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Functional, Structural and Thermal Properties of Protein Fractions from Cottonseed Meal

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ABSTRACT

Background: The increasing global demand for sustainable protein sources has focused interest on plant-derived alternatives, one of which is cottonseed meal (CSM), largely underutilized until now. Though, it possesses high-quality protein, but its' potential remains unexplored due to limited characterization of its constituent protein fractions.

Methods: In our study, CSM was fractionated using Osborne's sequential extraction method into albumin, globulin, prolamin and glutelin. Protein fraction yield, protein concentration and the functional properties of each fraction were evaluated. Additionally, the structural and thermal characteristics of the fractions were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and thermogravimetric analysis (TGA).

Result: Study findings revealed that CSM had a protein content of 46.16%. At the fraction level, globulin was detected with the highest yield (74.58%) and protein concentration (52.37%) among all four protein components. Albumin showed higher water-holding and emulsifying capacities as compared to ?? and glutelin had better foaming ability. FTIR and SEM analyses represented distinct structural and morphologicalstructures for all the fractioned proteins with each exhibiting unique secondary-structure features and surface microstructures reflective of their varying conformations, aggregation levels and functional behaviour, Maximum thermal stability was recorded in globulin. Overall, proteins fromCSM, especially globulin and albumin fractions, presented good functional and structural properties, thus making them potential ingredients for food formulations and protein-based value added food products.

Key words: Cottonseed meal, Functional properties, Protein, Structural properties, Thermal properties, Yield.

INTRODUCTION

The global population is expected to reach a level between 8.5 and 10 billion in 2050, with increased living standards and a shift in dietary preferences. This rapid population growth will intensify the need for affordable, nutritious and sustainable high-protein foods to meet global dietary requirements. Therefore, demand for sustainable and more reasonably priced protein sources than traditional animal origin proteins will continue to surge upwards (Ware *et al*., 2025). Within this context, plant-derived proteins have recently attracted interest worldwide because of their nutritive value, functional diversity, lower environmental footprint and economic viability. Among the plant sources, cotton holds a unique position as one of the most extensively cultivated plants in the world. The production of cotton generates large amounts of cottonseed as a by-product during fiber production, accounting for approximately 50% of the seed weight. Regardless of its abundance and nutritional richness, cottonseed remains largely underutilized. According Xie *et al*. (2023) during the year 2021-2022, global cotton production reached to a significant figure (43.8 million tonnes), highlighting its potential as an abundant protein feedstock. Cottonseed meal (CSM), the major by-product after oil extraction, contains about 23% protein, is a promising feedstock to meet increasing global protein demand (Sunilkumar *et al*., 2006).

CSM proteins have a balanced amino acid profile; and their functional properties of solubility, emulsification, foaming and gelation are desirable in many food systems (Hinze *et al*., 2015; Rigney, 2025). Such characteristics position cottonseed protein as an excellent player among established plant proteins *viz.* soy, pea and wheat gluten. Functional properties of CSM play a pivotal role for its' diverse applications in baked products, beverages, dairy alternatives and plant-based meat formulations, for obtaining desirable textural, stability and sensory qualities of the prepared product. The increasing consumer demand for protein-enriched foods, such as powders, bars and ready-to-drink formulations, has increased research into finding new and sustainable plant protein sources. More recently, proteins from unconventional sources, such as quinoa, jackfruit seeds and prickly pear seed cake have been studied (Mir *et al*., 2018; Ulloa *et al*., 2017;

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Borchani *et al*., 2021; Verma *et al*., 2022). In this context, valorization of cottonseed protein also presents an opportunity to convert an agricultural bye-product into a sustainable protein ingredient for value addition. Regardless of its nutritional potential, the value of cottonseed proteins has not been fully explored. Most literature has focused on general composition or oil extraction and specific information on physicochemical, structural and functional properties of these proteins is limited. The present study focuses on the systematic extraction and fractionation of proteins from CSM and comparative evaluation of their yield, functional, structural and thermal properties to ascertain the food applications of CSM as a low-cost, sustainable plant protein source.

MATERIALS AND METHODS

Raw material

The CSM was obtained from Kapeesh Industries, Khanna, Ludhiana, Punjab, India. All the chemicals and reagents were of analytical grade (Sigma-Aldrich) (Mention brand name).

