisons, the CBS extract was further examined for dose-dependent radical-scavenging activity (Figure 4). ABTS and DPPH radical-scavenging activities were measured at extract dosages of 10, 20, 40, 80, and 100 mg.

ABTS radical-scavenging activity increased progressively with extract dosage, from approximately 25% at 10 mg to 40% at 20 mg, 60% at 40 mg, 78% at 80 mg, and 84% at 100 mg (Figure 4A). The increase was most pronounced between 10 and 80 mg, over which the scavenging activity rose by approximately 53 percentage points. Increasing the dosage from 80 to 100 mg produced a smaller increase of approximately 6 percentage points. Thus, although ABTS scavenging activity continued to increase across the tested range, the response became less pronounced at the higher dosages.

A similar pattern was observed for DPPH radical-scavenging activity (Figure 4B). The activity increased from approximately 17% at 10 mg to 30% at 20 mg, 48% at 40 mg, 67% at 80 mg, and 74% at 100 mg. As observed by ABTS, the increase was substantial between 10 and 80 mg, whereas the additional increase from 80 to 100 mg was limited to approximately 7 percentage points. The reduced incremental response at the highest dosage was therefore observed in both assays, indicating that the radical-scavenging response was approaching a plateau within the upper range tested.

Across all extract dosages, ABTS scavenging activity remained higher than DPPH activity. This difference reflects the distinct reaction characteristics of the two assay systems and indicates that the antioxidant constituents present in the CBS extract did not respond equally to the two radicals. The higher ABTS response may be associated with the broader reactivity of the ABTS radical toward compounds capable of participating in electron- and hydrogen-transfer reactions, whereas DPPH is more strongly influenced by the hydrogen- or electron-donating characteristics of individual antioxidants. The present data, however, does not identify which specific phenolic, proteinaceous, or other constituents were responsible for the difference between the two assay responses.

The dose-response analysis extends the feedstock comparison presented in Figure 2. Whereas Figure 2 showed that the CBS extract had higher antioxidant capacity than the SCG extract on an equal extract-mass basis, Figure 4 demonstrates that the radical-scavenging activity of the selected CBS fraction increased consistently with the amount of extract added. The two datasets therefore represent sequential stages of the same comparison: initial screening of the two food-processing by-products followed by dose-dependent evaluation of the extract obtained from the better-performing feedstock. Overall, the CBS extract exhibited a clear dose-dependent increase in both ABTS and DPPH radical-scavenging activity over the 10-100 mg range. The smaller incremental increases between 80 and 100 mg in both assays indicate that the response began to level off at higher extract dosages. These results confirm that the antioxidant activity observed for the CBS extract in the initial feedstock comparison was retained across a range of extract dosages and was not limited to a single test concentration.

3.5. Lactose Content and FT-IR Characterization of the CBS Extract and Residual Matrix

To further characterize the composition of the cacao bean shell (CBS) extract, its lactose content was compared with that of skim milk powder, which was used as a representative dairy-derived protein material (Figure 5).

Skim milk powder contained approximately 0.52 g/g lactose, whereas lactose was not detected (N.D.) in the CBS extract. The clear detection of lactose in the reference material indicates that, under the analytical conditions used, the CBS-derived extract contained no detectable lactose (Misselwitz *et al.,* 2019; Catanzaro *et al.,* 2021).

This result provides additional compositional information distinguishing the CBS extract from dairy-derived protein ingredients. Given the prevalence of lactose intolerance, the absence of detectable lactose may be relevant when considering plant-derived protein-containing materials as alternatives to dairy-based ingredients. The present analysis, however, did not establish a method-specific limit of detection or quantification in relation to regulatory thresholds for lactose-free labeling. The CBS extract should therefore not be classified as a lactose-free ingredient solely based on this data. A more appropriate interpretation is that lactose was not detected under the analytical conditions applied in this study.

