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Phytochemical Composition, Anti-Nutritional Factors, Oil Quality Parameters, in Vitro Antioxidant Capacity and Mineral Composition of Cottonseed (*Gossypium hirsutum L.*)

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ABSTRACT

Cottonseed (Gossypium hirsutum L.) is an abundant agricultural by-product with nutritional and industrial potential, yet its safe utilisation in human diets is constrained by toxic compounds and limited biochemical data on locally sourced samples. This study determined the phytochemical constituents, anti-nutritional factors, oil quality parameters, in vitro antioxidant capacity, and mineral composition of cottonseed obtained from the Nigerian market. Phytochemicals were quantified using Folin-Ciocalteu, aluminium chloride colorimetric, and gravimetric procedures. Anti-nutritional factors were estimated titrimetrically. Oil quality was assessed through acid value, percentage free fatty acids (%FFA), and peroxide value. Antioxidant capacity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays in both seed extract and extracted oil, with ascorbic acid as the reference standard. Mineral analysis was performed by Atomic Absorption Spectrophotometry (AAS). Total phenolic content was the most abundant phytochemical (82.35 ± 0.40 g TAE/kg), followed by tannins (21.22 ± 1.78 g TAE/kg), saponins (9.45 ± 0.09 g/kg), flavonoids (9.16 ± 1.23 g QE/kg), and alkaloids (6.67 ± 0.34%). Phytate (2.12 ± 0.02%) was the dominant anti-nutrient. The acid value, %FFA, and peroxide value substantially exceeded Codex Alimentarius limits, indicating advanced rancidity. Calcium (178.8 mg/kg) and potassium (117.7 mg/kg) were the most abundant minerals; lead and cadmium were undetected. These findings indicate that Nigerian-market cottonseed contains biologically active phytochemicals and nutritionally relevant minerals, though phytate-reducing processing and full industrial oil refining are necessary before safe dietary use.

1. INTRODUCTION

Oilseeds are critical components of the global food system, serving as concentrated sources of dietary lipids, proteins, and industrially relevant bioactive compounds. Among these, *Gossypium hirsutum* L.

(upland cotton, family Malvaceae) is the most commercially dominant species, accounting for over 90% of worldwide cotton production (Ninkuu *et al.*, 2023). Although cultivated principally for its textile fibre, the cotton plant generates approximately 1.65 kg of cottonseed for every kilogram of lint produced, making cottonseed a highly abundant agricultural by-product (Kumar *et al.*, 2021). Raw cottonseed contains 18–25% oil and 20–30% crude protein, in addition to a diverse array of phytochemicals and minerals essential for metabolic regulation (Zubair *et al.*, 2021).

The lipid fraction of cottonseed is characterised by a high proportion of linoleic acid (50–60%), palmitic acid (22–26%), and oleic acid (15–20%), conferring favourable nutritional and physicochemical properties to the extracted oil (Zia *et al.*, 2022). Cottonseed is also a source of tocopherols, phytosterols, and other lipophilic antioxidants that protect the oil against oxidative deterioration during storage and thermal processing (Riaz *et al.*, 2021). The phytochemical fraction, which includes total phenolics, flavonoids, tannins, saponins, and alkaloids, contributes additional antioxidant, anti-inflammatory, and antimicrobial bioactivities that expand the therapeutic relevance of cottonseed derivatives (Morya *et al.*, 2022).

Despite these nutritional attributes, the integration of cottonseed into human diets is substantially constrained by its content of anti-nutritional factors, particularly phytic acid and oxalate. Phytic acid chelates essential divalent cations, notably iron, zinc, and calcium, forming insoluble metal-phytate complexes that escape intestinal absorption, thereby reducing the bioavailability of these critical micronutrients (Mostashari and Mousavi Khaneghah, 2024). Oxalate further limits calcium availability by precipitating insoluble calcium oxalate within the gastrointestinal tract, and its systemic absorption at elevated concentrations predisposes susceptible individuals to renal calculus formation (Wigner *et al.*, 2021). Additionally, cottonseed oil is inherently susceptible to both hydrolytic and oxidative rancidity owing to its high polyunsaturated fatty acid content; these degradation pathways are readily accelerated under conditions of inadequate post-harvest handling (Ahmed *et al.*, 2024).

