


COMPREHENSIVE REVIEW

A Comprehensive Review of Cowpea Proteins: Chemistry, Extraction, Techno-Functionality, Modification, and Food Applications

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Received: 16 February 2025 | **Revised:** 22 May 2025 | **Accepted:** 4 June 2025

Funding: This study was supported by USDA Agricultural Research Service Non-Assistance Cooperative Project (Grants 443993 and 439200) and USDA National Institute of Food and Agriculture Hatch project (Grant 7003330).

Keywords: cowpea protein | functional properties | plant-based proteins | protein modification

ABSTRACT

Cowpea (*Vigna unguiculata*) is a versatile legume crop recognized for its nutritional profile and potential as an alternative protein source in the food industry. The protein content of cowpea is about 20%–32%. The growing demand for sustainable and cost-effective plant proteins has increased research into cowpea protein (CP) chemistry, extraction methods, functional properties, and applications. CPs are mainly comprised of albumins, globulins, and glutelins, with globulins being the dominant fraction. Extraction methods, including wet techniques such as alkaline, acid, water, and salt extractions, as well as dry fractionation, significantly influence the yield, purity, and functional properties of CPs. The functional attributes can be further enhanced through enzymatic, physical, and chemical modifications. Despite notable progress, challenges such as poor functionality, resource-intensive extraction methods, and beany off-flavor limit their broader applications. Recent advancements in protein modifications and eco-friendly extraction technologies have opened new possibilities for CPs in novel applications, such as meat analogs, edible films, and encapsulation. However, the effects of individual modification methods and their combinations on functional properties and underlying mechanisms remain underexplored. This review provides a comprehensive analysis of the chemistry, processing methods, functionality, modifications, and food applications of CPs. It also highlights future trends and aims to guide further research while advancing processing technologies for CPs.

1 | Introduction

Proteins are crucial components in human nutrition, as they are required to structure and function organs, tissues, and cells. Dietary proteins can be sourced from animal and plant origins. Plant-based proteins have recently gained significant attention as alternatives to animal proteins. This shift is largely driven by their numerous advantages, including lower production costs, reduced environmental impact, and a lower risk of cardiovascular disease (Sá et al. 2020). Additionally, the superior functional properties of

plant proteins have made them an attractive ingredient for food products (Burger and Zhang 2019; Kumar et al. 2022).

Cowpea (*Vigna unguiculata*), also known as black-eyed pea, southern pea, China pea, crowder pea, bachapin bean, and cow gram, belongs to the Fabaceae family of herbaceous legumes (Sivakanthan et al. 2020). Legume proteins, particularly those from soybeans, have been extensively studied since the late 19th century. However, the protein composition and properties of cowpeas, which exhibit biochemical similarities with soybean



FIGURE 1 | Different types of cowpeas. Source: From Alfa et al. (2020).

proteins, received relatively little attention until the late 20th century (Sefa-Dedeh and Stanley 1979). In comparison to widely studied legumes such as soybean and pea, cowpea offers unique advantages, including adaptability to drought-prone and low-input environments, affordability, and potential for high protein yields with a favorable amino acid profile (Chibarabada et al. 2017). The remarkable adaptability of cowpeas to arid environments and low fertile soils led to their widespread cultivation in tropical regions of South Asia, Africa, and Latin America (Boukar et al. 2019; Gonçalves et al. 2016). Originating from sub-Saharan Africa, cowpea has spread globally to over 100 countries, with the Niger Republic and Nigeria being the leading producers, accounting for nearly 60% of the world's total cowpea production (Boukar et al. 2015; Gonçalves et al. 2016; Maqbool et al. 2021). Figure 1 shows three different types of cowpeas, including white, brown, and brown-black color. The global production of cowpeas has seen a substantial increase, from 1.3 million tons in 1981 to 8.9 million tons in 2019 (Affrifah et al. 2022). It highlights the growing importance and demand for this versatile legume.