Preparation of defatted CSM powder

The CSM was ground to a fine powder, then the obtained powder was defatted using hexane as the Soxhlet solvent for 8 hours at 60°C. The resulting defatted CSM powderwas dried at 40°C and stored in airtight pouches for further analysis.

Proximate analysis

The proximate composition of defatted CSM powder was determined using standard AOAC (2009) methods to detect moisture, crude protein, crude lipid, crude fiber and ash contents. Carbohydrate content was calculated by difference, subtracting the sum of moisture, protein, fat, fiber and ash from 100. All results were expressed on a dry-weight basis (g/100 g).

Osborne fractionation technique for CSM

Protein fractions were extracted from defatted CSM powder based on the modified Osborne and Voorhees (1894) sequential extraction procedure (albumin, globulin, prolamin and glutelin). For albumin, the defatted CSM powder was mixed with deionized water in a 1:10 w/v ratio and stirred overnight, after adjusting the pH to 7.0,. Then, the suspension was centrifuged at 8000 rpm for 15 minutes and the supernatant was collected (Albumin-1). The extraction was repeated twice to ensure maximum recovery. Then globulin, prolamin and glutelin were sequentially extracted with 0.5 N NaCl, 70% aqueous ethanol and 0.2% NaOH, respectively, under the same conditions of extraction and centrifugation. Supernatants were freeze-dried and kept in airtight containers at 4°C until use.

Estimation of protein yield and concentration

Protein yield was determined based as:

$$\text{Protein yield (\%)} = \frac{p}{s} \times 100$$

Where,

P= Weight of particular protein fraction (g).

S= Dry weight of CSM powder taken for protein extraction (g).

Protein concentration was estimated by using the Kjeldahl method.

Functional properties of CSM protein fractions

Water and oil holding capacity

Water and oil holding capacities were determined according to the procedure described by Tounkara *et al*. (2013). For water-holding capacity, 0.5 g protein sample was mixed with 10 mL of deionized water, vortexed for 30 seconds and then left at room temperature for 30 min prior to centrifugation (3000 rpm, 25 min). The supernatant was filtered and weight of the residue recorded. For oil-holding capacity, 1 g of protein was mixed in 10 g of refined soybean oil, vortexed for 5 minutes, incubated for 30 minutes and then centrifuged at 3000 rpm for 20 minutes.

$$\text{Water/Oil holding capacity (g/g)} = \frac{W_2 - W_1}{W_s}$$

Where,

W_1= Weight of centrifuged tube (g).

W_2= Weight of centrifuged tube after draining the supernatant (g).

W_s= Sample weight (g, db).

Emulsifying capacity

Emulsifying capacity was determined by the method of Kashyap *et al*. (2023). One gram of protein sample was dissolved in 50 mL of 0.5 N NaCl and mixed with 50 mL refined soybean oil. The emulsion was homogenized and heated at 90°C for 10 min, then centrifuged at 3000 rpm for 20 min.

$$\text{Emulsifying capacity (mL/g)} = \frac{V_1 - V_2}{W_s} \times 100$$

Where,

V_1= Volume of oil mixed to prepare emulsion (mL).

V_2= Volume of oil released after centrifugation (mL).

W_s= Sample weight (g,db).

Foaming capacity and foaming stability

The foaming properties were determined by the method described by Kashyap *et al*. (2023). One gram of protein was dissolved in 100 mL deionized water at pH 7.4 and stirred for 3 minutes. The mixture was transferred to a 250 mL graduated cylinder and the foam volume was recorded.

$$\text{Foam capacity (\%)} = \frac{V_2}{V_1} \times 100$$

Where,

V_1= Volume of protein sample solution (mL).

V_2= Volume of foam (mL).

The foam stability was measured at 10 min intervals over a period of 1 h under static conditions.

Wettability

Wettability was determined according to Akpossan *et al*. (2015). One gram of protein sample was put in a 10 mL graduated cylinder, inverted 10 cm above deionized water and released gently. The time taken for complete wetting of the sample was recorded as wettability time.

Structural properties of CSM protein fractions

Fourier transform infrared (FTIR) spectroscopy

According to Kashyap *et al*. (2023) Fourier transform infrared spectra were recorded on Perkin Elmer Spectrum (RX-I, FTIR, USA). The sample was scanned in the spectral range of 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}.