While extensive biochemical data on cottonseed have been reported for samples from Asia and the Americas, systematic data on the phytochemical profile, anti-nutritional factors, oil quality indices, antioxidant capacity, and mineral composition of cottonseed obtained from Nigerian markets remain sparse. Given that Nigeria is among the leading producers of cotton in sub-Saharan Africa and that cottonseed by-products enter local food and feed supply chains, such data are essential for evidence-based dietary and processing recommendations. This study therefore aimed to provide a comprehensive biochemical characterisation of Nigerian-market cottonseed and its mechanically extracted oil, with a view to establishing baseline data for safe dietary integration and targeted processing interventions.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Cottonseeds were obtained from the New Benin Market, Benin City, Edo State, Nigeria. Upon collection, seeds were manually sorted to remove debris, washed, oven-dried, and pulverised into a fine powder using a mechanical grinder. The powder was stored in labelled, airtight, moisture-free containers at room temperature before analysis. Oil was extracted from the seed powder by mechanical cold-pressing using a RAYLUX Automatic Oil Press Extractor. The crude extracted oil was stored in airtight amber glass bottles at 4°C until required (Edeoga *et al.*, 2005).

2.2. Qualitative Phytochemical Screening

Qualitative screening for alkaloids, flavonoids, phenolic compounds, tannins, and saponins was performed using Dragendorff's test, the alkaline reagent test, the ferric chloride test, and the froth test, respectively, following the standard procedures described by Harborne (1998), and Sofowora (1996).

2.3. Quantitative Phytochemical Analysis

2.3.1. Total phenolic content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method as described by Singleton and Rossi (1965). Briefly, 0.5 mL of methanol extract was reacted with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 765 nm. Results were expressed as grams of tannic acid equivalents per kilogram of dry weight (g TAE/kg), derived from a gallic acid calibration curve.

2.3.2. Flavonoid content

Flavonoid content was quantified using the aluminium chloride colorimetric method of Boham and Koupai-Abyazani (1994). One millilitre of methanol extract was mixed with 1.0 mL of 2% aluminium chloride in methanol. After 30 minutes of incubation, absorbance was read at 415 nm. Results were expressed as grams of quercetin equivalents per kilogram (g QE/kg) using a quercetin standard curve.

2.3.3. Tannin content

Total tannin content was estimated using the Folin–Denis colorimetric method (Bajaj and Devsharma, 1977). The extract was reacted with Folin-Denis reagent and 17% sodium carbonate solution, and absorbance was measured at 760 nm after a 20-minute colour development period. Results were reported as grams of tannic acid equivalents per kilogram (g TAE/kg).

2.3.4. Saponin content

Saponins were determined gravimetrically by the aqueous-ethanolic extraction method of Obadoni and Ochuko (2001). The seed powder was extracted with 20% aqueous ethanol at 55°C, and the saponin fraction was isolated through diethyl ether washing and n-butanol extraction. The final extract was evaporated to dryness, and the saponin content was reported in g/kg dry weight.

2.3.5. Alkaloid content

Total alkaloid content was estimated gravimetrically following Harborne (1998). Alkaloids were precipitated from an acidic ethanol extract by addition of concentrated ammonium hydroxide. The dried precipitate was weighed and the alkaloid content expressed as a percentage (%) of the dry sample weight.

2.4. Anti-nutritional Factor Determination

2.4.1. Phytate determination

Phytate was determined by the titrimetric method of Lolas and Markakas (1975). Approximately 4 g of sample was extracted in 2% hydrochloric acid and titrated against 0.00195 g Fe/mL iron (III) chloride solution using ammonium thiocyanate as indicator. Phytate content was expressed as a percentage using Equation (1):

$$\%\text{Phytic acid} = \frac{8.24 \times titre}{1000 \times sample\ weight} \times 100 \quad (1)$$