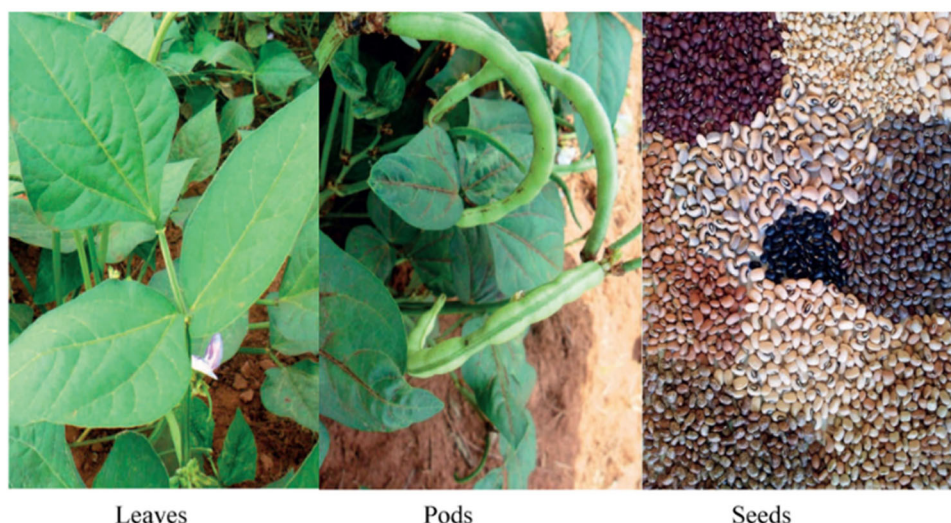
Recent studies have highlighted the nutritional value of various edible parts of the cowpea plant, including leaves, immature green pods, and seeds. In many parts of sub-Saharan Africa, cowpea leaves and immature pods are harvested and consumed as vegetables, owing to their considerable protein and micronutrient contents. Gerrano et al. (2017) reported protein concentrations of up to 28.54% in immature green pods, with significant variation observed across 22 cowpea genotypes. Additional studies confirmed that genotype-by-environment interactions significantly affect the protein and micronutrient composition of green pods, identifying stable high-protein genotypes suitable for cultivation across different regions (Gerrano et al. 2022). Although the young leaves of cowpeas are harvested and consumed for their protein and mineral content (Boukar et al. 2015), the primary protein content of cowpeas is found in the seeds housed within the pod

protective structure (see Figure 2). Therefore, the present study focuses specifically on the protein content of cowpea seeds.

As shown in Figure 3, the morphology of cowpea seeds includes three main components: seed coat, hilum, and cotyledon (Mwangwela 2008). The protein content of cowpea seeds varies between 20% and 32% depending on the specific variety (Awika and Duodu 2017; Gupta et al. 2010; Narayana and Angamuthu 2021; Nwokolo and Smartt 1996; Ragab et al. 2004; Rangel et al. 2004), which is much higher than the protein content of cereals (8%–15%) like rice and wheat (Amagliani et al. 2017; Jayathilake et al. 2018; Paina and Gregersen 2023), and comparable to or even higher than many other commonly consumed legumes such as lentils (20%–30%), chickpeas (15%–25%), and common beans (18%–30%) (Alfaro-Diaz et al. 2023; Grasso et al. 2022; Jarpa-Parra 2017).

Given their protein content comparable to certain types of meat, cowpea seeds are often referred to as the “poor person’s meat” due to their affordability (Som and Hazra 1993). In addition to their high protein content (20%–32%), cowpea seeds also include high carbohydrates (50%–60%), low fat (~1%), fiber, minerals (e.g., zinc, magnesium, phosphorus, potassium, and iron), vitamins (A, B complex, C, and K), and bioactive compounds (da Silva et al. 2021; Duraipandian et al. 2022; Esmaeilzadeh-Hosseini et al. 2023; Jimenez-Lopez 2021).