The lactose analysis serves as complementary characterization rather than as the primary basis for evaluating the CBS extract. Figures 1-4 first established the higher extract recovery, protein-equivalent content, and antioxidant activity of CBS relative to spent coffee grounds. The non-detection of lactose in Figure 5 subsequently provides an additional compositional characteristic of the selected CBS fraction without extending the interpretation to untested nutritional or health benefits.

Changes in the CBS matrix associated with extraction were further examined by comparing the FT-IR spectra of raw CBS and the residual material after extraction (Figure 6). The spectrum of raw CBS (Figure 6A) showed a broad absorption region around 3300 cm^{-1}, attributable primarily to O-H and N-H stretching, together with bands near 2920 and 2850 cm^{-1} corresponding to aliphatic C-H stretching. Additional bands were observed around 1730 cm^{-1}, associated with carbonyl C=O stretching; near 1620 cm^{-1}, where amide-related vibrations and aromatic C=C stretching may overlap; around 1460 cm^{-1}; and within the 1165–1030 cm^{-1} region, where C-O and C-O-C vibrations are prominent (Kacurakova *et al.,* 2000; Movasaghi *et al.,* 2008). This overall spectral pattern is consistent with the heterogeneous composition of plant-derived biomass containing polysaccharides, proteins, lipids, and phenolic constituents.

The residual matrix after extraction (Figure 6B) showed changes in the relative intensity and profile of several spectral regions compared with raw CBS. Changes were particularly evident in the regions indicated in Figure 6 as being associated with protein reduction and with phenolic compounds and other matrix constituents. These spectral differences are consistent with a compositional shift in the solid matrix during extraction, in which a portion of the extractable proteinaceous, phenolic, and other soluble constituents was transferred from the raw material into the recovered fraction. This interpretation is consistent with the substantial crude extract recovery observed for CBS in Figure 1 and the antioxidant activity measured in the recovered fraction in Figure 2.

Individual FT-IR bands should nevertheless be interpreted cautiously because CBS is a complex matrix containing proteins, carbohydrates, lignocellulosic components, lipids, and phenolic compounds with overlapping vibrational signals. For example, the region around 1620 cm^{-1} can contain contributions from both amide-related vibrations and aromatic C=C stretching, while the broad band near 3300 cm^{-1} includes overlapping O-H and N-H stretching modes (Kacurakova et al., 2000; Movasaghi et al., 2008). Accordingly, the spectral changes cannot be used independently to assign the removal of a specific component. Rather, the FT-IR data provides qualitative evidence of an extraction-related compositional shift that complements the quantitative recovery and functional measurements presented in Figures 1-4.

4. Conclusion

This study directly compared cacao bean shell (CBS) and spent coffee grounds (SCG) under identical extraction conditions to evaluate their practical recoverability as food-processing by-products. CBS showed approximately 3.6-fold higher crude extract recovery than SCG (0.18 vs 0.05 g/g) and a 1.6-fold higher protein-equivalent content (530 vs 330 mg BSA eq./g extract).

The same trend was observed for ABTS and DPPH antioxidant capacity and for the co-recovery of several mineral elements, including Mg, Ca, Cu, and Fe. The CBS extract also exhibited a clear dose-dependent increase in radical-scavenging activity over the 10–100 mg range. Lactose was not detected under the analytical conditions used, while FT-IR analysis showed extraction-related changes in the spectral profile of the CBS matrix, consistent with the transfer of proteinaceous, phenolic, and other extractable constituents into the recovered fraction.

Within the conditions tested, CBS therefore provided a more favorable feedstock than SCG for the recovery of a protein-rich functional fraction. The combined recovery of protein-equivalent material, antioxidant-active constituents, and selected minerals supports the use of CBS as a multicomponent resource rather than solely as a source of isolated protein. These results provide a practical basis for the valorization of cacao-processing residues within a circular use framework for food-processing by-products.