Scanning electron microscopy (SEM)

Surface morphology was analyzed in each protein fraction by a Scanning Electron Microscope (Hitachi SE 300H, Tokyo, Japan) according to the method of Kashyap *et al*. (2023). Samples were mounted on conductive adhesive stubs, sputter-coated with gold and observed at 2500× magnification with an accelerating voltage of 25 kV.

Thermal Property of CSM protein fractions

Thermo-gravimetric analysis (TGA)

The thermostability of the protein fractions was measured according to Kashyap *et al*. (2023) by using a Perkin Elmer TGA 4000 (USA). About 5 mg of protein powder was heated from 40°C to 600°C at 10°C/min with a nitrogen flow of 20 mL/min.

Statistical analysis

All analyses were performed in triplicate and the results were presented as mean ± standard deviation. Significant differences among the samples were tested by one-way ANOVA. Homogeneity among means was compared by the Duncan's multiple range test ($p<0.05$) using SPSS software (version 16.0, IBM, USA).

RESULTS AND DISCUSSION

Proximate composition of CSM

The proximate analysis of CSM showed that the major constituents in order of magnitude were protein (46.16±0.35%), carbohydrates (26.89±0.04%), crude fat (1.8±0.35%), ash (7.60±0.46%) and crude fiber (8.00±0.81%). This agrees with earlier works where 35-45% protein and 0.8-1.0% fat in CSM have been reported (Cheng *et al*., 2020; Kumar *et al*., 2021; Srinath *et al*., 2025). A high protein value indicates a promising plant protein source, while high ash values suggest the presence of essential minerals in foods.

Yield and concentration of different protein fractions

Fractionation of CSM proteins yielded four major classes globulin, albumin, glutelin and prolamin accounting for 94.55±1.05% of the total protein and 9.12±0.24 g/100 g total yield, representing 89.76% recovery of crude protein (16.21±0.14 g/100 g) (Table 1). The minor unextracted fraction (8-10%) eflects variations in solvent polarity, extraction pH, ionic strength, or particle size affecting solubility and diffusivity (Deb *et al*., 2022; Patel *et al*., 2025). Globulin was predominant (12.09±0.25 g/100 g; 55.37±1.32% protein), followed by albumin, glutelin and prolamin, a distribution consistent with oilseed protein profiles. The dominance of globulin and albumin aligns with previous findings (Gandhi *et al*., 2017), emphasizing their nutritional relevance as they are rich in essential amino acids and contribute to structural and enzymatic functions. Their high solubility and interfacial activity suggest strong potential for emulsified or protein-enriched food systems (Singh, 2019). Conversely, the lower glutelin and prolamin contents typically abundant in cereals indicate that CSM proteins are primarily salt and water-soluble, highlighting their distinct biochemical nature and superior functional adaptability compared to cereal storage proteins.

Functional properties of CSM protein fractions

Water holding capacity (WHC) and oil holding capacity (OHC)

The significant difference was seen among various CSM protein fractions for water and oil-holding capacities (Table 2). Albumin showed the highest WHC, which was followed by globulin, glutelin and prolamin. Higher WHC for albumin can be ascribed to its relatively flexible structure, higher surface polarity and exposure of hydrophilic groups, facilitating water-protein interactions and network formation during hydration (Kamani *et al*., 2024). The variability among the fractions arises due to the difference in molecular conformation, surface charge distribution and extent of protein denaturation during extraction (Dabbour *et al*., 2023).

The OHC values ranged from 1.71±0.01 to 2.51±0.01 g/g of albumin. The superior OHC of albumin indicates a greater availability of hydrophobic residues capable of binding nonpolar lipid chains, whereas the compact quaternary structure restricts such interactions in globulin. In contrast, the moderate OHC values of prolamin and glutelin indicate intermediate surface hydrophobicity (Borchani *et al*., 2021). Both WHC and OHC illustrate that albumin is an effective contributor to viscosity and texture enhancement both in aqueous systems and in lipid-based formulations, suggesting its potential use in bakery, confectionery and emulsified food applications.

Table 1: Protein content of fractions and protein fraction yield.

