Original Article

Open Access
Correspondence;

Citation: *Ezeja Enechojo Peace, Adie Peter Agorye, Ogo Ogo (2026) Nutritional Characterization of African Locust Bean (Parkia biglobosa) Seed Protein Isolates Recovered by Green Extraction Methods*

Date of Publication:
Vol. 9 (1), 2026

pp 358-368

NUTRITIONAL CHARACTERIZATION OF AFRICAN LOCUST BEAN (*Parkia biglobosa*) SEED PROTEIN ISOLATES RECOVERED BY GREEN EXTRACTION METHODS

Ezeja Enechojo Peace[1,2]; Adie Peter Agorye[2]; Ogo Ogo[3]
[1]Department of Nutrition and Dietetic, Faculty of Agriculture, University of Nigeria, Nsukka
[2]Department of Chemistry, Faculty of Sciences, Rev. Fr. Moses Orshio Adasu University, Makurdi, Nigeria
[3]Department of Biochemistry, College of Health Sciences, Rev. Fr. Moses Orshio Adasu University, Makurdi, Nigeria
*Corresponding Author: peace.ezeja@unn.edu.ng

ABSTRACT

African locust bean (Parkia biglobosa) is an underutilised West African legume consumed mainly as a fermented condiment, and its potential as a source of plant protein isolate remains largely unexplored. Conventional alkaline solubilisation-isoelectric precipitation exposes protein to harsh pH conditions that can degrade amino acids and reduce protein quality, prompting interest in greener alternatives such as natural deep eutectic solvents (NADES) and ultrasound-assisted extraction. This study characterised the proximate composition of African locust bean flour and comparatively evaluated the amino acid profile and anti-nutrient content of protein isolates recovered by three methods: conventional alkaline solubilisation-isoelectric precipitation (ALK), natural deep eutectic solvent extraction (NDS), and ultrasound-assisted NADES extraction (ULT) methods. African locust bean seeds were processed into flour and protein was extracted by the three methods. Proximate composition of the seed flour, amino acid profiles and antinutrient contents of the isolated proteins were determined using standard methods, and data were analysed using SPSS version 23, with significance accepted at $p < 0.05$. African locust bean seed flour contained 28.67% protein, 28.23% fat, 8.40% fibre, 6.34% moisture, 1.94% ash and 26.43% carbohydrate. Total amino acid content was highest in NDS (31.42 g/100 g), followed by ULT (26.19 g/100 g) and ALK (25.14 g/100 g), and anti-nutrient levels declined substantially from flour to isolates, with tannins and trypsin inhibitor undetectable in most isolates. Natural deep eutectic solvent extraction produced protein isolates with the highest and best-preserved amino acid content alongside the most comprehensive anti-nutrient reduction, positioning it as a promising green alternative to conventional alkaline extraction for valorising African locust bean as a plant protein source.

KEYWORDS: **African Locust Bean, Protein Isolate, Green Extraction, Nutritional Characterization.**

INTRODUCTION

Rising global population and the environmental cost of animal protein production have intensified interest in valorising underutilised plant protein sources capable of meeting nutritional demand sustainably (Kumoro *et al.,* 2024).

African locust bean (*Parkia biglobosa*), locally known as "dawadawa" after its fermented seed product, is an indigenous leguminous tree widely distributed across the West African savanna, where its seeds provide an important, affordable source of dietary protein, particularly for rural households (Termote *et al.,* 2022). Despite its nutritional and socioeconomic importance, the seed remains comparatively underexploited relative to major grain legumes such as soybean and remains largely restricted to traditional fermentation and household consumption (Kabir and Rukayat, 2024). Much of the existing literature on *P. biglobosa* has focused on its fermented product or the proximate composition of the whole seed rather than on its protein fraction as an isolate.

Protein isolates, concentrated protein fractions from which fat, fibre and carbohydrate have been removed, are of particular interest for food fortification because they offer higher protein density and more standardised functional properties than whole flours. The conventional method for producing plant protein isolates, alkaline solubilisation followed by isoelectric precipitation, achieves high protein yields but requires substantial acid and alkali, generates saline wastewater, and exposes protein to conditions that promote unfolding, aggregation and amino acid degradation (Kumoro et al., 2024).

