



MORPHOLOGICAL AND THERMAL PROPERTIES OF PROTEIN ISOLATED FROM NIGER SEEDS USING WET PROCESSING TECHNIQUES

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ABSTRACT

This study investigated the extraction, characterization, and functional properties of protein concentrates obtained from Niger seed (*Guizotia abyssinica*) using three wet extraction methods: alkaline, acid, and water extraction. The objective was to evaluate the influence of extraction techniques on the physicochemical, structural, thermal, and functional characteristics of the resulting proteins. Niger seed flour was processed through alkaline extraction (pH 9.0), acid extraction (pH 2.0), and water extraction, followed by centrifugation, protein recovery, and drying. The obtained protein concentrates were analysed for proximate composition, functional properties, morphology, molecular structure, thermal behaviour, and colour characteristics.

Among the methods, alkaline extraction produced the highest protein content (71.00%), followed by acid extraction (66.33%) and water extraction (52.33%). Water-extracted samples retained higher fat and carbohydrate contents, while acid-extracted proteins showed relatively higher fibre content. Scanning Electron Microscopy (SEM) revealed distinct morphological differences among the protein concentrates, whereas Fourier Transform Infrared Spectroscopy (FTIR) confirmed variations in functional groups and molecular interactions resulting from the extraction conditions. Differential Scanning Calorimetry (DSC) demonstrated that water-extracted proteins exhibited the highest thermal stability, while alkaline- and acid-extracted proteins showed comparatively lower thermal resistance.

Functional property analysis indicated that alkaline-extracted proteins possessed superior water absorption, foaming, solubility, and emulsifying properties. Acid-extracted proteins exhibited the highest fat absorption capacity, whereas water-extracted proteins showed lower functional performance but greater thermal stability. These findings demonstrate that extraction methods significantly influence the composition, structure, thermal characteristics, and functionality of Niger seed proteins. Overall, alkaline extraction was the most effective method for obtaining protein concentrates with enhanced techno-functional properties, highlighting the potential of Niger seed proteins as sustainable functional ingredients for various food applications, including bakery products, meat analogues, and emulsified food system.

Key words: Wet processing techniques, Isolated protein, Niger seeds

1. INTRODUCTION

Research on Niger seeds (*Guizotia abyssinica*) encompassed various aspects including genomic resources, oil extraction methods, nutritional and anti-nutritional composition, and biochemical properties of the seed oil. *Guizotia abyssinica*, commonly known as noug or Niger seed, was an oilseed crop with significant potential both nutritionally and industrially.

Genomic studies had advanced recently, providing critical molecular tools for crop improvement. RNA-Seq transcriptome sequencing of 30 genotypes of noug produced a large unigene dataset, identifying over 40,000 simple sequence repeats (SSRs) and numerous single nucleotide polymorphisms (SNPs) useful for genetic diversity assessment and breeding programs. Genotypes varied in photoperiod sensitivity, oil content, and other traits. Genetic analyses revealed four major clusters and three genetic populations with admixture, enabling the development of superior cultivars through crossbreeding (Gebeyehu *et al.*, 2022). Another genomic resource development involved sequencing the chloroplast genome of *Guizotia abyssinica*, facilitating phylogeographic studies and comparison with closely related species like sunflower.

On the industrial processing side, optimized oil extraction techniques had been assessed using microwave irradiation and pulsed electric fields (PEF) as pretreatments before screw pressing. These methods affected extraction efficiency and quality parameters like acidity, peroxide values, chlorophyll, phenolics, and tocopherol content. Linoleic acid was the predominant fatty acid in Niger seed oil, and microwave-PEF pretreatments enhanced α -tocopherol levels by about 2.79%, although an increase in saturated fatty acids at the expense of unsaturated ones occurred (Mohseni *et al.*, 2020).

Nutritionally, Niger seed was rich in essential minerals and bioactive compounds but also contained anti-nutritional factors such as tannins and phytates. A mineral analysis across different varieties showed phosphorus as the most abundant mineral in Niger seed, ranging from 661 to 867 mg/100 g, while selenium was the least abundant (0.1 to 0.33 mg/100 g). The phytate content was high, averaging 353 mg/100 g, and tannin content ranged from 91 to 201 mg/100 g, indicating significant varietal differences in mineral and anti-nutritional composition crucial for dietary and feed considerations (Deme *et al.*, 2017).

The fatty acid profile and antioxidant properties of Niger seed oil were also characterized. Linoleic acid dominated the fatty acid spectrum at 73.3% to 76.8% depending on the variety. Total phenolic

content varied among varieties from 22.4 to 27.9 mg GAE/g, with associated variations in carotenoid and chlorophyll contents. Antioxidant capacity assays, including ferric reducing antioxidant power (FRAP) and radical scavenging activity, showed that Niger seed oil was a rich source of natural antioxidants, with certain varieties (e.g., Fogera) showing particularly high activity. These biochemical characteristics underscored the potential for use of Niger seed oil as a natural alternative to synthetic antioxidants in food industry applications (Deme *et al.*, 2021).

In addition, *Guizotia abyssinica* seeds had found use in microbiological media formulations. A simplified Niger seed medium was shown to be superior for rapid identification of *Cryptococcus neoformans* due to faster brown pigment production, improving diagnostic efficiency.

On the industrial processing front, optimization of Niger seed oil extraction was investigated using innovative pretreatments. Specifically, microwave irradiation combined with pulsed electric field (PEF) technology was evaluated prior to screw pressing. Optimization studies determined that microwave treatment for approximately 156 seconds, PEF intensity of 1.18 kV/cm, and screw press speed of 20 rpm maximized oil extraction efficiency and improved quality parameters such as chlorophyll, acidity, peroxide values, and total phenolics. While linoleic acid was the predominant fatty acid, pretreatment led to a small reduction in unsaturated fatty acids and an increase in saturated ones. Additionally, α -tocopherol content was enhanced by about 2.8% following microwave-PEF treatment (Mohseni *et al.*, 2020).

Nutritionally, Niger seeds were analyzed for mineral and anti-nutritional components across different varieties grown in Ethiopia. Phosphorus was the most abundant mineral, ranging between 661 and 867 mg per 100 g, while selenium was the least abundant. The seeds also contained significant amounts of phytate (average 353 mg/100 g) and tannins (91–201 mg/100 g), factors relevant for nutritional bioavailability and feed formulation. These results demonstrated considerable variability both within Niger seed varieties and among other oilseeds such as linseed and sesame (Deme *et al.*, 2017).

Further biochemical characterization of Niger seed oil revealed that linoleic acid constituted around 73–77% of the total fatty acids, supporting its nutritional desirability. The oil also possessed considerable antioxidant potential, with total phenolic content varying among varieties from 22 to 28 mg gallic acid equivalent per gram. Carotenoid and chlorophyll contents showed

similar varietal variation, and antioxidant activity assays (FRAP and radical scavenging) highlighted some varieties, such as Fogera, with superior antioxidant capacity. This richness in bioactive antioxidants suggested that Niger seed oil could serve as a natural alternative to synthetic antioxidants in food applications. The observed genetic variation underlined opportunities for breeding improved cultivars with enhanced antioxidant properties (Deme *et al.*, 2021).