Sample	Protein (%)	Protein fraction yield (g/100 g of defatted CSM powder)	Protein fraction yield (%)
Albumin	24.74±0.25^{b}	1.70±0.03^{c}	7.40
Globulin	52.37±1.32^{a}	12.09±0.25^{a}	74.58
Prolamin	6.41±0.27^{d}	0.51±0.07^{d}	3.14
Glutelin	11.03±0.31^{c}	1.91±0.01^{b}	11.78

Emulsifying capacity

Table 2 presented the emulsifying capacity of the respective protein fractions from CSM. Interestingly enough, albumin exhibited highest emulsifying capacity followed in efficiency by glutelin, globulin and prolamin. Of significance here is that the difference in emulsifying capacity between albumin and glutelin was not quite significant ($p<0.05$). These protein fractions stabilize emulsions during homogenization by lowering interfacial tension to produce smaller droplets and by increasing repulsive forces to prevent aggregation (Rudra *et al*., 2016). The relatively greater emulsion capacities of these two fractions could be due to the higher proportion of small, soluble protein particles, based on their particle size distribution. Smaller particle-sized proteins have been shown to have greater emulsifying activity based on their better ability to quickly adsorb at the oil-water interface, thus stabilizing emulsions (Chen *et al*., 2022).

Foaming capacity and foaming stability

The CSM protein fractions exhibited significant differences in foaming properties, indicating structural and interfacial heterogeneity among the extracted proteins. FC values ranged from 16.00±0.01% for prolamin to 26.62±0.03% for glutelin (Table 2). A higher FC value for glutelin indicates a balanced composition of hydrophobic and hydrophilic domains that favor rapid diffusion and adsorption at the air-water interface, enhancing foam generation. Globulin had a moderate FC of 20.98±0.03%, while albumin had a relatively lower FC of 19.50± 0.01% but with relatively good stability due to its flexible and compact molecular structure in general (Chen *et al*., 2022). Globulin exhibited the highest foam stability, showing the capability to form cohesive and viscoelastic interfacial films by resisting coalescence and liquid drainage (Fig 1). On the contrary, despite the higher FC of glutelin, it showed rapid foam collapse, possibly due to insufficient interfacial rigidity or weak intermolecular crosslinking. Prolamin exhibited poor FC and stability, consistent with its limited solubility and tendency toward aggregation in aqueous environments. These observations suggest that globulin and albumin stabilize foam while glutelin enhances foam formation, implying complementary functionality for fractions of CSM in both aerated and whipped food products.

Wettability

Wettability of CSM protein fractions reflected their hydration and dispersibility characteristics, influenced by particle size, surface polarity and hydrophobicity. Significant differences ($p<0.05$) occurred among the fractions, where globulin exhibited the shortest wetting time (9.65±0.14 s), reflecting rapid hydration and superior dispersibility. Albumin and glutelin exhibited intermediate levels of wettability at 13.27±0.07 s and 10.60±0.01 s, respectively, while prolamin demonstrated the longest wetting time of 15.31±0.04 s, attributed to an aggregated structure and high hydrophobic amino acid content that restrict water entrance. These observations are in good agreement with those made in the case of some plant proteins, in which higher surface polarity and increased porosity enhance the dynamics of hydration (Akpossan *et al*., 2015). In general, globulin and glutelin

Table 2: Functional properties of cotton seed meal protein fractions.

Properties	Protein fractions			
	Albumin	Globulin	Prolamin	Glutelin
WHC (g/g)	1.88±0.02^{a}	1.43±0.29^{b}	0.67±0.02^{d}	0.93±0.04^{c}
OHC (g/g)	2.51±0.01^{a}	1.84±0.02^{b}	1.71±0.012^{d}	1.94±0.01^{c}
EC (mL/g)	56.83±0.61^{a}	48.36±0.11^{c}	31.07±0.05^{d}	56.35±0.05^{b}
FC (%)	19.5±0.008^{c}	20.98±0.03^{b}	16.00±0.01^{d}	26.62±0.03^{a}
Wettability	13.27±0.07^{b}	09.65±0.14^{d}	15.31±0.04^{a}	10.60±0.01^{c}