These drawbacks have driven interest in greener extraction alternatives, particularly natural deep eutectic solvents (NADES): biodegradable, low-toxicity liquids formed from hydrogen-bond donors and acceptors such as choline chloride and glycerol, which solubilise protein under considerably milder conditions than alkaline extraction and better preserve protein structure (Kumoro *et al.,* 2024; Hanafi et al., 2024). NADES efficiency can be further enhanced by coupling with ultrasound, where acoustic cavitation disrupts cell walls and increases mass transfer, potentially improving protein recovery within a shorter extraction time (Rahman & Lamsal, 2021).

Legume seeds, including *P. biglobosa*, also contain anti-nutritional factors such as tannins, phytic acid and trypsin inhibitors that bind protein and minerals, inhibit digestive enzymes, and can reduce protein digestibility and amino acid bioavailability if not adequately removed (Gilani et al., 2012; Samtiya et al., 2020). Because these compounds differ in physicochemical properties, the extraction method is expected to influence not only protein yield and amino acid retention but also the residual anti-nutrient burden of the isolate.

Although alkaline, NADES and ultrasound-assisted extraction have each been investigated individually in various oilseed and legume systems, direct comparisons of how these methods affect the amino acid profile and anti-nutrient content of protein isolates from the same starting material remain scarce, and none has previously been reported for *P. biglobosa*.

This study therefore characterised the proximate composition of African locust bean flour and comparatively evaluated the amino acid profile and anti-nutrient (tannin, phytate and trypsin inhibitor) content of protein isolates recovered using conventional alkaline extraction, NADES extraction, and ultrasound-assisted NADES extraction methods, with a view to identifying the method best suited to producing a nutritionally superior, food-safe

protein isolate from this underutilised legume.

MATERIALS AND METHODS

Sample Collection and Preparation

African locust bean (ALB) seeds were sourced from Orba market in Udenu Local Government Area, Enugu state, Nigeria. The seeds were manually cleaned, soaked in clean water for 36 hours (water changed every 12 hours), and manually de-pulped after the first 12 hours before soaking for the remaining 24 hours. The seeds were then boiled for 1 hour, cooled, manually de-hulled, and dried to constant weight at 55°C. The dried seeds were milled into fine powder and stored in an airtight container until analysis.

Proximate Analysis of ALB Flour

Proximate composition (moisture, protein, crude fat, fibre, ash and carbohydrate) was determined according to AOAC (2023). Total protein (Kjeldahl) was calculated as %N × 6.25; moisture by drying at 105°C for 8 h; crude fat using a Dionex ASE 350 system; crude ash by ashing at 550°C overnight; and crude fibre by sequential acid/alkali digestion, filtration and ashing of a defatted sample. Carbohydrate content was calculated by difference: Total carbohydrates (%) = 100 − [crude protein + moisture + fibre + crude fat + crude ash].

ALB flour was defatted with hexane at a ratio of 1:10 (w/v) in three cycles for 1 hour.

Synthesis of natural deep eutectic solvent (NADES)

Natural deep eutectic solvent (NADES) was prepared according to the methods described by Kibar and Karabulut (2025) by combining choline chloride (ChCl) with glycerol at a molar ratio of 1: 2 (ChCl: glycerol). The mixture was stirred at 80 °C for approximately 2 h in a sealed beaker until a clear, homogeneous liquid was formed.

The NADES was subsequently diluted with 40% (v/v) distilled water to reduce viscosity and improve mass transfer while maintaining the eutectic hydrogen-bond network (Vilkovská et al., 2020). The prepared solution was cooled to room temperature (approximately 22 °C) and stored until use.