2.4.2. Oxalate determination

Total oxalate was determined by permanganate titrimetry following Day and Underwood (1986). The sample was extracted in 1.5 N sulphuric acid and the hot filtrate titrated against 0.1 N potassium permanganate to a persistent faint pink endpoint. Oxalate content was calculated as:

$$\text{Oxalate (mg/g)} = \text{titre} \times 0.9004 \quad (2)$$

2.5. Oil Quality Parameters

2.5.1. Acid value and percentage free fatty acids

The acid value (AV) was determined by titration against 0.1 N KOH in a neutralised ethanol-diethyl ether solvent, with phenolphthalein as indicator per AOCS Official Method Cd 3d-63 (AOCS, 2017) (Equation 3).

$$\text{AV (mg KOH/g)} = \frac{(\text{V} \times \text{N} \times 56.1)}{W} \quad (3)$$

where V = volume of KOH (mL), N = normality, and W = sample weight (g).

The percentage free fatty acids (%FFA, as oleic acid) was derived from the acid value using the conversion factor 0.503 per AOCS Official Method Ca 5a-40 (AOCS, 2017).

2.5.2. Peroxide value

The peroxide value (PV) was determined by iodometric titration per AOCS Official Method Cd 8-53 **(AOCS, 2003)**. The oil was dissolved in glacial acetic acid-chloroform (3:2 v/v) and reacted with saturated potassium iodide solution. Liberated iodine was titrated against 0.01 N sodium thiosulfate using starch indicator Equation (4).

$$\text{PV(meq/kg)} = \frac{[(\text{Vs} - \text{Vb}) \times \text{N} \times 1000]}{W} \quad (4)$$

2.6. In Vitro Antioxidant Assays

2.6.1. DPPH radical scavenging assay

DPPH radical scavenging activity was assessed for both the seed extract and the extracted oil following Aliya *et al.* (2024) and Musa *et al.* (2016), with minor modifications. Two millilitres of each sample was mixed with 2 mL of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 518 nm and percentage inhibition calculated as Equation (5):

$$\% \text{ Scavenging} = [(A_0 - A_1) / A_0] \times 100 \quad (5)$$

Ascorbic acid served as the reference standard.

2.6.2. Ferric reducing antioxidant power assay

The FRAP assay was conducted on the extracted oil only, following the method of Aliya *et al.* (2024). FRAP reagent (2.85 mL) was mixed with 150 µL of each sample concentration, incubated for 30 minutes, and absorbance measured at 593 nm. Results were expressed as percentage ferric-reducing activity relative to ascorbic acid standards.

2.7. Mineral Analysis by Atomic Absorption Spectrophotometry

Mineral composition of both pulverised seed and extracted oil was determined by Atomic Absorption Spectrophotometry (AAS), following wet acid digestion with concentrated HNO_3 and $HClO_4$ (4:1 v/v) (Bankaji *et al.*, 2023). The digest was diluted to volume with deionised water and analysed at element-specific wavelengths. Concentrations of calcium (Ca), potassium (K), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and cadmium (Cd) were interpolated from standard calibration curves and expressed as mg/kg.

2.8. Statistical Analysis

All analyses were performed in triplicate and results expressed as mean ± standard error of mean (SEM). Data were processed using SPSS version 23. One-way ANOVA was employed to compare means, and differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Composition of Cottonseed

The quantitative phytochemical analysis of cottonseed extract is presented in Table 1. Total phenolic content (TPC) was the most concentrated class of secondary metabolites, at 82.35 ± 0.40 g TAE/kg, and was significantly higher than all other quantified phytochemicals ($p < 0.05$). Phenolic compounds are biosynthesised through the shikimate and phenylpropanoid pathways and exert antioxidant effects principally through hydrogen donation from their phenolic hydroxyl groups to reactive oxygen species, as well as through chelation of redox-active iron and copper ions that would otherwise catalyse Fenton-type hydroxyl radical generation (Dominguez-López *et al.*, 2024). The TPC recorded in this study is higher than values commonly reported for cottonseed extracts prepared under comparable extraction conditions (Morya *et al.*, 2022; Suleiman *et al.*, 2024), a difference most likely attributable to cultivar-specific genotype, seed maturation stage at harvest, and the polarity of the extraction solvent, all of which are established determinants of extractable phenolic yield in oilseeds (Pérez *et al.*, 2023).