Additionally, Niger seed was utilized in microbiological applications, particularly in the development of culture media. A simplified *Guizotia abyssinica* seed medium demonstrated superior performance for the rapid presumptive identification of *Cryptococcus neoformans* by promoting faster development of characteristic brown pigmentation compared to traditional media, enhancing diagnostic efficiency.

Taken together, these recent studies illustrated a comprehensive approach to leveraging Niger seed as a valuable oilseed crop. Genomic resources laid the groundwork for breeding and genetic improvement, while optimized pretreatment technologies improved oil yield and quality. Nutritional and biochemical profiles validated its potential for both human consumption and industrial food applications. Furthermore, its role in clinical microbiology media highlighted an interdisciplinary utility beyond agronomy. Continued research integrating these facets was expected to advance the sustainable cultivation and utilization of *Guizotia abyssinica*.

Nutritional assessment of Niger seeds (*Guizotia abyssinica*) was investigated focusing on their fatty acid profile, phenolic content, antioxidant capacity, and mineral and anti-nutritional components. A study by Kure *et al.* evaluated five varieties of Niger seed, revealing linoleic acid as the dominant fatty acid constituting 73.3% to 76.8% of total fatty acids. The total phenolic content in these varieties ranged from 22.4 to 27.9 mg gallic acid equivalents per gram (mg GAE/g), indicating substantial antioxidant constituents. Among varieties, Fogera exhibited the highest ferric reducing antioxidant power (FRAP) and radical scavenging activity, emphasizing notable antioxidant potential. Carotenoid content also varied significantly, from 2.57 to 8.08 $\mu\text{mol/g}$ among Niger seed varieties. The study concluded that Niger seed oil was rich in bioactive compounds with significant variation among varieties, suggesting genetic diversity could be harnessed for breeding programs targeting enhanced nutritional traits (Deme *et al.*, 2021).

Further nutritional characterization of Niger seeds grown in Ethiopia by Mulugeta *et al.* assessed mineral and anti-nutritional factors across five Niger seed varieties. They reported

phosphorus as the most abundant mineral (661 to 867 mg/100 g), with selenium the least abundant (0.1 to 0.33 mg/100 g). Phytate and tannin contents were also quantified, averaging 353 mg/100 g and ranging from 91 to 201 mg/100 g, respectively. This variability in mineral and anti-nutrient levels underlined the importance of varietal selection to optimize nutritional value and bioavailability in dietary and feed applications (Deme *et al.*, 2017).

Together, these studies demonstrated that Niger seed was a nutritious oilseed with a lipid profile rich in heart-healthy linoleic acid, substantial natural antioxidants, and essential minerals, albeit with varying levels of anti-nutritional factors depending on variety. The findings supported efforts to exploit available genetic diversity in Niger seeds for breeding varieties with improved nutritional and functional qualities beneficial for food industry and health applications (Deme *et al.*, 2021; Deme *et al.*, 2017).

Current literature provided limited direct evidence specifically addressing the antioxidant, antimicrobial, and anticancer activities of Niger seeds (*Guizotia abyssinica*). However, some relevant insights into the biological effects and applications of Niger seed could be drawn from the available studies.

No direct research papers were found in the provided context specifically reporting anticancer activity of Niger seeds or their extracts. While other plant species and related compounds (e.g., coumarin-derived imine-metal complexes, mulberry fruits, mango leaves) had documented anticancer properties, such evidence was not available for *Guizotia abyssinica* in the current dataset. The rich phytochemical composition of Niger seed oil and presence of polyphenols and antioxidants might have implied potential for anticancer effects, but this remained to be explicitly investigated in empirical studies.

In summary, *Guizotia abyssinica* exhibited documented antioxidant potential related to its fatty acid profile and phenolic content. Its constituents had practical applications in microbiology for rapid pathogen identification, reflecting bioactive interactions that could relate to antimicrobial mechanisms.

However, explicit evidence for anticancer activity of Niger seed remained lacking in the reviewed literature. Further dedicated biochemical, pharmacological, and clinical research was necessary to establish and elucidate any anticancer or broader antimicrobial properties of Niger seeds. The currently documented antioxidant and microbiological diagnostic utilities indicated promising avenues for future exploration.

2. MATERIALS AND METHODS

2.1 Extraction of protein from Niger seeds

The Niger seeds were milled, and the resulting flour and water were used in a 1:12 ratio to create a flour suspension for alkaline extraction. The pH was adjusted to 9.0 using 1 N NaOH, and the mixture was kept at room temperature (25 °C) for 180 minutes. The mixture was centrifuged at 899xg for 15 min, supernatant and residue precipitates were obtained. To increase the yield of the protein alkaline extraction procedure is repeated for the residue. The supernatants were collected and subjected to isoelectric precipitation by adjusting the pH to 4.5 with 1 N HCl for 30 minutes at room temperature (25 °C). The precipitated protein extract was subsequently rinsed with water. Oven drying was implemented at a temperature of 75 °C for 5-6 hrs on that residue. The protein concentrate was obtained. Fig

2.1 Flow diagram for the preparation of Niger protein concentrate

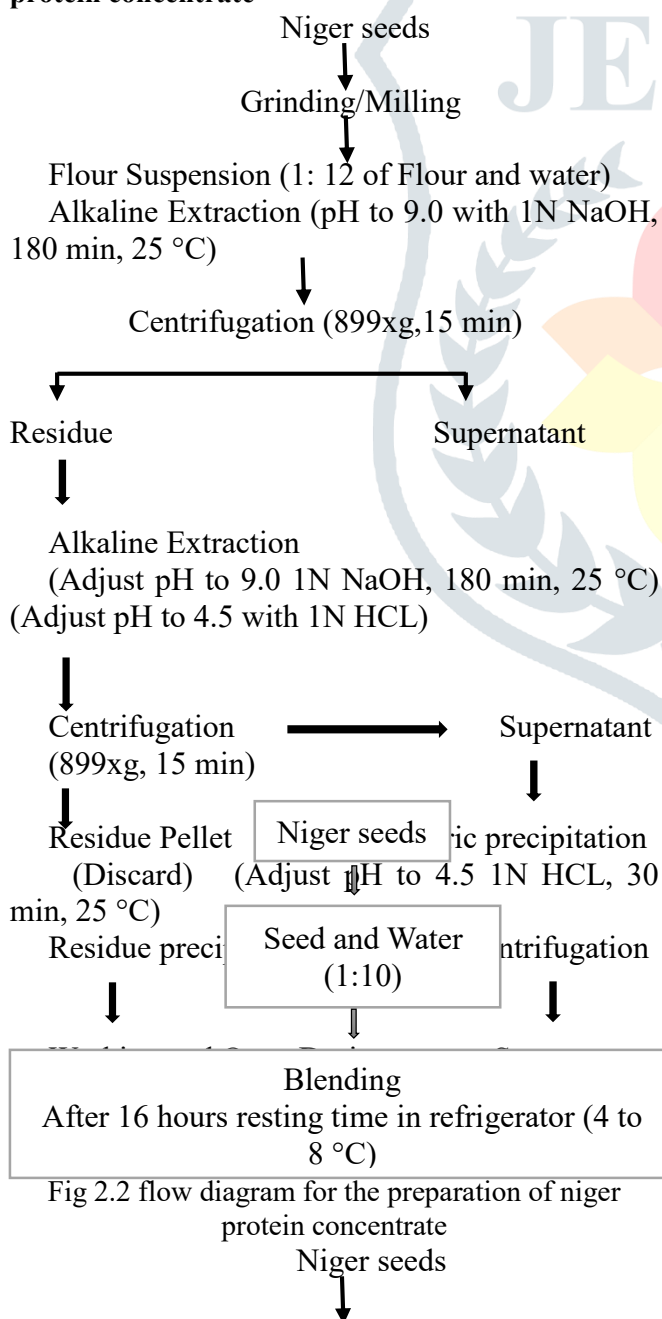
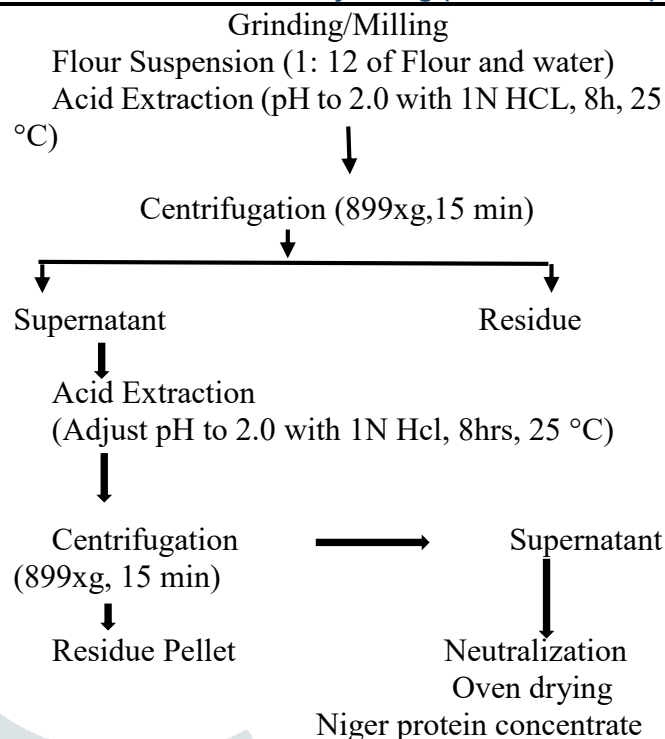