Cowpea protein (CP) is an excellent source of essential amino acids, such as lysine and histidine (Jimenez-Lopez 2021; Matemú et al. 2021). The protein digestibility of CPs (72%–87%) is considered high and generally exceeds that of other common legumes such as bean, lentil, and chickpea, which typically show digestibility values ranging from 70%–80% (Rangel et al. 2004; Seabra et al. 2001; Tekka et al. 2020). Moreover, CPs, their hydrolysates, or certain peptide fractions have demonstrated sev-



Leaves

Pods

Seeds

FIGURE 2 | Picture of cowpea leaves, pods, and grains. Source: From Abebe and Alemayehu (2022).

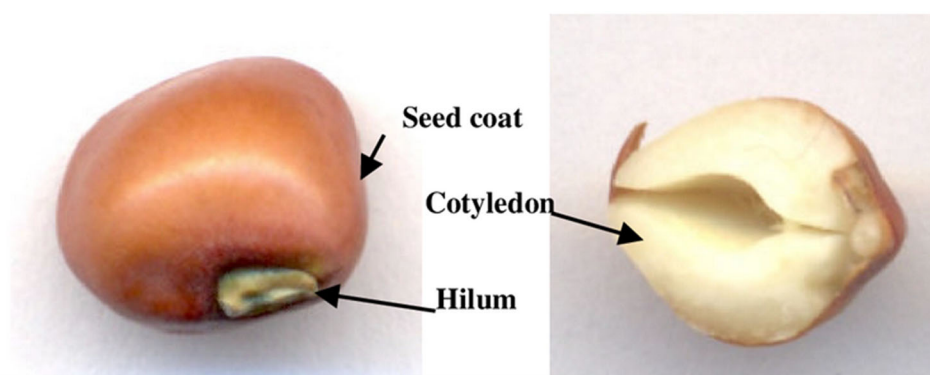


FIGURE 3 | The morphology of cowpea seeds. Source: From Mwangwela (2008).

eral health-promoting effects, including anti-oxidative (Gómez et al. 2021; M. R Segura Campos et al. 2010), non-toxicity (Rangel et al. 2004), anti-obesity (Frota et al. 2008; Marques et al. 2015), anticancer (Philadelpho et al. 2023), anti-diabetes (Barnes et al. 2015; de Souza Rocha et al. 2014), hypocholesterolemic (Marques et al. 2018), anti-inflammatory, and antihypertensive (Marques et al. 2018; Maira R Segura Campos et al. 2010; M. R. Segura Campos et al. 2013; M. R. Segura-Campos et al. 2011) activities. These bioactivities are also reported for other legume proteins such as soy, chickpea, and lentil; however, some studies have shown that cowpea-derived proteins and peptides exhibit comparable or even superior potency in specific functions, such as antioxidant and antihypertensive effects (Garcés-Rimón et al. 2022; Marathe et al. 2011). Despite these advantages, CP remains underutilized in food applications compared to soy and pea proteins, which dominate the plant-based market. A clearer understanding of CP's structure, functionality, and health-promoting properties could help expand its application in sustainable food systems.

Although a few studies have summarized the nutritional, functional, and health benefits of CPs (Awika and Duodu 2017; da Silva et al. 2021; Guang et al. 2012), these reviews have certain limitations. Some primarily focus on specific aspects such as bioactive peptides or health implications. In contrast, others provide only

a general overview without an in-depth discussion of extraction methods, structural characteristics, or modifications. Additionally, earlier reviews may not incorporate recent advancements in protein extraction technologies, structural characterization techniques, and novel food applications. Given the increasing interest in plant-based proteins for sustainable food systems, this article addresses these gaps by reviewing six key areas: (i) protein composition and structure; (ii) extraction methods; (iii) techno-functional properties; (iv) modification methods; (v) potential novel food applications; and (vi) future trends. By offering a detailed and up-to-date overview, this review serves as a valuable resource for researchers and industry professionals, facilitating further innovation within the plant-based food sector.