Table 1: Phytochemical composition of cottonseed (*Gossypium hirsutum* L.) extract

Parameter	Concentration
Total phenolic content (g TAE/kg)	82.35 ± 0.40^{a}
Total tannin content (g TAE/kg)	21.22 ± 1.78^{b}
Saponin content (g/kg)	9.45 ± 0.09^{c}
Flavonoid content (g QE/kg)	9.16 ± 1.23^{c}
Alkaloid content (%)	6.67 ± 0.34^{c}

Values are expressed as Mean ± SEM (n = 3). Values with different superscript letters differ significantly at $p < 0.05$

Total tannin content was the second most abundant phytochemical class (21.22 ± 1.78 g TAE/kg). Tannins are high-molecular-weight polyphenolic compounds that form stable cross-linked complexes with dietary proteins via hydrogen bonding and hydrophobic interactions, which reduces digestive enzyme accessibility and overall protein digestibility in the gastrointestinal tract. Their phenolic hydroxyl groups also contribute to radical scavenging and metal chelation activity (Cosme *et al.*, 2025). The tannin concentration recorded falls within the range described for raw tropical oilseeds (Shakeel *et al.*, 2025). Saponin and flavonoid contents were statistically similar (9.45 ± 0.09 and 9.16 ± 1.23 g/kg, respectively; $p > 0.05$), both well within values documented for cottonseed by Suleiman *et al.* (2024). Saponins are amphiphilic glycosides that lower micellar cholesterol solubilisation in the intestinal lumen, thereby reducing cholesterol absorption (Usman *et al.*, 2023). Flavonoids, sharing the conserved C_6-C_3-C_6 carbon backbone, contribute to oxidative defence through hydrogen atom donation, pro-oxidant metal chelation, and inhibition of lipid peroxidation propagation (Stachelska *et al.*, 2025). Alkaloids were the least abundant phytochemical (6.67 ± 0.34%), consistent with values reported by Suleiman *et al.* (2024), and are unlikely to produce acute toxic effects under normal dietary conditions. Nonetheless, reduction of alkaloid content through standard food processing prior to consumption would remain advisable.

3.2. Anti-nutritional Factor Composition

Table 2 presents the anti-nutritional factor concentrations in cottonseed. Phytate was the dominant anti-nutrient at 2.12 ± 0.02%, significantly higher than oxalate (0.44 ± 0.00 mg/g; $p < 0.05$). Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is the principal phosphorus storage compound in plant seeds, and its six phosphate groups carry a high negative charge density over a broad physiological pH range. Within the aqueous environment of the small intestine, the deprotonated phosphate groups bind to essential multivalent cations iron, zinc, calcium, and magnesium forming insoluble metal-phytate coordination complexes (phytin) that resist hydrolysis by mammalian intestinal enzymes, thereby substantially reducing mineral bioavailability (Mostashari and Mousavi Khaneghah, 2024). The phytate level observed is consistent with the range documented for raw cottonseed by Abera *et al.* (2023) and

Morya *et al.* (2022), with inter-study variation most likely attributable to differences in cultivar genotype, soil phosphorus status, and seed maturation stage. It is biochemically noteworthy that phytic acid's iron-chelating property can suppress the Fenton reaction, yielding a secondary antioxidant function within the intact seed by limiting hydroxyl radical generation from redox-active iron (Pujol *et al.*, 2023).

Table 2: Anti-nutritional factor composition of cottonseed (*Gossypium hirsutum* L.) extract

Parameter	Concentration
Oxalate (mg/g)	0.44 ± 0.00^{a}
Phytate (%)	2.12 ± 0.02^{b}

Values are expressed as Mean ± SEM (n = 3). Values with different superscript letters differ significantly at $p < 0.05$