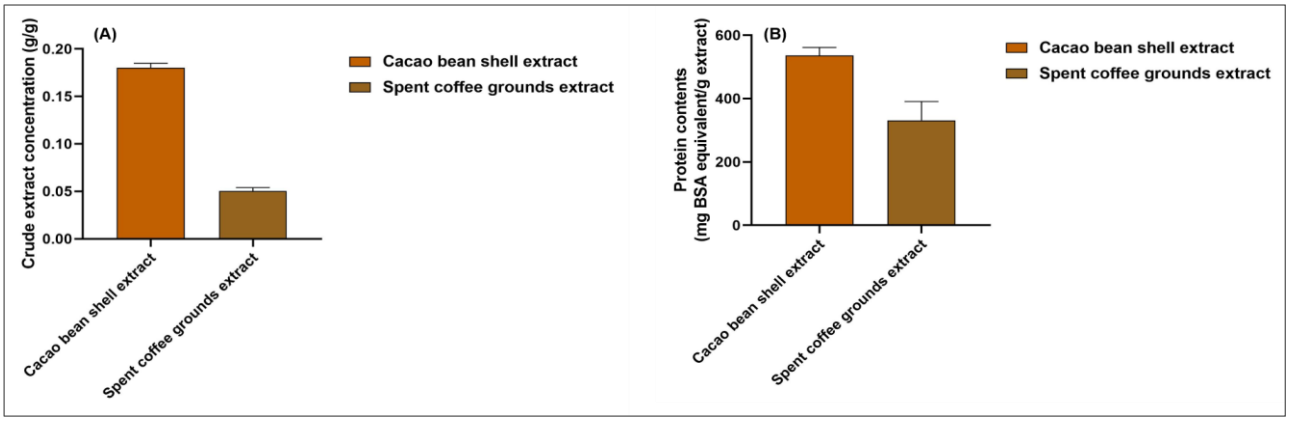

Figure 1. Crude extract recovery and protein-equivalent content of cacao bean shell and spent coffee grounds; (A) Crude extract recovery from cacao bean shell (CBS) and spent coffee grounds (SCG), expressed as g extract per g raw material. (B) Protein-equivalent content of the recovered extracts, expressed as mg bovine serum albumin (BSA) equivalent per g extract.