2 | Protein Composition and Structure

Understanding the functional, structural, and nutritional properties of proteins requires a detailed analysis of their amino acid profile and protein composition and structure. The amino acid profile is essential for assessing nutritional quality, especially the presence of essential amino acids. Meanwhile, protein composition refers to the types and relative abundance of different protein fractions or subunits, which influence functional properties

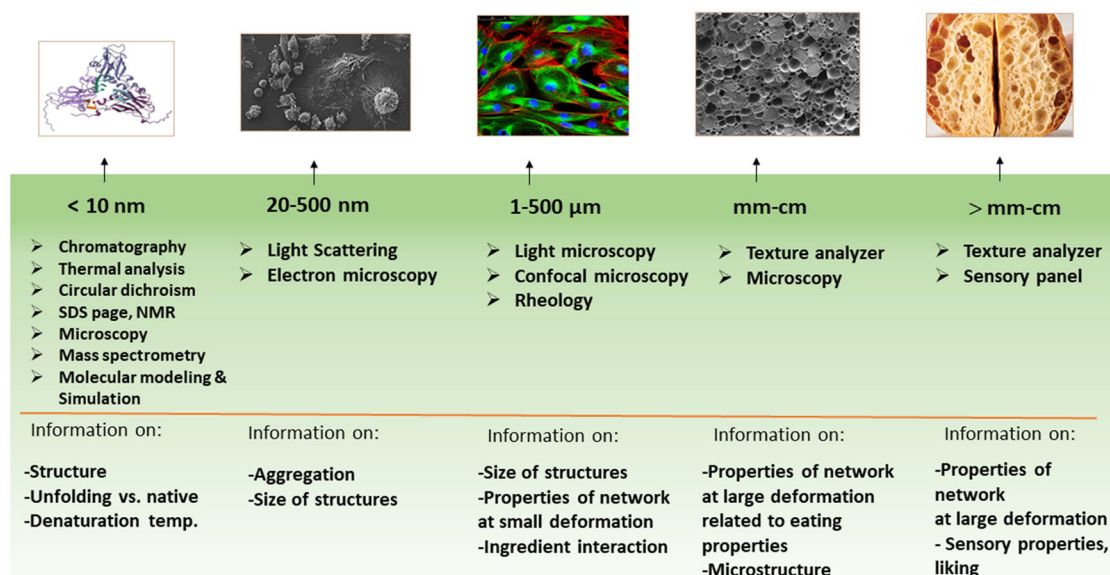


FIGURE 4 | Analytical techniques for determining protein structures. SDS, sodium dodecyl sulfate.

such as solubility and gelation. Techniques such as amino acid profiling and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) are commonly employed to determine the amino acid composition and identify protein bands in CPs. This section provides an overview of CP protein fractions, amino acid profiles, and the application of advanced analytical techniques, such as Fourier transform infrared (FTIR) spectroscopy, fluorescence spectroscopy, scanning electron microscopy (SEM), and circular dichroism (CD) to analyze the compositional and structural properties of CPs (see Figure 4).

2.1 | Protein Fractions

The CPs are classified into four main fractions based on the Osborne classification system: salt-soluble globulins, water-soluble albumins, basic and acid-soluble glutelins, and alcohol-soluble prolamins. Among these, globulins (50%–70%) and albumins (20.10%–24.80%) are the dominant storage proteins in CPs and are readily digestible by proteases (Ragab et al. 2004; Tekas et al. 2020; Vasconcelos et al. 2010). Similarly, globulins and albumins are recognized as primary storage proteins in other legumes, such as lupin seed flour (Shrestha et al. 2021). However, the hierarchy of protein fractions in CPs is not generally consistent from the literature. Some studies, such as that by Gupta et al. (2010), identified glutelins as the second most abundant protein fraction after globulins. In their analysis of two cowpea varieties (CS-88 and V-240), the major protein fractions were reported as globulins (55.6%–58.8%), followed by glutelins (14.4%–15.6%), albumins (8.2%–11.9%), and prolamins (2.3%–5.0%). These discrepancies highlight the influence of genetic variation and varietal differences on CP composition. Additionally, environmental factors, cultivation conditions, and genetic background play critical roles in shaping the protein composition and content of cowpea genotypes, as noted by Vasconcelos et al. (2010). This variability underscores the importance of genotype-specific analyses when evaluating CPs for food applications.