Oxalate was detected at a considerably lower level (0.44 ± 0.00 mg/g). Although intestinal absorption of soluble oxalates can precipitate calcium oxalate within the renal tubules and trigger a cascade of oxidative and inflammatory events — including NADPH oxidase activation, reactive oxygen species generation, and nuclear factor kappa B-mediated cytokine secretion — that predispose susceptible individuals to renal calculus formation (Wigner *et al.*, 2021), the concentration recorded here is well below the clinically significant threshold cited in food safety assessments (Morya *et al.*, 2022). Phytate therefore represents the anti-nutritional factor of greatest practical relevance in this cottonseed sample. Processing interventions such as fermentation, soaking, germination, or enzymatic phytase supplementation effectively hydrolyse the phosphate-ester bonds of phytic acid to yield lower-order inositol phosphates with reduced mineral-binding capacity, and are recommended to enhance mineral bioavailability prior to dietary incorporation (Lin *et al.*, 2024).

3.3. Comparison of Total Phenolic Content Between Cottonseed and Extracted Oil

A statistically significant difference was observed in total phenolic content between the whole cottonseed extract and the extracted oil (Table 3; $p < 0.05$), with the oil retaining only approximately 6% of the phenolic concentration present in the intact seed. This large differential follows directly from the chemical polarity of the phenolic compounds predominant in cottonseed. Phenolic acids, flavonoids, and tannins are predominantly hydrophilic or moderately polar species that partition preferentially into aqueous phases during oil extraction; the great majority therefore remain in the defatted seed meal rather than partitioning into the lipid phase (Pérez *et al.*, 2023; Dominguez-López *et al.*, 2024). Only lipid-soluble phenolic derivatives, principally tocopherols and phenolic lipids, are co-extracted with the oil fraction. Comparable reductions in phenolic content between whole seeds and their expressed oils have been documented for sunflower, sesame, and soybean (Mostashari and Mousavi Khaneghah, 2024; Salimath *et al.*, 2021), confirming that this result is consistent with the general behaviour of oilseed polyphenols during mechanical extraction. Notwithstanding its markedly lower phenolic concentration, the oil-phase phenolics are structurally embedded within the triacylglycerol matrix in close proximity to the unsaturated fatty acyl chains, and are therefore well positioned to intercept peroxyl radicals and decompose lipid hydroperoxides during the initiation and propagation stages of autoxidation, providing direct in situ oxidative protection within the oil itself (Salimath *et al.*, 2021).

Table 3: Total phenolic content of cottonseed and extracted cottonseed oil

Sample	Total phenolic content (g TAE/kg)
Cottonseed oil	4.94 ± 0.19^{a}
Cottonseed extract	82.35 ± 0.40^{b}

Values are expressed as Mean ± SEM (n = 3). Values with different superscript letters differ significantly at $p < 0.05$

3.4. In Vitro Antioxidant Activity of Cottonseed and Cottonseed Oil

The in vitro antioxidant capacity of cottonseed extract and oil is presented in Table 4. In the DPPH assay, antioxidant molecules donate a hydrogen atom or electron to the stable 2,2-diphenyl-1-picrylhydrazyl radical, reducing it from its violet radical form to the yellow diphenylpicrylhydrazine product; the resulting fall in absorbance at 518 nm is directly proportional to the extent of radical scavenging (Gulcin and Alwasel, 2023). The cottonseed oil (51.39 ± 6.23%) exhibited a higher DPPH scavenging activity than the whole seed extract (38.79 ± 3.41%), despite its markedly lower TPC. This apparent paradox is explained by the nature of the antioxidants concentrated in the oil fraction. Cottonseed oil is enriched in lipid-soluble antioxidants, primarily alpha- and gamma-tocopherol, which function as efficient hydrogen atom donors and react readily with the DPPH radical through single-electron transfer. Riaz *et al.* (2021) and Salimath *et al.* (2021) have established that tocopherols, in combination with phytosterols and residual phenolic lipids, account for the substantial radical scavenging capacity of cottonseed oil in hydrophobic assay systems.