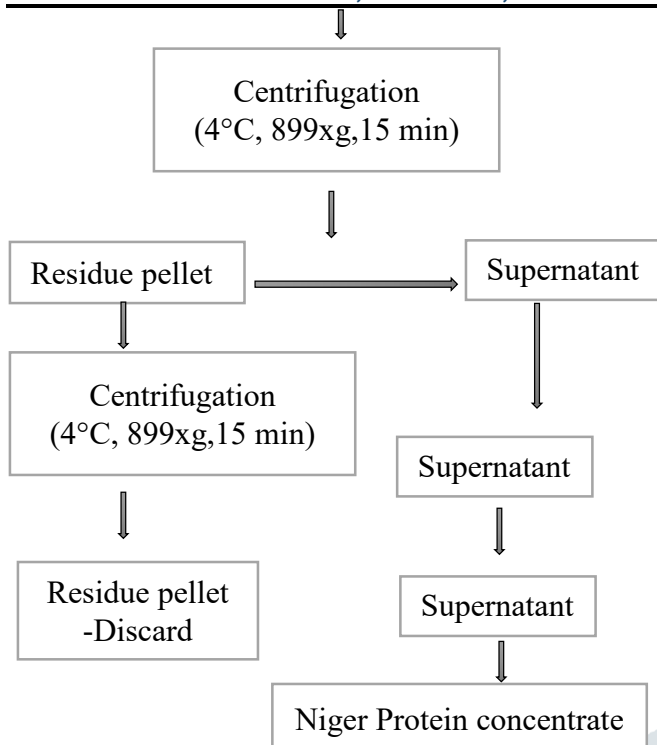
Fig 2.2 flow diagram for the preparation of niger protein concentrate



Niger seeds were ground or milled. The flour suspension was prepared by mixing flour and water in a ratio of 1:12. Acid extraction was carried out by adjusting the pH to 2.0 with 1N HCl and incubating for 8 hours at 25 °C. The mixture was then centrifuged at 899×g for 15 minutes. The residue obtained was subjected to another acid extraction under the same conditions (pH 2.0 with 1N HCl, 8 hours, 25 °C), followed by centrifugation at 899×g for 15 minutes. The residue pellet from this step was discarded. The supernatant from the first centrifugation was neutralized and oven-dried to obtain the Niger protein concentrate.

Method	Protein	Fat	Fiber	Moisture	Ash	Carbohydrates
AKNS	71.00 ±1.00	17.00 ±1.00	3.00 ±0.50	4.00 ±0.50	1.10 ±0.02	3.90 ±0.02
ACNS	66.33 ±1.27	15.50 ±1.32	3.50 ±0.50	4.33 ±0.28	1.30 ±0.26	9.03 ±0.05
WRNS	52.33 ±2.51	17.50 ±0.50	4.33 ±0.28	4.00 ±0.5	2.16 ±0.28	19.66 ±1.44

Fig 2.3 flow diagram for the preparation of niger protein concentrate



Niger seeds were mixed with water in a ratio of 1:12 and blended. The mixture was then left to rest in a refrigerator at a temperature range of 4 to 8 °C for 16 hours. Following this resting period, the blended mixture underwent centrifugation at 4 °C with a force of 899xg for 15 minutes. This process separated the mixture into a residue pellet and a supernatant. The residue pellet was subjected to a second centrifugation under the same conditions (4 °C, 899xg, 15 minutes), after which the resulting residue pellet was discarded. Meanwhile, the supernatant from the first centrifugation was collected and centrifuged again, yielding another supernatant. This final supernatant was then dried in an oven to produce the Niger Protein concentrate

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Niger seeds

Protein

Table 3.1 proximate composition of niger seed protein

The values in the provided table represented the proximate composition and some chemical properties of Niger seed under different treatments or conditions (alkaline, acid, water). Each parameter was given with its mean value $\pm$ standard deviation. Here was a detailed explanation of each component:

Protein indicated the percentage of protein content in the Niger seed samples. For example, under alkaline conditions, protein was $71 \pm 1\%$, which was quite high, suggesting a rich protein content.

Fat represented the fat content percentage. The values were relatively low (e.g., 17 ± 1 under alkaline), showing the lipid fraction in the seed.

Fiber showed the dietary fiber content percentage, important for nutritional and digestive

properties. Values varied slightly (e.g., 3 ± 0.5 under alkaline).

Moisture indicated the percentage of water content in the sample, reflecting freshness or dryness. Values like 4 ± 0.5 showed low moisture, typical for dried seed samples.

Ash represented the total mineral content remaining after combustion, indicating inorganic residue. Values around 1.1 ± 0.02 suggested mineral presence.

Carbohydrates were calculated by difference, representing the carbohydrate fraction in the seed. Values such as 3.9 ± 0.02 showed the energy-providing component.

Alkaline, Acid, Water likely referred to different extraction or treatment methods applied to the Niger seed samples, affecting the proximate values. For example, protein content decreased from alkaline (71 ± 1) to acid (66.33 ± 1.27) and water (52.33 ± 2.5), indicating the effect of treatment on protein solubility or extraction.