Protein extraction

For each extraction method, defatted (with hexane at a ratio of 1:10 (w/v) in three cycles for 1 hour) ALB flour (approximately 25 g) was mixed with 250 mL of extraction solvent at a 1:10 (w/v) solid-to-solvent ratio and processed as described as follows: before extraction, all the suspensions were centrifuged at 4,000 rpm for 40 min at 25 °C. Protein precipitates were then recovered, washed three times each with 40 mL distilled water (4,000 rpm, 4 °C, 30 min per wash), freeze-dried, weighed, and stored in an airtight container until analysis. The different extraction methods (conventional alkaline extraction, NADES extraction, and ultrasound-assisted NADES) differed only in the extraction and precipitation steps, as follows.

Natural deep eutectic solvent (NDS) extraction

The ALB–NADES suspension was stirred for 1 h at room temperature using a magnetic stirrer. Protein precipitation was induced by adding distilled water to the supernatant at a 5:1 (v/v) water-to-NADES ratio, followed by overnight cold antisolvent precipitation at 4 °C.

Ultrasound-assisted NADES (ULT) extraction

This procedure was adapted from Kibar and Karabulut (2025), with minor

modifications. The ALB–NADES suspension was first stirred for 30 min at room temperature, then subjected to ultrasonic extraction using a probe-type processor (Sonics, 750 W, USA; 13 mm tip) operating at 400 W, 30% amplitude, in pulsed mode (5 s on/5 s off) for 30 min, with the suspension temperature maintained at 25 ± 5 °C using an ice bath. Protein precipitation followed the same 5:1 (v/v) water-to-NADES antisolvent procedure described above.

Conventional alkaline (ALK) extraction

Defatted ALB flour was suspended in distilled water (1:10 w/v), and the pH was adjusted to 10.0 with 1 M NaOH. The suspension was stirred at 50 °C for 1 h to solubilise the protein, and precipitation was induced by adjusting the resulting supernatant to pH 5.0 with 1 M HCl.

Amino acid profile determination

The amino acid profile was determined according to Kibar and Karabulut (2025). Protein isolates were hydrolysed in 6 M HCl at 110 °C for 24 h in sealed, nitrogen-purged glass tubes containing the kit antioxidant system (Reagent-2, Jasem Amino Acid Analysis Kit, Germany) to minimise oxidative degradation. Hydrolysates were centrifuged (13,000 rmp for 5 min), diluted 800-fold, and combined with a stable-isotope-labelled internal standard mixture and Reagent-1 (Jasem kit) prior to analysis.

Amino acids were separated on an Agilent 1260 Infinity HPLC fitted with a Jasem amino acid analytical column (3.0 × 100 mm, 3 µm; amine/weak-cation-exchange hybrid) at 30 °C, using a 7.5-min water/formic-acid–acetonitrile gradient at 0.7 mL/min, and detected on an Agilent 6460 triple quadrupole with positive-mode electrospray ionisation. Quantification used multiple-reaction monitoring with isotope-labelled internal standards and seven-point calibration curves (0.5–100 µM; $R^2 \geq 0.995$), with quality-control samples included in each batch.

Antinutrient determinations

Trypsin inhibitors, tannins and phytic acid in the ALB protein isolates were determined using established methods, as described below.

Tannins

Tannin content was determined by the Folin–Ciocalteu method with PVPP clarification (Makkar, 2003). Samples (0.2 g) were extracted in 70% aqueous acetone (2 h, room temperature) and centrifuged. Total phenolics in the extract were measured by reaction with Folin–Ciocalteu reagent and sodium carbonate (absorbance at 725 nm); non-tannin phenolics were measured after PVPP treatment of a parallel aliquot. Tannin content was calculated as the difference between total and non-tannin phenolics and expressed as mg tannic acid equivalent per 100 g dry weight.

Phytic acid

Phytate content was determined using the Megazyme Phytic Acid Assay Kit (K-PHYT). Samples (1.0 g) were extracted in 0.66 M HCl (3 h, room temperature), and an aliquot of the clarified extract was hydrolysed with phytase and alkaline phosphatase (40 °C, 1 h) to release inorganic phosphate. Phosphorus was quantified colorimetrically (655 nm) against a phosphorus standard curve, and the result was multiplied by 3.55 to obtain phytic acid content (McKie & McCleary, 2016).