Table 4: DPPH radical scavenging activity and FRAP reducing activity of cottonseed extract, cottonseed oil, and ascorbic acid reference standard

Sample	DPPH (% Inhibition)	FRAP (% Reducing activity)
Cottonseed extract	38.79 ± 3.41[a]	—
Cottonseed oil	51.39 ± 6.23[a]	35.18 ± 5.79[a]

Values are expressed as Mean ± SEM (n = 3). Values with different superscript letters within each assay column differ significantly at $p < 0.05$. — indicates not determined

The FRAP reducing activity of cottonseed oil (35.18 ± 5.79%) was lower than its DPPH scavenging value. The FRAP assay measures electron-transfer-driven reduction of the ferric tripyridyltriazine complex to its ferrous form under strictly acidic conditions (Knez *et al.*, 2025). Tocopherols, which function most efficiently as hydrogen atom donors in lipophilic environments, are comparatively less effective as direct electron donors under the acidic aqueous conditions of the FRAP system, which partially accounts for the lower value recorded. In all assays, ascorbic acid exceeded both fractions, confirming that the antioxidant capacity of cottonseed and its oil is moderate relative to a standard chemical antioxidant, consistent with moderate activities reported for cottonseed fractions by Suleiman *et al.* (2024) and Morya *et al.* (2022). Nevertheless, this moderate antioxidant capacity carries nutritional relevance, given that chronic imbalance between reactive oxygen species production and biological antioxidant defence is mechanistically linked to the pathogenesis of cardiovascular disease, type 2 diabetes mellitus, and carcinogenesis through lipid peroxidation, protein carbonylation, and deoxyribonucleic acid strand damage (Nuñez-Selles *et al.*, 2025).

3.5. Oil Quality Parameters of Extracted Cottonseed Oil

The oil quality assessment (Table 5) revealed that all three parameters substantially exceeded the maximum permissible limits established by the Codex Alimentarius Commission for refined vegetable oils intended for human consumption (Codex Alimentarius Commission, 2023). The acid value (23.30 ± 3.02 mg KOH/g) and %FFA (11.72 ± 1.51%) represent a many-fold excess of the Codex maximum of 0.6 mg KOH/g for refined oils. Elevated acid value and %FFA are biochemical indicators of advanced hydrolytic rancidity, arising from cleavage of ester bonds at the sn-1, sn-2, and sn-3 glycerol positions of triacylglycerol molecules. This hydrolysis is catalysed principally by endogenous seed lipases activated upon structural disruption of the seed during mechanical milling, and is further accelerated by exogenous microbial lipases introduced under conditions of inadequate post-harvest storage (Reyes-Reyes *et al.*, 2022). The elevated free fatty acid concentrations observed are most readily attributable to the storage and handling conditions typical of locally traded Nigerian cottonseed — seeds are frequently insufficiently dried prior to storage, held for extended periods in non-hermetic containers, and exposed to the elevated temperature and humidity characteristic of the tropical environment, all of which sustain lipase activity and accelerate moisture-mediated ester bond hydrolysis (Zubair *et al.*,

2021). Furthermore, the mechanical milling extraction method used does not incorporate the alkali neutralisation step central to industrial oil refining, so the values obtained reflect the quality of crude, unrefined oil under prevailing local market conditions. Elevated free fatty acid concentrations also act as pro-oxidants by catalysing the decomposition of lipid hydroperoxides, thereby accelerating the secondary phases of oxidative rancidity (Ahmed *et al.*, 2024).

Table 5: Oil quality parameters of mechanically extracted crude cottonseed oil

Parameter	Value
Acid Value (mg KOH/g)	23.30 ± 3.02^{a}
Percentage Free Fatty Acids (%FFA)	11.72 ± 1.51^{b}
Peroxide Value (meq/kg)	99.58 ± 0.80^{c}

Values are expressed as Mean ± SEM (n = 3). Values with different superscript letters differ significantly at $p < 0.05$. Codex Alimentarius maximum limits for refined vegetable oils: AV ≤ 0.6 mg KOH/g; PV ≤ 1 meq/kg