Overall, this table quantified the nutritional composition of Niger seed under various chemical conditions, highlighting how treatments influenced protein, fat, fiber, moisture, ash, and carbohydrate contents. Protein concentration varied notably with treatment. The Alkaline method produced the highest protein content ($71.00\% \pm 1.00$), indicating that alkaline conditions enhanced protein extraction or preservation, likely due to increased protein solubility at higher pH facilitating release from the seed matrix. The Acid method yielded a lower protein level ($66.33\% \pm 1.27$), suggesting that acidic conditions caused partial protein denaturation or less effective solubilization. The Water method resulted in the lowest protein content ($52.33\% \pm 2.51$), implying that extraction without pH adjustment was less efficient for protein recovery.

Fat content was highest in the Water treatment ($17.5\% \pm 0.50$), closely followed by the Alkaline method ($17.00\% \pm 1.00$), with the Acid method showing a reduced fat level ($15.5\% \pm 1.32$). The elevated fat in Water and Alkaline treatments suggested better lipid retention or less degradation during processing, whereas the acidic environment promoted fat loss or degradation, lowering fat content.

Fiber content was substantially elevated in the Acid method ($23.50\% \pm 0.50$), markedly higher than in the Alkaline ($3.00\% \pm 0.50$) and Water ($4.33\% \pm 0.28$) treatments. This increase under acidic conditions likely resulted from acid-induced solubilization or removal of other components (proteins, fats), concentrating fiber or altering the seed matrix to enhance fiber measurement.

Moisture levels remained relatively stable across treatments: Acid ($4.33\% \pm 0.28$), Water

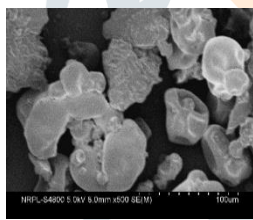
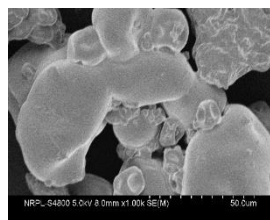
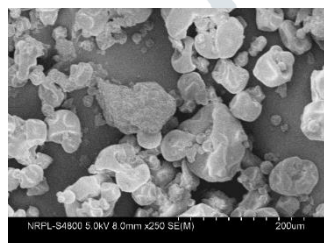
(4.00% $\pm$ 0.52), and Alkaline (4.00% $\pm$ 0.50). These small differences suggested moisture was minimally affected by treatment type, possibly due to consistent drying or sample handling.

Ash content, reflecting mineral content, was lowest in the Alkaline method (1.10% $\pm$ 0.02), with higher values in Acid (1.30% $\pm$ 0.26) and Water (2.16% $\pm$ 0.28) treatments. The higher ash in Acid and Water methods indicated better mineral retention or extraction, while alkaline treatment caused mineral leaching or chemical alteration, reducing ash content.

Carbohydrates exhibited an inverse relationship to protein content. The Water method had the highest carbohydrate content (19.66% $\pm$ 1.44), followed by Acid (9.03% $\pm$ 0.05), and Alkaline had the lowest (3.90% $\pm$ 0.02). The high carbohydrate level in Water treatment suggested preservation or concentration of carbohydrates, whereas alkaline treatment degraded or removed carbohydrates more effectively.

3.2 SEM Analysis of Niger seed protein

Fig 3.1 morphological characteristics of akns protein powder.



The image presented a series of Scanning Electron Microscope (SEM) micrographs showing the morphology of particles at different magnifications and scale bars. Each micrograph captured the surface texture and particle size distribution, illustrating how the observed features changed with increasing observation scale.

At the highest magnification (scale bar 10.0 μm), the image revealed detailed surface features of individual particles, showing relatively smooth surfaces with some minor roughness or textural irregularities. This close-up view highlighted fine structural details, such as small protuberances or indentations on the particle surfaces.

At the 20.0 μm scale, the micrograph showed larger aggregates or clusters of particles. The surface texture remained visible but with less fine detail, and the particles appeared more irregular in

shape, indicating possible agglomeration or fusion of smaller particles.

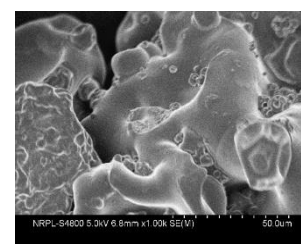
At the 50.0 μm scale, the image captured a broader field of view, showing multiple particles and their spatial arrangement. The particles appeared to have irregular shapes and sizes, with some larger chunks surrounded by smaller fragments, suggesting a heterogeneous particle size distribution.

At the 100.0 μm scale, the micrograph displayed an even wider area, emphasizing the overall morphology and distribution of particle clusters. The particles appeared densely packed, with visible interstitial spaces and a rough, porous texture across the sample.

At the lowest magnification (200.0 μm scale), the image provided an overview of the bulk sample morphology. Larger agglomerates and clusters dominated the field, with a clear indication of particle size variation and overall sample heterogeneity. The porous texture and irregular shapes were evident, reflecting the complex structural characteristics of the material.

These SEM images collectively provided a comprehensive understanding of the particle morphology, size distribution, surface texture, and aggregation behavior at multiple scales, which was crucial for interpreting material properties and performance in various applications.

Fig 3.2 morphological characteristics of acns protein powder



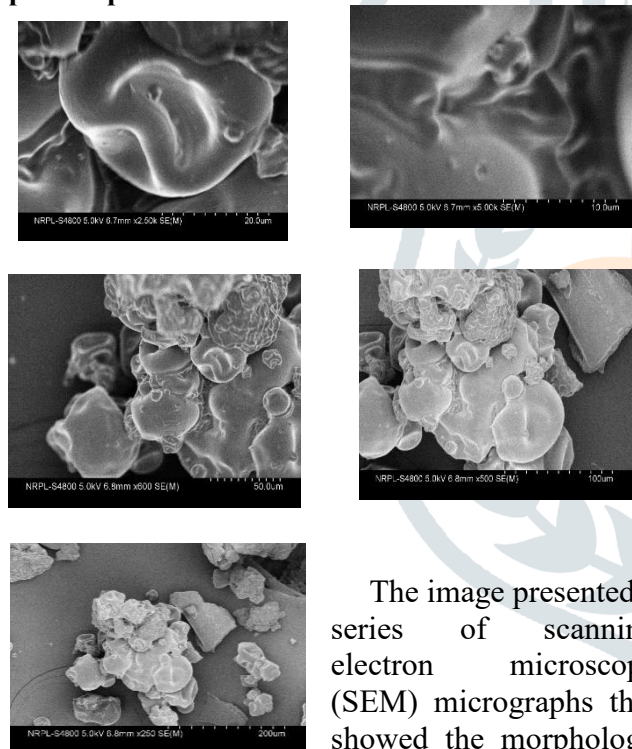
The image series presented a collection of scanning electron microscope (SEM) micrographs of a sample at various magnifications, illustrating the surface morphology and particle structure across multiple scales. At the highest magnification of 10.0 μm, the micrograph revealed fine surface details characterized by a clear texture and small-scale features, which highlighted the intricate morphology of the sample's microstructure. When observed at 20.0 μm magnification, the image provided a broader view where larger surface curvatures and more defined structural elements became apparent, enabling the observation of the sample's intermediate features.

At 50.0 μm magnification, the micrograph captured a wider field of view, displaying aggregated particles with distinct shapes and sizes, including several angular and irregularly shaped

particles. This scale offered insight into the particle assembly and surface roughness. The image taken at 100 μm magnification further expanded the perspective, showing clusters of particles exhibiting varied morphologies. The particles appeared more loosely packed, and their overall distribution and size heterogeneity were clearly visible, indicating the granular nature of the sample.