Trypsin inhibitor

Trypsin inhibitor activity (TIA) was determined spectrophotometrically based on inhibition of trypsin activity (Kakade et al., 1974). Sample (1 g) was extracted in 0.01 M NaOH (3 h, room temperature) and

centrifuged. The extract was incubated with standard trypsin solution and the substrate BAPNA (37 °C, 10 min); the reaction was stopped with 30% acetic acid, and released p-nitroaniline was measured at 410 nm. TIA was calculated from the absorbance decrease relative to an inhibitor-free control and expressed as trypsin inhibitor units per milligram of sample.

Data Analysis

Data were analysed using IBM SPSS Statistics version 23. One-way ANOVA with Turkey's test was used to evaluate significance at $p < 0.05$. Results are presented as mean ± standard deviation (n = 3).

RESULTS

Proximate composition of African Locust Bean Flour

The proximate composition of African locust bean (*Parkia biglobosa*) flour (Table 1) revealed moisture content of 6.34 ± 0.05%, protein content of 28.67 ± 0.07%, ash content of 1.94 ± 0.03%, crude fat content of 28.23 ± 0.08%, crude fibre content of 8.40 ± 0.05%, and carbohydrate content of 26.43 ± 0.09%.

Table 1: Results of Proximate analysis of African locust bean flour

Parameter	Mean ± SD (%)
Moisture	6.34 ± 0.05
Protein	28.67 ± 0.07
Ash	1.94 ± 0.03
Crude fat	28.23 ± 0.08
Crude fibre	8.40 ± 0.05
Carbohydrates	26.43 ± 0.09

Values are presented as mean ± standard deviation (n = 3). Means within the same column bearing the different superscript letter are significantly different at P< 0.05, where ALK =Alkaline Solubilization-Isoelectric Precipitation, NDS= Natural Deep Eutectic Solvent, ULT= Ultrasound Assisted Natural Deep Eutectic Solvent Extraction Methods.

Amino Acid Composition of Protein Isolates

Table 2 shows the amino acid composition of the isolates obtained using the three extraction methods. Glutamic acid was the most abundant amino acid in all samples (9.06–11.19 g/100 g protein), followed by aspartic acid (4.40–5.25 g/100 g protein). Most amino acids generally followed the trend NDS ≥ ALK ≈ ULT, although leucine followed ULT ≈ NDS > ALK, phenylalanine followed ULT > NDS > ALK, glycine and threonine followed ULT > NDS ≈ ALK, valine and tryptophan followed NDS > ALK > ULT, and alanine followed NDS ≈ ALK > ULT. Glutamine was detected in ALK and NDS but not in ULT. Total amino acid content was highest in NDS (31.42 g/100 g), followed by ULT (26.19 g/100 g) and ALK (25.14 g/100 g).

Table 3: Amino acid composition of African locust bean protein isolate samples (g/100g protein).