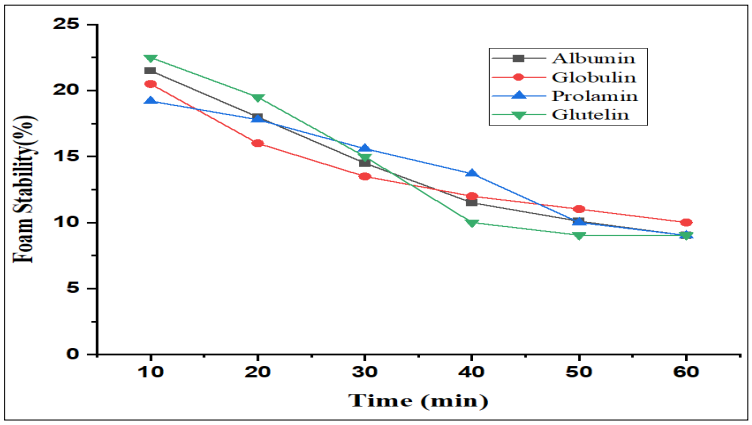


Fig 1: Foaming stability of CSM protein fractions.

showed rapid wettability and, hence, a greater potential for instant or dispersible food applications.

Fourier transform infrared spectroscopy

The FTIR spectra of albumin, globulin, glutelin and prolamin fractions of CSM proteins showed different absorption bands, thus confirming their intact secondary structures (Fig 2). The broad peak at 3300-3400 cm^{-1} was assigned to O-H and N-H stretching, showing the presence of hydrogen bonding and typical protein groups such as hydroxyls and amines. The Amide I band, around 1650 cm^{-1}, which represents C=O stretching of peptide linkages, suggested the presence of α-helix and β-sheet, while Amide II (~1540 cm^{-1}) was due to N-H bending and C-N stretching, further confirming the protein nature of the samples (Kong and Yu, 2007). The additional peaks between 1000-1200 cm^{-1} due to C-N and C-O stretching have formed the fingerprint region that differentiated fractions. Albumin exhibited broader and less defined peaks, indicating a more heterogenous structure, whereas globulin had a lower intensity band, suggesting a compact conformation. These show successful extraction and different secondary structures which influence functional behavior in food matrices (Zeng *et al*., 2011). These are important because they influence how each protein behaves functionally, which has direct implications for their use in food applications and other industrial products.

Scanning electron microscopy

SEM micrographs of CSM protein fractions are presented in Fig 3 and have shown different morphologies related to their functional properties. Albumin had a dense smooth surface, indicating an aggregated or denatured protein, matching its low solubility. Globulin had a rough and irregular granular appearance with apparent porosity that favored hydration and oil-binding potential. Glutelin presented spherical, moderately aggregated particles with uniform packing and is associated with improved dispersion and emulsification capability (Wu *et al*., 2022). Prolamin displayed porous, flaky and loosely aggregated structures, reflecting weak intermolecular interaction but high water absorption upon hydration. These morphological differences are consistent with previous reports on plant proteins (Sun and Arntfield, 2010) and directly impact the solubility, emulsification and foaming properties of interest in food applications.

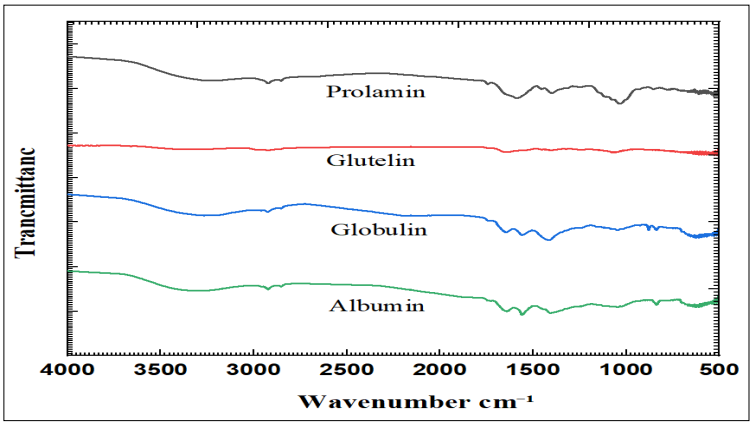


Fig 2: FTIR spectroscopy of cottonseed meal protein fractions.