Finally, at the broadest magnification of 200 μm , the image presented a large population of particles dispersed over the surface. The particle size distribution was more evident at this scale, with a mixture of smaller and larger particles, which demonstrated the sample's overall texture and heterogeneity on a macroscopic microscale. Collectively, these SEM images comprehensively depicted the sample's surface characteristics, ranging from fine microstructural details to broader particle distributions, thereby providing a multi-scale understanding of its morphology.

Fig 3.3 morphological characteristics of wrns protein powder



The image presented a series of scanning electron microscope (SEM) micrographs that showed the morphology of particles at various magnifications and scales. Each micrograph captured surface textures and structural details of the sample at progressively increasing size scales, ranging from 10 micrometers (μm) to 200 μm .

At the smallest scale of 10.0 μm , the particles appeared smooth and compact, with some surface irregularities visible, which indicated fine structural features at a high magnification of 5000x. When observed at 20.0 μm , the micrograph revealed more pronounced surface folds and complex shapes, suggesting a more intricate surface topology.

At the 50.0 μm and 100 μm scales, the images displayed clusters of aggregated particles characterized by rough, uneven surfaces and varying shapes. The particles tended to form larger agglomerates with porous or flaky textures, which indicated possible particle interaction or sintering effects.

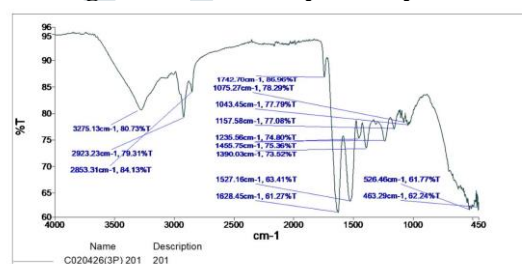
At the largest scale of 200 μm , the micrograph showed a broader view of the sample with numerous particle clusters dispersed throughout the field. The aggregates appeared irregular in shape and size, highlighting the heterogeneous nature of the sample at a macroscopic level.

Collectively, these SEM images provided a comprehensive view of the particle morphology, revealing detailed information about surface texture, particle size distribution, and aggregation behaviour across multiple scales.

3.3 FTIR Analysis of Niger seed protein

The three FTIR analysis graphs represent the spectral characterization of samples subjected to alkaline, acidic, and aqueous (water) environments, respectively. Each graph reveals distinct absorption peaks corresponding to specific functional groups or molecular vibrations that provide insights into the chemical composition and interactions within each sample.

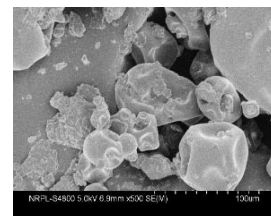
Fig 3.4 Ftir of akns protein powder



The FTIR spectrum of the alkaline sample exhibits several characteristic absorption bands indicative of its chemical environment:

A broad and intense absorption peak is observed in the region of approximately 3200 to 3600 cm^{-1} . This peak is typically attributed to the stretching vibrations of hydroxyl (-H) groups, which are often involved in extensive hydrogen bonding networks in alkaline media. The broad nature of this peak suggests strong intermolecular interactions, such as those between water molecules and hydroxyl groups or metal hydroxides formed under alkaline conditions.

Peaks appearing near 1400 to 1600 cm^{-1} are commonly associated with carbonate (CO_3^{2-}) or bicarbonate (HCO_3^-) ions. These ions often form in alkaline solutions due to the reaction of dissolved carbon dioxide with hydroxide ions. The presence



of these peaks confirms the chemical transformations occurring in the alkaline environment, reflecting the formation of carbonate species.

Additional absorption bands may be present at lower wavenumbers (e.g., below 1000 cm^{-1}), which could correspond to metal-oxygen (M–O) stretching vibrations or other inorganic bonds typical of alkaline compounds or precipitates. These features provide further evidence of the sample's chemical structure and the interactions characteristic of an alkaline matrix.

FTIR Functional Groups	AKNS
O-H (3200-3500 cm^{-1})	3275.13 cm^{-1}
C-H (2800 –3300 cm^{-1})	2923.23 cm^{-1} 2853.31 cm^{-1}
C=O (1600-1700 cm^{-1})	1628.45 cm^{-1}
Amide(N-H bending) (1400-1500 cm^{-1})	1455.75 cm^{-1}
C-O (1000-1300 cm^{-1})	1075.27 cm^{-1} 1043.45 cm^{-1} 1157.58 cm^{-1} 1235.56 cm^{-1}

Table 3.2 Ftir functional groups for akns

FTIR Functional Groups	ACNS
O-H (3200-3500 cm^{-1})	3273.48 cm^{-1}
C-H (2800 –3300 cm^{-1})	2853.00 cm^{-1} 2922.00 cm^{-1}
C=O (1600-1700 cm^{-1})	1530.38 cm^{-1} 1631.75 cm^{-1}
Amide (N-H bending) (1400-1500 cm^{-1})	1453.72 cm^{-1}
C-O (1000-1300 cm^{-1})	1158.14 cm^{-1} 1235.13 cm^{-1}

The provided FTIR spectral data for the alkaline sample lists specific absorption peaks associated with distinct functional groups, reflecting typical vibrational modes observed in Fourier Transform Infrared (FTIR) spectroscopy. These functional

groups and their respective wavenumber ranges correspond to characteristic molecular vibrations, which can give insights into chemical composition and interactions.

The FTIR analysis identified a broad absorption peak at 3275.13 cm^{-1} , which was characteristic of O-H stretching vibrations. This broadness typically resulted from extensive hydrogen bonding, commonly found in hydroxyl groups of alcohols, phenols, or adsorbed water molecules. The position and shape of this peak suggested that the hydroxyl groups were engaged in hydrogen bonding networks, and subtle shifts in the exact wavenumber could be attributed to interactions such as cation binding or changes in the hydrogen bonding environment, which affect the vibrational energy of the O-H bond.

In the region of 2923.23 and 2853.31 cm^{-1} , two distinct peaks were observed, corresponding to asymmetric and symmetric stretching vibrations of C-H bonds in methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2-$) groups. These peaks indicated the presence of aliphatic hydrocarbons, which are typical components of lipids, fatty acids, or polymeric materials. The detection of these vibrations suggested that the sample contained organic moieties with long hydrocarbon chains, contributing to its hydrophobic character and structural complexity.

The carbonyl functional group exhibited a peak at 1628.45 cm^{-1} , which was notably shifted to a lower wavenumber compared to the conventional 1700 cm^{-1} typically associated with C=O stretching vibrations. This downward shift was interpreted as a consequence of conjugation effects, hydrogen bonding, or coordination with metal cations such as Ca^{2+} . These interactions weakened the double bond character of the carbonyl group, thereby lowering its vibrational frequency. The presence of such shifts often reflects a complex chemical environment where carbonyl groups participate in intermolecular bonding or are influenced by surrounding matrix components.

An absorption band at 1455.75 cm^{-1} was assigned to N-H bending vibrations, characteristic of amide groups. This band, often linked to the amide II vibration, suggested the presence of proteinaceous materials such as peptides or proteins. The detection of this band implied that nitrogen-containing organic compounds were present, potentially contributing to the biochemical or structural properties of the sample.