Amino acid	ALK	NDS	ULT
Aspartic acid	$4.40^{a} \pm 1.02$	$5.25^{a} \pm 0.36$	$4.50^{a} \pm 0.10$
Glutamic acid	$9.62^{a} \pm 2.20$	$11.19^{a} \pm 0.70$	$9.06^{a} \pm 0.22$
Serine	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.01$	$0.04^{b} \pm 0.00$
Glutamine	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.01$	—
Histidine	$1.24^{a} \pm 0.10$	$1.32^{a} \pm 0.09$	$1.16^{a} \pm 0.03$
Glycine	$0.61^{a} \pm 0.08$	$0.61^{a} \pm 0.04$	$0.78^{b} \pm 0.02$
Threonine	$0.04^{a} \pm 0.01$	$0.03^{a} \pm 0.00$	$0.30^{b} \pm 0.01$
Arginine	$2.87^{a} \pm 0.32$	$2.97^{a} \pm 0.17$	$2.90^{a} \pm 0.07$
Alanine	$2.19^{a} \pm 0.27$	$2.25^{a} \pm 0.13$	$0.01^{b} \pm 0.00$
Tyrosine	$1.79^{a} \pm 0.21$	$1.83^{a} \pm 0.10$	$1.73^{a} \pm 0.05$
Cystine	$0.29^{a} \pm 0.04$	$0.27^{a} \pm 0.27$	$0.23^{a} \pm 0.21$
Valine	$0.40^{b} \pm 0.04$	$0.53^{a} \pm 0.06$	$0.34^{b} \pm 0.05$
Methionine	$0.03^{a} \pm 0.01$	$0.04^{a} \pm 0.01$	$0.03^{a} \pm 0.01$
Tryptophan	$0.33^{c} \pm 0.04$	$0.44^{a} \pm 0.02$	$0.19^{b} \pm 0.02$
Phenylalanine	$0.98^{a} \pm 0.27$	$1.07^{a} \pm 0.07$	$1.30^{a} \pm 0.04$
Isoleucine	$0.05^{a} \pm 0.01$	$0.06^{a} \pm 0.00$	$0.04^{b} \pm 0.00$
Leucine	$0.14^{c} \pm 0.03$	$3.40^{a} \pm 0.06$	$3.53^{a} \pm 0.11$
Lysine	$0.06^{a} \pm 0.00$	$0.06^{a} \pm 0.00$	$0.05^{b} \pm 0.00$
Total amino acids	**25.14**	**31.42**	**26.19**

Values are presented as mean ± standard deviation (n = 3). Means within the same column bearing the different superscript letter are significantly different at **P<** 0.05, where ALK =Alkaline Solubilization-Isoelectric Precipitation, NDS= Natural Deep Eutectic Solvent, ULT= Ultrasound Assisted Natural Deep Eutectic Solvent Extraction Methods.

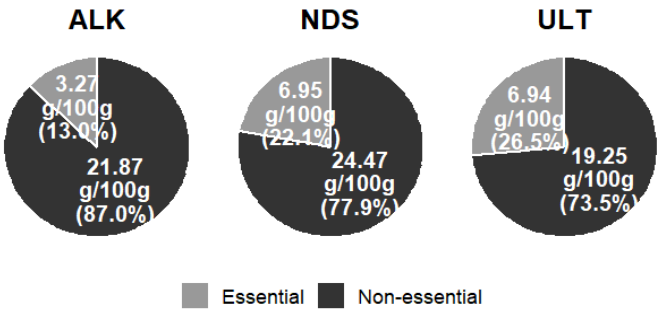


Figure 1: Essential and non-essential amino acid proportion

Anti-Nutrient Content of Flour and Protein Isolates

Table 3 shows that African locust bean flour (ALBF) had the highest tannin content (1.627 ± 0.564 mg/100 g), followed by ALK (0.643 ± 0.326 mg/100 g), while tannins were not detected (ND) in the NDS and ULT isolates. Phytate content decreased from 0.843 ± 0.110% in ALBF to 0.139 ± 0.072%, 0.170 ± 0.014%, and 0.135 ± 0.037% in ALK, NDS, and ULT, respectively. Trypsin inhibitor content was 0.167 ± 0.023 TIU in ALBF, was not detected in ALK and NDS, and was 0.130 ± 0.000 TIU in ULT.

Table 4: Anti-nutrient content of African locust bean flour and protein isolates

Sample	Tannins (mg/100 g)	Phytate (%)	Trypsin inhibitor (TIU)
ALBF	$1.627^{a} \pm 0.564$	$0.843^{a} \pm 0.110$	$0.167^{a} \pm 0.023$
ALK	$0.643^{a} \pm 0.326$	$0.139^{b} \pm 0.072$	ND
NDS	ND	$0.170^{b} \pm 0.014$	ND
ULT	ND	$0.135^{b} \pm 0.037$	$0.130^{a} \pm 0.000$