The peroxide value (99.58 ± 0.80 meq/kg) was dramatically in excess of both the Codex Alimentarius maximum of 10 meq/kg for refined oils and the more lenient threshold of 15 meq/kg for cold-pressed oils, and is indicative of advanced oxidative deterioration. This degree of hydroperoxide accumulation is attributable to the high proportion of linoleic acid (C18:2) in cottonseed oil (50–60% of total fatty acids), as confirmed by He *et al.* (2022). Linoleic acid contains two double bonds with an intervening bis-allylic methylene carbon whose C–H bond dissociation energy is low due to resonance stabilisation of the resulting carbon-centred radical across the two adjacent π-bond systems, rendering hydrogen abstraction thermodynamically favourable and the acyl chain highly susceptible to autoxidation (Gharby *et al.*, 2025). Autoxidation proceeds through three stages: initiation in which external energy or catalytic transition metals abstract a bis-allylic hydrogen to generate a lipid alkyl radical; propagation in which the alkyl radical reacts at near diffusion-controlled rates with dissolved molecular oxygen to form a peroxyl radical that abstracts hydrogen from neighbouring polyunsaturated acyl chains in an autocatalytic cycle; and termination in which radical-radical coupling depletes the reactive intermediates (Riaz *et al.*, 2021). High storage temperatures, light exposure, and trace catalytic iron accelerate each step of this cascade, accounting for the magnitude of hydroperoxide accumulation observed. These findings are consistent with Karim *et al.* (2020), who reported very high peroxide values far in excess of regulatory limits for unrefined cottonseed samples. In aggregate, the acid value and peroxide value data establish conclusively that the crude, unrefined oil sourced from the Nigerian market is not suitable for direct human consumption and must undergo full industrial refining — encompassing degumming, alkali neutralisation, bleaching, and high-temperature deodorisation supplemented with approved antioxidant stabilisers to retard further oxidative deterioration during storage.

3.6. Mineral Elemental Composition of Cottonseed and Extracted Oil

The mineral composition of cottonseed and extracted oil is shown in Table 6. In the seed, calcium (178.8 mg/kg) was the most abundant element, followed by potassium (117.7 mg/kg) and iron (33.3 mg/kg). This order of mineral abundance is consistent with the pattern documented for Nigerian and West African cottonseed by Suleiman *et al.* (2024) and broadly in agreement with mineral profiles reported for near-isogenic cotton lines by Bellaloui *et al.* (2021), who demonstrated that genetic traits associated with leaf morphology directly influence the rate of calcium deposition into the developing seed. Calcium is a functionally critical macronutrient whose physiological roles encompass the structural mineralisation of the skeletal system, regulation of voltage-gated calcium channels that govern excitation-contraction coupling in cardiac and skeletal muscle, and activation of calcium-dependent intracellular signaling enzymes including calmodulin-dependent kinases and protein kinase C (Beshaw *et al.*, 2022). However, the effective intestinal absorption of calcium will be reduced in vivo by the phytate and oxalate anti-nutrients present in the seed matrix, both of which form insoluble calcium complexes within the intestinal lumen (Lin *et al.*, 2024; Abera *et al.*, 2023).

Table 6: Mineral elemental composition of cottonseed and extracted cottonseed oil

Mineral element	Cottonseed (mg/kg)	Extracted oil (mg/kg)
Calcium (Ca)	178.8	0.98
Potassium (K)	117.7	1.081
Iron (Fe)	33.3	ND
Zinc (Zn)	1.401	0.23
Manganese (Mn)	0.133	0.08
Copper (Cu)	0.085	ND
Nickel (Ni)	0.061	0.008
Lead (Pb)	ND	ND
Cadmium (Cd)	ND	ND

ND = Not Detected (below instrument detection limit). Values are single determinations per sample type

Potassium is the principal intracellular cation in mammalian cells, maintaining osmotic balance, membrane potential, and the activity of the Na^+/K^+-ATPase pump fundamental to neuronal action potential generation and transmission (Beshaw *et al.*, 2022). Iron (33.3 mg/kg) is of particular nutritional relevance in the Nigerian context, where iron deficiency anaemia remains a major public health problem. Iron is incorporated into haem-containing proteins, including haemoglobin, myoglobin, and the cytochrome chain enzymes of the mitochondrial inner membrane, and supports oxygen transport and oxidative phosphorylation through reversible Fe^{2+}/Fe^{3+} redox cycling. In line with concentrations documented for sesame and other major oilseeds by Beshaw *et al.* (2022), the iron concentration detected in this cottonseed represents a potentially useful dietary source of this element, provided that the phytate burden is reduced by appropriate processing before consumption.