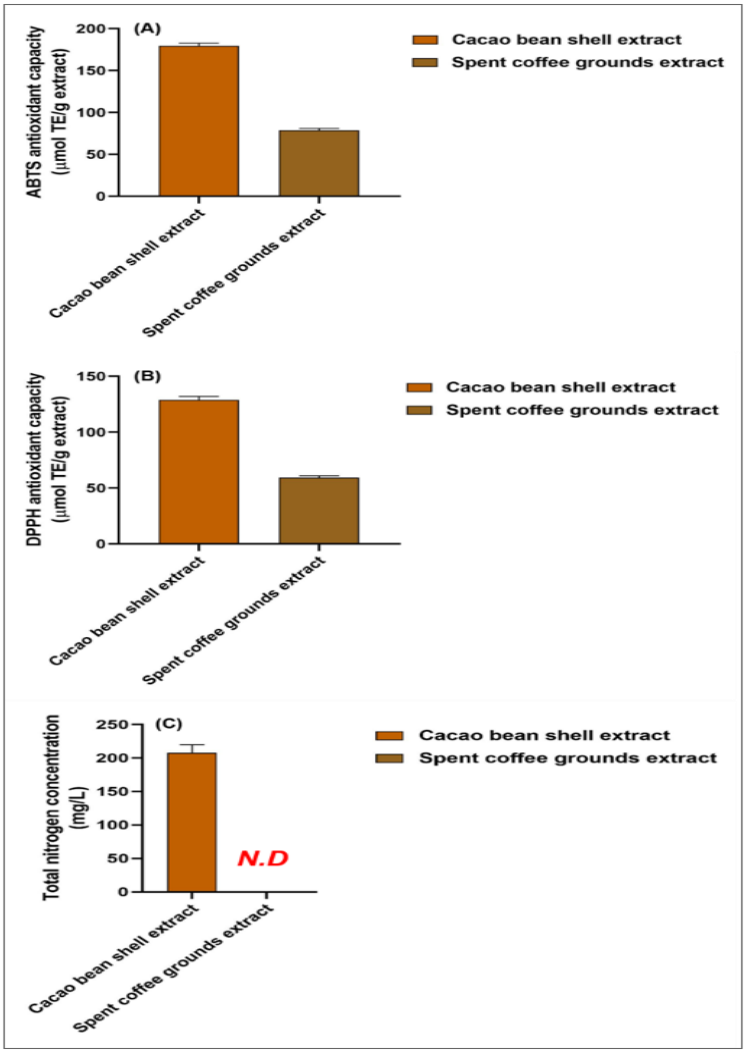

Figure 2. Antioxidant capacity and total nitrogen concentration of cacao bean shell and spent coffee grounds extracts; (A) ABTS antioxidant capacity and (B) DPPH antioxidant capacity of cacao bean shell (CBS) and spent coffee grounds (SCG) extracts, expressed as µmol Trolox equivalent (TE) per g extract. (C) Total nitrogen concentration of the recovered extracts.

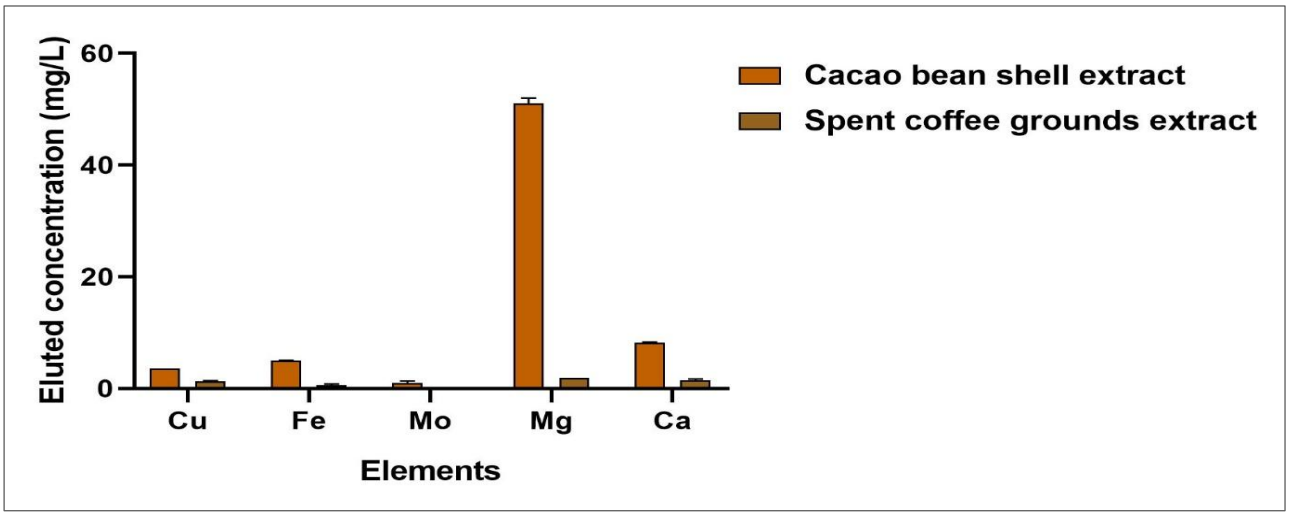

Figure 3. Mineral element concentrations in cacao bean shell and spent coffee grounds extracts.