Values are presented as mean ± standard deviation (n = 3). Means within the same column bearing the different superscript letter are significantly different at ***P****< 0.05, where ALK =Alkaline Solubilization-Isoelectric Precipitation, NDS= Natural Deep Eutectic Solvent, ULT= Ultrasound Assisted Natural Deep Eutectic Solvent Extraction Methods.*

DISCUSSION

The moisture content (6.34%) was lower than the 20.20% reported for raw *P. biglobosa* seeds by Gberikon et al. (2023), likely reflecting differences in drying and storage conditions. Protein content (28.67%) was higher than the 20.56% reported by Oshido *et al.* (2024) but lower than the 33.50% reported by Soetan *et al*. (2014), while fat content (28.23%) exceeded the 20.00% reported by Gberikon *et al.* (2023) but was lower than the 49.20% reported by Soetan *et al.* (2014); such differences likely reflect variation in genotype, geographical origin, maturity and processing. Ash content (1.94%) was lower than values reported by Oshido *et al.* (2024) and Gberikon *et al.* (2023), while crude fibre (8.40%) was slightly higher than the 6.00% reported by Oshido *et al.* (2024). Carbohydrate content (26.43%), calculated by difference, was correspondingly lower than values reported in both studies. Overall, the proximate composition obtained falls within the broad range previously reported for *P. biglobosa*, with variation attributable to genetic background, geography, maturity, processing and analytical methodology. The relatively high protein and fat contents indicate considerable potential for *P. biglobosa* flour as a protein- and energy-rich food ingredient.

The predominance of glutamic acid, aspartic acid and arginine is consistent with previous reports on *P. biglobosa* protein isolates (Osunsanmi et al., 2024; Masters & Kelly, 2024) and with other grain legumes such as soybean and cowpea, in which acidic amino acids typically dominate total amino acid content. Although absolute concentrations of most individual amino acids were lower than reported by Osunsanmi et al. (2024), the qualitative distribution was preserved across all three extraction treatments, with quantitative differences likely reflecting variation in genotype, maturity, isolation protocol,

solvent and pH, drying conditions, and analytical methodology.

Viewed as a whole, the amino acid profile was best preserved by NDS, intermediate under ULT, and most degraded under ALK, reflecting the harshness of the extraction environment rather than amino-acid-specific chemistry alone. NDS operates under mild, near-neutral conditions, avoiding alkaline-induced side reactions such as lysinoalanine formation and cross-linking that degrade specific residues (Hanafi et al., 2024), consistent with its highest total amino acid content (31.42 g/100 g) and balanced retention across residues. ALK, by contrast, showed the lowest total recovery, driven largely by near-total loss of leucine, consistent with alkaline-induced unfolding and side-chain modification of buried residues (Hewage *et al.*, 2024; Đermanović et al., 2025).

ULT produced a distinctly bidirectional effect reflecting the dual physical and oxidative nature of ultrasonic cavitation: cavitation-induced unfolding enhanced extractability of residues in flexible or buried regions (e.g., phenylalanine, glycine, threonine), consistent with the established mechanical action of ultrasound-assisted extraction (Rahman & Lamsal, 2021), while the same process generated hydroxyl radicals that oxidatively degraded exposed residues (e.g., valine, tryptophan, alanine) and promoted glutamine deamidation (Custodio-Mendoza et al., 2024). This is also evident at the essential/non-essential level: ULT recorded the highest essential amino acid fraction (26.5%) but an absolute essential amino acid content statistically unchanged from NDS (6.94 g/100 g), indicating that its apparent advantage largely reflected oxidative depletion of the non-essential pool rather than genuine enrichment (Wang et al., 2021; Matthews *et al.*, 2025).