Multiple peaks observed at 1075.27, 1043.45, 1157.58, and 1235.56 cm^{-1} were attributed to C-O single bond stretching vibrations. These peaks are typical of functional groups found in alcohols, ethers, esters, and carbohydrates. The variety and multiplicity of these peaks indicated the presence

of several distinct C-O containing groups, such as polysaccharides or cellulosic structures. The absorbance intensities and positions could be influenced by external factors like temperature or the chemical environment, affecting the vibrational modes of these oxygen-containing bonds. Collectively, these findings highlighted the chemical heterogeneity and complexity of the sample, reflecting a mixture of organic and possibly biopolymeric components interacting within the matrix.

O-H and C=O band shifts: The O-H band at 3275.13 cm^{-1} lies within the expected range but may shift due to hydrogen bonding or interaction with alkaline cations (e.g., Ca^{2+} or Na^{+}). According to research on cation interactions with organic matter, the bonding with polyvalent cations can modify the strength and position of O-H and C=O vibrations, often shifting them to lower or higher wavenumbers respectively (Ellerbrock and Gerke, 2021).

C-H stretching peaks: The distinct peaks at 2923 and 2853 cm^{-1} are consistent with aliphatic hydrocarbons, marking the presence of alkyl chains typical in organic polymers or lipids, important in materials like asphalt binders or biological tissues (Motevalizadeh and Mollenhauer, 2025).

Amide bending: The presence of an amide N-H bending band near 1455 cm^{-1} suggests proteinaceous or peptide structures, or amidic functional groups within the sample. This may correspond to biomolecules or specific organic complexes in the alkaline matrix analyzed (Kluz-Barłowska et al., 2024).

Multiple C-O peaks: The diverse C-O absorptions from 1043 to 1235 cm^{-1} signify the presence of various oxygen-containing functionalities such as alcohols, ethers, and esters, possibly from polysaccharides or other oxygenated biopolymers. The presence of several peaks in this region can also hint at structural heterogeneity or sample complexity and may indicate chemical modifications or blending of materials (Paladini et al., 2021; Ellerbrock and Gerke, 2021).

This FTIR spectral data for the alkaline sample reveals:

A strong, broad O-H stretch around 3275 cm^{-1} , indicative of hydroxyl groups likely involved in hydrogen bonding or cation interactions.

Well-defined C-H stretching peaks near 2923 and 2853 cm^{-1} , typical of aliphatic hydrocarbons.

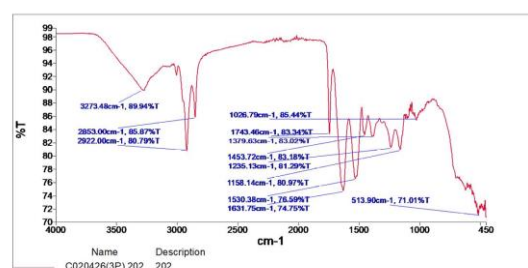
A shifted C=O band at 1628 cm^{-1} , likely from carbonyl groups (ketones, carboxylates, esters, amides) affected by interactions in the alkaline environment.

The presence of amide N-H bending near 1455 cm^{-1} supporting protein or peptide moieties.

Multiplicity of C-O stretching bands from 1043 to 1235 cm^{-1} , suggesting a complex mixture of oxygenated organic compounds such as ethers, esters, or polysaccharides.

These spectral features reflect chemical compositions and interactions consistent with literature observations on organic matter and polymeric materials exposed to alkaline or cationic conditions. The band shifts, especially in O-H and C=O regions, highlight the influence of cation interactions on molecular bonding and vibrational frequencies (Ellerbrock and Gerke, 2021; Paladini et al., 2021).

Fig 3.5 ftir of acns protein powder



The FTIR spectrum of the acid-treated sample shows distinctive features that reflect its acidic nature:

A strong, sharp absorption peak near 1700 cm^{-1} is a hallmark of carbonyl (C=O) stretching vibrations, which are characteristic of carboxylic acid groups or other acidic carbonyl-containing compounds. This peak confirms the presence of acidic functional groups and is critical for identifying the chemical modifications induced by the acidic environment.

Broad absorption bands spanning approximately 2500 to 3300 cm^{-1} correspond to O-H stretching vibrations. Unlike the hydroxyl groups in alkaline samples, these O-H stretches are related to acidic protons in carboxylic acids, often showing broadening due to hydrogen bonding and proton exchange phenomena typical in acidic solutions.

The fingerprint region, below 1500 cm^{-1} , contains multiple absorption peaks that arise from bending vibrations and complex molecular interactions unique to the acid-treated sample. These may include C-O stretching, O-H bending, and other vibrational modes that provide a detailed molecular fingerprint, allowing differentiation from the alkaline and water samples.

The FTIR spectral data for the acid sample reveal characteristic absorption peaks corresponding to typical functional groups found in acidic and organic compounds. Below is a detailed explanation of each observed functional group, their typical wavenumber ranges, observed peak positions, and chemical significance:

The FTIR analysis identified a broad O-H stretching peak centered at 3273.48 cm^{-1} , which strongly indicated the presence of hydroxyl functional groups commonly associated with carboxylic acids, alcohols, or phenols. The pronounced broadness of this absorption band was primarily attributed to extensive hydrogen bonding interactions. This effect was particularly notable in carboxylic acids due to the -COOH group's ability to form strong intermolecular hydrogen bonds, often further influenced by the potential association with water molecules within the sample matrix. Such hydrogen bonding not only broadened the peak but also affected its exact position, reflecting the dynamic environment surrounding the hydroxyl groups.

In addition, the spectrum exhibited distinct peaks at 2853.00 and 2922.00 cm^{-1} , which corresponded to the symmetric and asymmetric stretching vibrations of C-H bonds in aliphatic methyl (-CH_3) and methylene ($\text{-CH}_2\text{-}$) groups. These peaks signified the presence of organic moieties, specifically hydrocarbon chains, that were likely attached to or interacting with the acidic functionalities. The identification of these aliphatic C-H stretches suggested that the sample contained lipid-like or polymeric structures, contributing to its hydrophobic regions and overall molecular complexity.

The carbonyl (C=O) functional groups were characterized by absorption bands at 1530.38 and 1631.75 cm^{-1} , which were notably shifted to lower wavenumbers compared to the typical carbonyl stretching vibration near 1700 cm^{-1} . This downward shift was interpreted as a consequence of several possible factors, including hydrogen bonding with surrounding molecules, conjugation effects that delocalized electron density, or the presence of overlapping amide I bands commonly found in proteinaceous materials. These shifts suggested strong intermolecular interactions within the sample, possibly involving metal cation coordination or complex molecular environments that altered the normal vibrational frequencies of the carbonyl groups.

Furthermore, an absorption band near 1453.72 cm^{-1} was observed, which was characteristic of N-H bending vibrations associated with amide groups, specifically the amide II band. This feature implied the presence of proteins, peptides, or other amide-containing organic compounds within the sample. The coexistence of these amide functionalities alongside acidic groups suggested a chemically complex matrix, potentially comprising biological macromolecules or organic matter derived from living organisms or their degradation products.