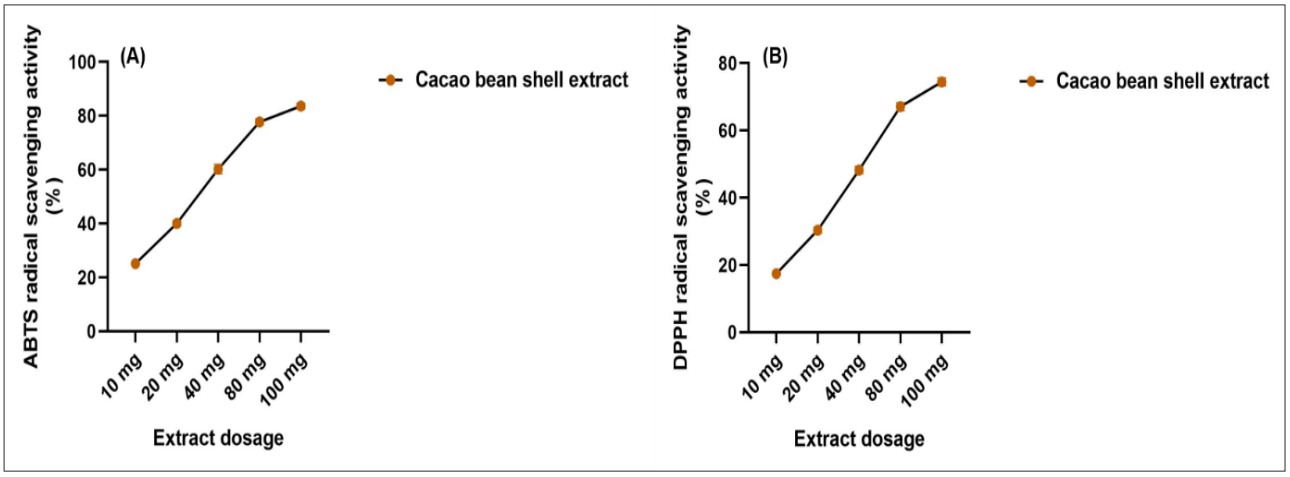

Figure 4. Dose-dependent radical-scavenging activity of cacao bean shell extract; (A) ABTS and (B) DPPH radical-scavenging activities of cacao bean shell (CBS) extract at extract dosages of 10, 20, 40, 80, and 100 mg. Radical-scavenging activity is expressed as a percentage of the corresponding radical removed under the assay conditions.