Anti-nutrient concentrations declined substantially from flour to all three isolates, but the pattern of removal differed by class. Tannin removal was most complete under NDS and ULT, where compounds were undetectable, while ALK retained a measurable residue, best explained by a separation effect, as NADES-based systems selectively partition low-molecular-weight phenolics away from protein, whereas tannins co-solubilise with protein at high pH and fail to separate completely during isoelectric precipitation (Hanafi *et al.*, 2024; Đermanović *et al.*, 2025). Phytate reduction was essentially uniform across all three methods, reflecting its high water solubility and pH/ionic-strength-dependent behaviour, which allows it to be washed away during any aqueous isolation procedure regardless of solvent identity (Zhao, 2023); the small residual phytate (~0.13–0.17%) likely reflects a fraction complexed with protein near the isoelectric point. Trypsin inhibitor was fully inactivated in ALK and NDS but remained detectable in ULT; because trypsin inhibitors are compact, disulfide-stabilised proteins requiring strong pH or heat-driven denaturation for inactivation (Hu et al., 2025), the predominantly mechanical action of ultrasonic cavitation was evidently insufficient to fully denature them, despite enhancing mass transfer and cell disruption.

CONCLUSION

This study demonstrated that the method used to recover protein isolates from African locust bean seed markedly influences both amino acid retention and anti-nutrient content. Natural deep eutectic solvent extraction, conducted under mild, near-neutral conditions, yielded the isolate with the highest total amino acid content and the most complete removal of tannins, phytate and trypsin inhibitor activity, confirming its potential as a green alternative to conventional alkaline

extraction for producing high-quality protein isolates from this underutilised legume.

Ultrasound-assisted natural deep eutectic solvent extraction offered a favourable essential amino acid fraction but exhibited a bidirectional pattern of amino acid gain and oxidative loss, and left trypsin inhibitor activity only partially inactivated. Conventional alkaline extraction, by contrast, produced the isolate with the lowest total amino acid content, driven largely by near-total loss of leucine, consistent with alkaline-induced protein degradation. Further work is needed to optimise ultrasound-assisted conditions and to evaluate the functional and sensory properties of the isolates in real food systems.

ACKNOWLEDGMENTS

The author acknowledges the German Academic Exchange Service (DAAD) for providing funding for this research.

REFERENCES

AOAC (2023). Official method 986.25: Proximate analysis of milk-based infant formula. In: Official Methods of Analysis of AOAC INTERNATIONAL. Oxford University Press.

Custodio-Mendoza, J. A., Pokorski, P., Aktaş, H. and Kurek, M. A. (2024). Rapid and efficient high-performance liquid chromatography-ultraviolet determination of total amino acids in protein isolates by ultrasound-assisted acid hydrolysis. *Ultrasonics Sonochemistry*, 111: 107082.

Đermanović, B., Marić, A., Sakač, M., Tomić, D., Dragojlović, D., Popović, L., Šarić, B. and Jovanov, P. (2025). Impact of alkaline and deep eutectic solvent extraction on rapeseed protein isolates characteristics and in vitro digestibility. *Current Research in Food Science*, 101075.

Gberikon, G. M., Orsar, T. and Agbakwuru, C. J. (2023). Analysis of thermophilic and halophilic bacteria in traditionally processed locust beans sold in Benue State. *FUDMA Journal of Sciences*, 7(3): 112-119.

Gilani, G. S., Xiao, C. W. and Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*, 108(Suppl. 2): S315-S332.

Hanafi, M. A., Anwar, F. and Saari, N. (2024). Valorization of biomass for food protein via deep eutectic solvent extraction: Understanding the extraction mechanism and impact on protein structure and properties. *Food Frontiers*, 5(3): 1265-1301.

Hewage, A., Olatunde, O. O., Nimalaratne, C., House, J. D., Aluko, R. E. and Bandara, N. (2024). Improved protein extraction technology using deep eutectic solvent system for producing high purity fava bean protein isolates at mild conditions. *Food Hydrocolloids*, 147: 109283.

Hu, X., Zheng, L., Wang, Y., Liu, T., Zhang, H. and Li, J. (2025). Advancements in inactivation of soybean trypsin inhibitors. *Foods*, 14(6): 975.

Kabir, A. M. and Rukayat, I. O. (2024). Transforming locust bean processing: A path to profit for elderly women's microbusiness in Sokoto, Nigeria. *African Journal of Social Sciences and Humanities Research*, 7(4): 77-88.