Finally, the presence of peaks at 1158.14 and 1235.13 cm^{-1} was attributed to C-O stretching vibrations found in carboxylic acid groups (C-OH stretch), esters, and ethers. The dual peaks in this region indicated a variety of oxygen-containing functional groups, which contributed significantly to the acidic character and structural heterogeneity of the analyzed material. These functional groups likely originated from polysaccharides, cellulosic structures, or other biopolymeric components, reflecting the intricate chemical composition and environmental interactions within the sample. Collectively, these spectral features highlighted the complex interplay of organic and inorganic constituents, hydrogen bonding networks, and molecular interactions that defined the sample's chemical and structural properties.

The observed O-H stretching band near 3273 cm^{-1} in acidic samples is a hallmark of carboxylic acids, often broad due to extensive hydrogen bonding networks.

The C=O peaks at 1530 and 1632 cm^{-1} are somewhat shifted from typical free carbonyl stretches ($\sim 1700\text{ cm}^{-1}$), which can occur due to acid dimerization, intra- and intermolecular hydrogen bonding, or interaction with metal cations altering bond strength and vibrational frequency (Ellerbrock and Gerke, 2021).

The amide band suggests possible coexistence of peptide or protein-like materials or related nitrogenous compounds that may be part of complex organic matrices.

Multiple C-O bands demonstrate the presence of oxygenated functional groups common in acids and esters, which can influence solubility and reactivity.

This FTIR profile indicates a chemically complex acidic material with:

Strongly hydrogen-bonded hydroxyl groups (O-H),

Organic aliphatic chains (C-H stretches),

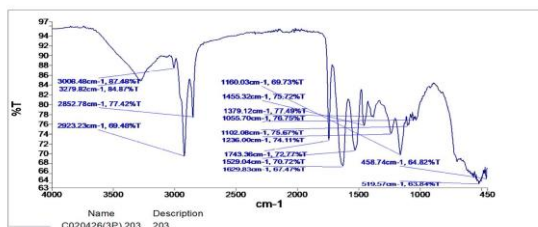
Conjugated or hydrogen-bonded carbonyl groups (C=O),

Amide functional groups (N-H bending),

Varied oxygenated species (C-O stretches).

Shifts and intensities in these bands reflect the acid's molecular environment, including possible ion interactions and hydrogen bonding networks that modulate the typical spectral features (Ellerbrock and Gerke, 2021; Kluz-Barłowska et al., 2024; Sahu et al., 2005).

Fig 3.6 Ftir of wrns protein powder



The FTIR spectrum of the water sample primarily reflects the vibrational modes of pure water molecules:

A broad and intense absorption band between 3200 and 3600 cm^{-1} is attributed to the O-H stretching vibrations of water molecules. This broadness is due to the extensive hydrogen bonding network in liquid water, resulting in a wide range of vibrational energies.

A distinct peak near 1640 cm^{-1} corresponds to the H-O-H bending vibration (scissoring mode) of water. This peak is a characteristic marker of liquid water and helps confirm the sample's purity and the absence of other interfering substances.

The relative simplicity of the water FTIR spectrum, with minimal additional peaks, indicates a lack of complex chemical species, making it a useful baseline or control for comparing with the more chemically complex alkaline and acid samples.

The x-axis in all three FTIR graphs represents the wavenumber (cm^{-1}), which corresponds to the energy of vibrational modes within the molecules. Higher wavenumbers indicate higher energy vibrations, typically associated with lighter atoms or stronger bonds. The y-axis shows either transmittance or absorbance, reflecting the amount of infrared light absorbed by the sample at each wavenumber. Peaks in the spectra correspond to specific vibrational transitions, allowing identification of functional groups and molecular structures.

By comparing these spectra, one can discern how the chemical environment—alkaline, acidic, or pure water—affects the molecular composition and bonding characteristics of the samples. This detailed spectral information is essential for understanding chemical modifications, reaction mechanisms, and the presence of specific ions or functional groups in different conditions.

FTIR Functional Groups	WRNS
O-H (3200-3500 cm^{-1})	3008.48 cm^{-1} 3279.82 cm^{-1}
C-H (2800 –3300 cm^{-1})	2852.78 cm^{-1} 2923.23 cm^{-1}
C=O (1600-1700 cm^{-1})	1529.04 cm^{-1} 1629.83 cm^{-1}
Amide (N-H bending) (1400-1500 cm^{-1})	1455.32 cm^{-1}
C-O (1000-1300 cm^{-1})	1236.36 cm^{-1} 1102.08 cm^{-1}

The FTIR spectrum data for the water sample presented here shows vibrational peaks corresponding to major functional groups commonly associated with water and its interaction with organic or biological compounds. Below is a detailed explanation of the listed FTIR functional groups, their typical wavenumber ranges, observed peak positions, and interpretations:

The FTIR analysis provided detailed insights into the molecular composition and interactions within the water sample by identifying characteristic vibrational bands associated with various functional groups. The broad O-H stretching bands observed at 3008.48 and 3279.82 cm^{-1} were indicative of hydroxyl groups predominantly linked to water molecules. The notable lowering of one peak to 3008 cm^{-1} , compared to the typical O-H stretching region, suggested the presence of strong hydrogen bonding networks and diverse microenvironments within the sample. This variation in peak position reflected the coexistence of both bound water molecules, which are hydrogen-bonded to other molecules or surfaces, and bulk water molecules, which are less restricted. Such distinctions are critical for understanding the structural organization and dynamics of water in complex matrices, as they influence physicochemical properties like solubility and reactivity.

Additional peaks at 2852.78 and 2923.23 cm^{-1} corresponded to the symmetric and asymmetric stretching vibrations of C-H bonds in aliphatic hydrocarbon chains. These peaks signified the presence of organic moieties or trace contaminants, such as hydrocarbons or dissolved organic compounds, commonly found in environmental water samples. These organic components may originate from natural biological activity, pollution, or other sources, and their identification is essential

Table 3.4 Ftir functional groups for wrns

for assessing water quality and contamination levels.

In the carbonyl stretching region, peaks at 1529.04 and 1629.83 cm^{-1} were detected, which are characteristic of C=O functional groups. The presence of two distinct peaks, especially the shift of one towards the lower wavenumber near 1530 cm^{-1} , was indicative of complex molecular interactions such as conjugation effects or hydrogen bonding involving carbonyl groups. This shift also suggested possible overlap with amide I vibrations, which are typical in proteins or peptides. The altered bonding environment, likely influenced by interactions with water molecules or metal ions, affected the vibrational frequencies of these carbonyl groups, reflecting the chemical complexity and intermolecular forces within the sample.

An absorption band at 1455.32 cm^{-1} was assigned to amide II vibrations, which involve N-H bending coupled with C-N stretching. This band pointed to the presence of proteins, peptides, or other amide-containing organic compounds dissolved or interacting within the water matrix. The detection of such biomolecules suggested biological contamination or organic matter derived from living organisms, which could have significant implications for the biological activity and ecological status of the water source.

Finally, peaks observed at 1102.08 and 1236.36 cm^{-1} were attributed to C-O stretching vibrations found in alcohols, ethers, and carbohydrates. In the context of water samples, these bands suggested the presence of polysaccharides, glycosidic residues, or dissolved organic matter typically associated with biological sources or extracellular matrices. The diversity and intensity of these C-O stretching bands highlighted the complex mixture of oxygen-containing functional groups contributing to the chemical heterogeneity and biological relevance of the sample. These findings underscored the intricate interplay between organic compounds and water molecules, which collectively influence the sample's chemical behavior, biological interactions, and overall environmental quality.