Zinc, manganese, and copper were detected at lower but physiologically relevant concentrations. Zinc is a structural and catalytic cofactor for over three hundred metalloenzymes and is essential for deoxyribonucleic acid synthesis, immune cell proliferation, and the structural integrity of zinc-finger transcription factors (Mititelu *et al.*, 2025). Manganese is the obligate metal cofactor for mitochondrial manganese-superoxide dismutase, which catalyses dismutation of the superoxide anion radical in the mitochondrial matrix as a primary component of endogenous mitochondrial antioxidant defence (Mititelu *et al.*, 2025). Copper at the concentration recorded is required for lysyl oxidase activity, which catalyses collagen and elastin cross-linking in connective tissue, and for the cytoplasmic copper-zinc superoxide dismutase that scavenges superoxide radicals in the cytosol (Beshaw *et al.*, 2022). Nickel was detected at a low concentration unlikely to represent a toxicological concern at this level.

In the extracted oil, all mineral concentrations were markedly lower than in the seed, with potassium (1.081 mg/kg) and calcium (0.98 mg/kg) predominating. This outcome is expected on physicochemical grounds, as mineral ions are ionic and hydrophilic species that do not dissolve appreciably in the apolar triacylglycerol continuum of a vegetable oil. The trace mineral levels detected in the oil fraction are most likely attributable to carry-over within phospholipid micelles or metalloprotein fragments co-extracted with the lipid phase during mechanical pressing. The non-detection of lead and cadmium in both the pulverised cottonseed and the extracted oil constitutes an important food safety finding. Lead and cadmium are non-essential heavy metals whose toxicity operates through distinct mechanisms: lead inhibits delta-aminolaevulinic acid dehydratase in haem biosynthesis and displaces calcium at calmodulin binding sites, causing neurological damage and cardiovascular disease (Mititelu *et al.*, 2025), while cadmium accumulates in the proximal renal tubule over prolonged exposure, causing nephropathy and disrupted calcium homeostasis (Scutarașu and Trincă, 2023). The absence of both metals indicates that the cottonseed was cultivated and handled under conditions that did not result in significant heavy metal bioaccumulation. This finding is consistent with Bereda (2025), who similarly documented non-detection of lead and cadmium in locally sourced Nigerian oilseed samples, and confirms that the extracted oil would satisfy the European Commission Regulation (EU) 2023/915 maximum of 0.10 mg/kg for lead in edible fats and oils.

4. CONCLUSION

Nigerian-market cottonseed (*Gossypium hirsutum* L.) contains a diverse and biologically active phytochemical profile, with total phenolics and tannins as the dominant classes, alongside nutritionally relevant minerals, particularly calcium, potassium, and iron. The detected moderate antioxidant capacity in both the seed extract and the extracted oil reflects the combined contributions of polyphenols and lipid-soluble tocopherols, respectively. However, phytate represents the most significant anti-nutritional concern owing to its capacity to form insoluble coordination complexes with essential mineral cations, thereby reducing their intestinal bioavailability. Processing interventions such as fermentation, soaking, germination, or enzymatic phytase supplementation are strongly recommended to degrade phytic acid and enhance the mineral bioavailability of this seed before dietary incorporation. The substantially elevated acid value, percentage free fatty acids, and peroxide value of the crude extracted oil —all far in excess of Codex Alimentarius limits for refined edible vegetable oils demonstrate advanced hydrolytic and oxidative rancidity attributable to inadequate post-harvest handling under local tropical market conditions. Full industrial refining, including alkali neutralisation, bleaching, and high-temperature deodorisation, supplemented with antioxidant stabilisation, is therefore necessary to render the oil safe and suitable for food and industrial use. The non-detection of lead and cadmium in both the seed and oil confirms that the cottonseed was grown and handled without significant toxic heavy metal bioaccumulation, which is a positive food safety indicator for this commodity within the Nigerian market context.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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