Kakade, M. L., Rackis, J. J., McGhee, J. E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of

an improved procedure. *Cereal Chemistry*, 51(3), 376–381.

Karabulut, G., Sanli, I. and Ozkan, G. (2025). Green extraction using natural deep eutectic solvents for the recovery of proteins from sunflower meal. *International Journal of Biological Macromolecules*, 147556.

Kibar, B. E. and Karabulut, G. (2025). Ultrasound-assisted deep eutectic solvent extraction versus alkaline extraction: Functional and structural properties of hazelnut proteins. *Food Chemistry*: X, 103080.

Kumoro, A. C., Wardhani, D. H., Kusworo, T. D., Djaeni, M., Ping, T. C. and Alhanif, M. (2024). Deep eutectic solvents and natural deep eutectic solvents for extraction and purification of proteins from animal and botanical sources—A review. *International Journal of Technology*, 15(5): 1420-1437.

Makkar, H. P. S. (2003). Measurement of total phenolics and tannins using Folin-Ciocalteu method. In: Quantification of tannins in tree and shrub foliage: A laboratory manual Makkar, H. P. S. (Ed.) *Springer Netherlands*, 49-51.

Masters, E. T. and Kelly, B. A. (2024). Protein quality of African locust bean—A high-value gathered tree food contributing protein and palatability to plant-based diets. *International Journal of Food Science*, 2024: 1596212.

Matthews, J. J., Arentson-Lantz, E. J., Moughan, P. J., Wolfe, R. R., Ferrando, A. A. and Church, D. D. (2025). Understanding dietary protein quality: Digestible indispensable amino acid scores and beyond. *The Journal of Nutrition*, 155(10): 3152-3167.

McKie, V. A. and McCleary, B. V. (2016). A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. *Journal of AOAC INTERNATIONAL*, 99(3): 738-743.

Oshido, B. A., Aondoaver, S. and Oshido, J. I. (2024). Amino acid profile and proximate analysis of fermented African locust bean (*Parkia biglobosa*) seeds. *International Research Journal of Pure and Applied Chemistry*, 25(5): 123-131.

Osunsanmi, F. O., Ogunyinka, B. I., Oyinloye, B. E., Opoku, A. R. and Kappo, A. P. (2024). Effect of processing methods on amino acid and fatty acid composition of Parkia biglobosa seeds. *Applied Sciences*, 14(22): 10106.

Rahman, M. M. and Lamsal, B. P. (2021). Ultrasound-assisted extraction and modification of plant-based proteins: Impact on physicochemical, functional, and nutritional properties. *Comprehensive Reviews in Food Science and Food Safety*, 20(2): 1457-1480.

Samtiya, M., Aluko, R. E. and Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production, Processing and Nutrition*, 2: 6.

Soetan, K. O., Akinrinde, A. S. and Adisa, S. B. (2014). Comparative studies on the proximate composition, mineral and anti-nutritional factors in the seeds and leaves of African locust bean (Parkia biglobosa). *Annals. Food Science and Technology*, 15(1): 70-74.

Termote, C., Odongo, N. O., Dreyer, B. S., Guissou, B., Parkouda, C. and Vinceti, B. (2022). Nutrient composition of Parkia biglobosa pulp, raw and fermented seeds: A

systematic review. *Critical Reviews in Food Science and Nutrition*, 62(1): 119-144.

Vilkovská, M., Płotka-Wasylka, J. and Andruch, V. (2020). The role of water in deep eutectic solvent-based extraction. *Journal of Molecular Liquids*, 304: 112747.

Wang, Q., Wang, Y., Huang, M., Hayat, K., Kurtz, N. C., Wu, X., Ahmad, M. and Zheng, F. (2021). Ultrasound-assisted alkaline proteinase extraction enhances the yield of pecan protein and modifies its functional properties. *Ultrasonics Sonochemistry*, 80: 105789.

Zhao, Y., Tian, R., Xu, Z., Jiang, L. and Sui, X. (2023). Recent advances in soy protein extraction technology. *Journal of the American Oil Chemists' Society*, 100(3): 187-195.