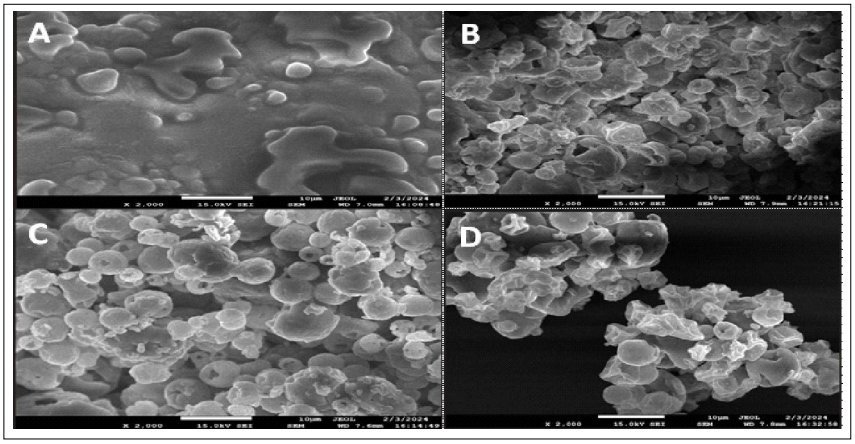


Fig 3: SEM images of the cotton seed meal protein fraction Albumin (A), globulin (B), glutelin (C), Prolamin (D).

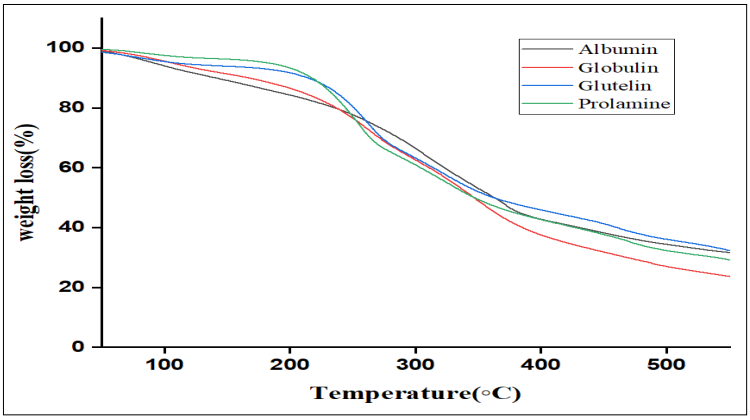


Fig 4: Thermogravimetric analysis of CSM protein fractions.

Thermogravimetric analysis

TGA profiles of CSM protein fractions over the temperature range of 0-600°C showed distinct thermal degradation characteristics (Fig 4). An initial weight loss below 200°C corresponded to the evaporation of moisture. Relatively higher weight losses were observed in prolamin and glutelin, which may be attributed to a higher moisture content or higher water retention in their structures. The major decomposition took place between 200-400°C, reflecting peptide bond cleavage and protein denaturation as stated by Kumar *et al*. (2021). Accordingly, albumin showed the fastest degradation, marking low thermal stability, while globulin degraded more gradually and developed a secondary peak in degradation near 400°C, confirming superior thermostability as explained by Taarji *et al*. (2024). Glutelin and prolamin developed intermediate profiles with greater initial moisture losses but moderate structural resistance. Residual mass at 600°C was highest for globulin, perhaps due to mineral and non-volatile residues as stated by Ortega *et al*. (2024). Globally, thermal behavior suggests that globulin is most suitable for high-temperature applications, while albumin does better under mild conditions.

CONCLUSION

The extraction, fractionation and characterization of cottonseed meal protein isolates (CSMPI) demonstrated that CSM is an excellent source of high-quality plant proteins with functional and nutritional potential. Conventional extraction yielded substantial protein recovery, suggesting that advanced methods could further enhance the yield. Thermal analysis revealed moderate denaturation temperatures, desirable for nutritional applications. FTIR spectra confirmed typical amide I, II and III protein bands, with β-sheet as the predominant secondary structure. Functional assessments showed favorable foaming and emulsifying capacities comparable to other plant proteins. SEM micrographs exhibited distinct microstructural variations among fractions, explaining their functional differences. Notably, the high albumin content underscores CSM's potential to reduce reliance on animal-derived albumin. Overall, cottonseed meal protein fractions exhibit desirable functional and structural properties suitable for food, nutraceutical and pharmaceutical applications, though further optimization of extraction and evaluation under varied processing conditions remain necessary.

Conflict of interest

The authors have no conflicts of interest to declare.

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