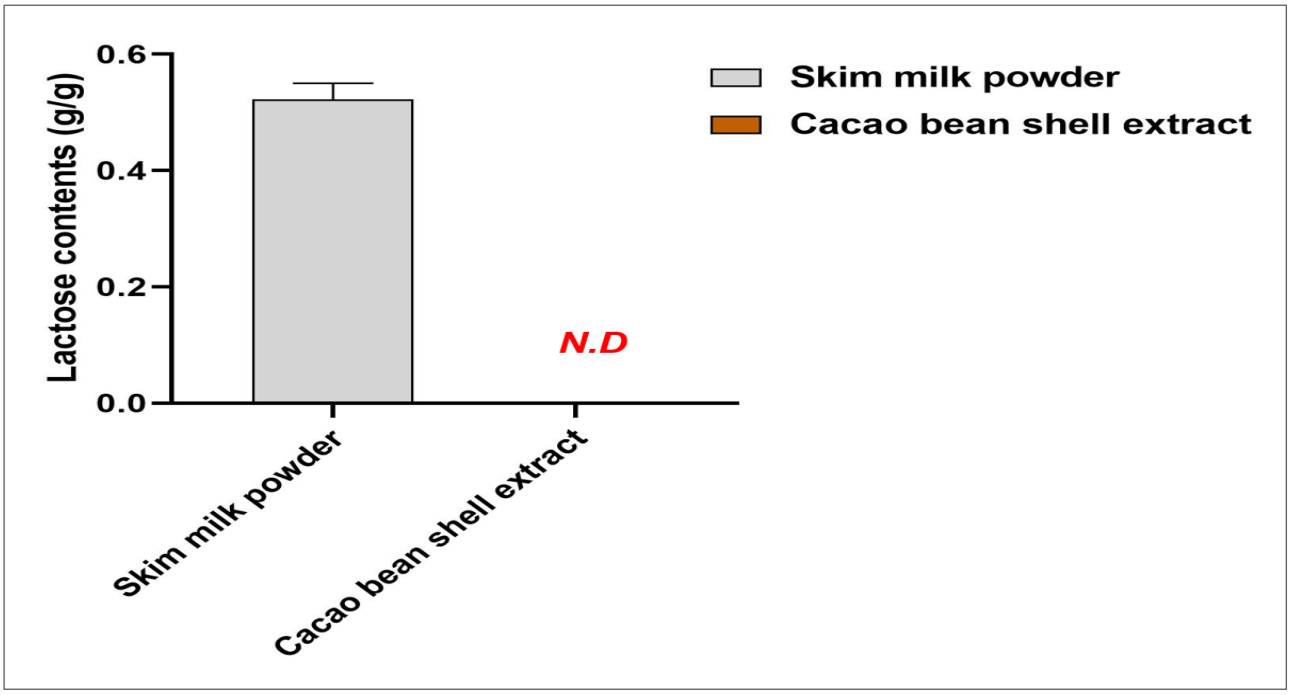

Figure 5. Lactose content of skim milk powder and cacao bean shell extract.

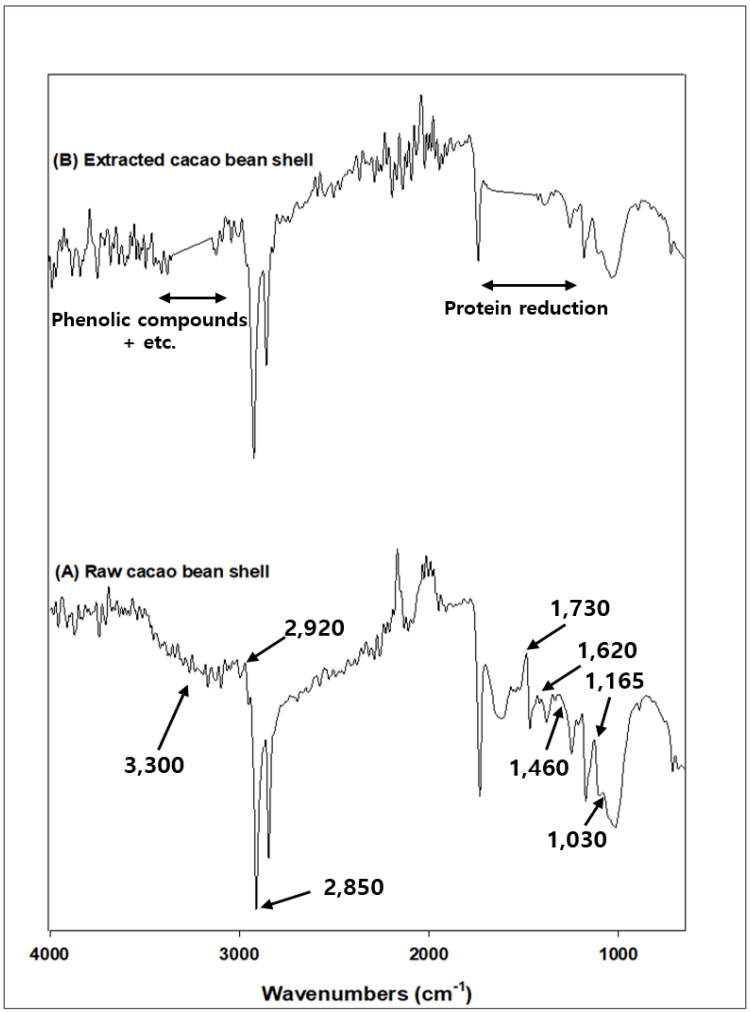

Figure 6. FT-IR spectral profiles of cacao bean shell before and after extraction; (A) Raw cacao bean shell (CBS) and (B) CBS after extraction.

Declarations

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