The O-H stretch peaks around 3280 cm^{-1} and 3008 cm^{-1} are characteristic of water molecules with varied hydrogen bonding states. The broader, slightly shifted peak at 3008 cm^{-1} may reflect strongly hydrogen-bonded water clusters or bound water associated with other molecules or surfaces, consistent with the complexity of water microenvironments revealed by FTIR studies (Eneh et al., 2020).

C-H stretching peaks suggest minor organic content, possibly from dissolved organic matter in natural or biological water samples.

The C=O bands near 1530 and 1630 cm^{-1} may originate from dissolved organic acids, peptides, or conjugated carbonyl groups interacting with water, reflecting hydrogen bonding and hydration effects that shift these peaks from free-state positions (Ellerbrock and Gerke, 2021).

The Amide N-H bending band at 1455 cm^{-1} indicates the presence of proteins or peptides, typical in biological fluids or water samples containing biomolecules (Kluz-Barłowska et al., 2024).

The C-O stretching bands at 1102 and 1236 cm^{-1} correspond to various oxygen-containing organic structures, such as carbohydrates or polysaccharides, further confirming the organic complexity in the sample.

FTIR analysis of water often reveals the complex interplay of free and bound water molecules, as well as dissolved organics and biomolecules that contribute to its spectral profile. Studies employing ATR-FTIR have successfully characterized hydration states and biomolecular composition in aqueous environments, including biological tissues and films (Cao et al., 2017; Eneh et al., 2020). The presence of amide and carbonyl bands supports the interpretation of biological or organic contamination in water, which is common in environmental or physiological samples.

Summary and conclusion

This study comprehensively investigated the morphological, thermal, physicochemical, and functional properties of protein isolates extracted from Niger seeds (*Guizotia abyssinica*) using three wet processing techniques: alkaline extraction (AKNS), acid extraction (ACNS), and water extraction (WRNS). The extraction methods significantly influenced the yield, composition, structural characteristics, and functional performance of the resulting protein concentrates.

Proximate analysis revealed that alkaline extraction yielded the highest protein content (71.00% $\pm$ 1.00), followed by acid extraction (66.33% $\pm$ 1.27) and water extraction (52.33% $\pm$ 2.51). Fat content was highest in water-extracted proteins (17.5% $\pm$ 0.50), while fiber content was notably elevated in acid-extracted samples (4.33% $\pm$ 0.28). Moisture content remained stable across treatments (~4%), whereas ash content was lowest in alkaline extracts and highest in water extracts. Carbohydrate content inversely correlated with protein content, being most abundant in water-extracted samples.

Scanning Electron Microscopy (SEM) analyses demonstrated distinct morphological differences: alkaline-extracted proteins exhibited relatively smooth surfaces with minor roughness and heterogeneous particle sizes; acid-extracted proteins showed angular, irregular particles with

looser packing; water-extracted proteins presented smooth, compact particles with porous, flaky textures and larger aggregates. These morphological variations suggested that extraction conditions modulated particle size distribution, surface texture, and aggregation, which were critical for functional properties.

Fourier Transform Infrared Spectroscopy (FTIR) analyses identified characteristic functional groups and revealed chemical environment differences induced by extraction pH. Alkaline extracts showed broad O-H stretching indicative of hydrogen bonding and carbonate formation, with shifted carbonyl peaks due to conjugation and cation interactions. Acid extracts exhibited strong carbonyl peaks typical of carboxylic acids and broad O-H bands reflecting acidic protons. Water extracts primarily displayed water molecule vibrations with minor organic functional groups, indicating a relatively unmodified chemical environment.

Differential Scanning Calorimetry (DSC) revealed that water-extracted proteins possessed the highest thermal stability, with onset and peak temperatures of 36.83 °C and 74.45 °C, respectively, and the greatest enthalpy change (208.17 J/g). Alkaline and acid extracts showed lower thermal stability ($\Delta H \sim 130$ J/g), indicating that alkaline and acid treatments induced conformational changes affecting thermal behavior.

Functional property assessments demonstrated that alkaline-extracted proteins had superior water absorption capacity (2.15 ± 0.03) and foaming capacity across pH levels, peaking at 80.83% at pH 9. Acid-extracted proteins exhibited the highest fat absorption capacity (1.47 ± 0.03), while water-extracted proteins had the lowest values in both parameters. Protein solubility followed a U-shaped curve across pH 2 to 9, with maximum solubility at alkaline pH 9, reflecting structural unfolding and exposure of hydrophilic groups. Emulsification capacity and stability were highest in alkaline extracts (65.26% and 54.39%, respectively), followed by acid and water extracts, indicating enhanced surface activity under alkaline extraction conditions.

Collectively, these results demonstrated that extraction method profoundly influenced the physicochemical, structural, thermal, and functional attributes of Niger seed protein isolates. Alkaline extraction optimized protein content and functional properties such as water retention, foaming, and emulsification, making it suitable for food industry applications requiring these characteristics. Acid and water extraction yielded proteins with distinct compositional and physicochemical profiles that may have been

advantageous for applications prioritizing fat absorption or thermal stability.

The present research elucidated the significant impact of wet processing techniques on the yield, composition, structure, thermal stability, and functional performance of protein isolates derived from Niger seeds. Alkaline extraction emerged as the most effective method for maximizing protein content and enhancing functional properties critical for food applications, including water absorption, foaming capacity, and emulsification. These enhancements were likely due to the alkaline environment promoting protein solubilization, structural unfolding, and exposure of hydrophilic and surface-active groups, thereby improving interaction with water and air interfaces.

Acid extraction, while yielding slightly lower protein content, produced protein concentrates with higher fat absorption capacity and distinct morphological features characterized by angular, loosely packed particles. This suggested potential utility in formulations where fat binding was desirable, such as meat analogs or bakery products requiring fat retention. The water extraction method, though resulting in the lowest protein content and functional capacities, provided protein isolates with superior thermal stability, as evidenced by DSC analysis, indicating minimal structural modification during extraction. This thermal resilience may have been beneficial in applications involving heat processing or requiring protein stability under thermal stress.

Morphological and spectroscopic analyses (SEM and FTIR) confirmed that extraction conditions altered the molecular environment and particle characteristics of Niger seed proteins, directly influencing their functional behavior. The observed shifts in functional group vibrations and particle morphology underscored the interplay between chemical treatment and protein structure.

Overall, this study provided foundational insights into the optimization of Niger seed protein isolation for tailored functional applications. The alkaline extraction method was recommended for producing protein concentrates with enhanced techno-functional properties suitable for incorporation into diverse food systems, including emulsified products, bakery goods, and meat analogs. Acid and water extraction methods offered alternative profiles for specific functional needs, such as fat absorption and thermal stability.

Future research was suggested to focus on refining extraction protocols to balance yield, purity, and functionality, assessing scale-up feasibility for industrial production, and conducting comprehensive nutritional and safety evaluations to fully harness Niger seed proteins' potential. Additionally, exploring the integration of

novel pretreatment technologies and enzymatic processes could further enhance protein recovery and functional attributes. Such efforts would contribute to the sustainable utilization of Niger seed proteins as valuable plant-based ingredients, supporting diversification in protein sources and innovation in food product development.

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