2.1.1 | Globulins

Globulins, highly soluble in diluted salt solutions, are the dominant protein fraction in CPs, similar to other legume seeds, comprising 38.4%–58.80% of the total protein. These proteins are commonly classified into fractions with sedimentation coefficients (S20) of 7–8S and 11–12S, referred to as vicilin and legumin, respectively. Within the globulin fraction, two primary groups have been identified: vicilin/ β -vicilin (7S) and legumin (11S) (Vasconcelos et al. 2010). Among these, vicilin is the predominant storage protein, making up approximately 88% of the total globulin (Loushigam and Shanmugam 2023). Advanced analyses, such as ultracentrifugation and electrophoresis, have revealed three salt-soluble protein (SP) fractions in CPs with sedimentation coefficients ranging from 2.7S to 12.1S (Sefa-Dedeh and Stanley 1979). Further research identified specific variants of vicilin, including α -vignin and β -vignin, with S20 values of 16.5S and 13S, respectively (Martin Alain 2023). The most prominent proteins in CPs are the 8S and 11–12S globulins, characterized by large molecular weights (MWs), aligning with the presence of vicilin and legumin (Sefa-Dedeh and Stanley 1979). These findings are supported by other studies, such as Derbyshire et al. (1976), who observed an 11.3S protein fraction.

SDS–PAGE analysis of CP reveals four major protein bands with MWs of 65, 60, 56, and 50 kDa, along with several minor bands ranging from 28 to 42 kDa. The vicilin (7S) subunit typically exhibits two or three bands within the 49–63 kDa range (Chan and Phillips 1994). Similarities to soybean protein bands have also been noted, with CPs displaying major bands at 40, 60, and 66.2 kDa, and a minor band at 30 kDa (Horax et al. 2004). Recent studies confirm the presence of three major bands in the 47–58 kDa range and additional prominent bands at 50 and 52 kDa, attributed to 7S globulin-3 and 8S globulin, respectively (Martin Alain 2023). Other researchers, such as Loushigam and Shanmugam (2023), have also identified major bands at 55 and 12 kDa.

Studies on cowpea cultivars reinforce these findings. For instance, an analysis of Brazilian cowpea varieties revealed at least 16 globulin bands using 2-mercaptoethanol and SDS, with prominent bands in the 44–63 kDa range (Vasconcelos et al. 2010). Similar results were reported by Araújo et al. (2002) for other cultivars. Rangel et al. (2004) identified a major protein fraction at 52 kDa and a minor fraction at 30 kDa, the latter precipitating with vicilin at its isoelectric point (pH 4.5). Gel filtration chromatography using Sephadex G-200 identified MW ranges from 13.5 to 50 kDa and a significant fraction exceeding 600 kDa, which was likely caused by protein aggregation (Sefa-Dedeh and Stanley 1979).

2.1.2 | Albumins

Albumins, a water-soluble fraction, are the second most predominant protein available in CPs, accounting for 8.20%–22.5% of the total protein content (Gupta et al. 2010; Tekka et al. 2020). In addition to their role as storage proteins, albumins also function as metabolic and enzymatic proteins, including protease inhibitors, lipoxygenases, and lectins (Park et al. 2010). SDS-PAGE analysis of the albumin fraction reveals four prominent protein bands with MW of 99, 91, 32, and 30 kDa, alongside minor bands at 28 and 52–83 kDa (Chan and Phillips 1994).

2.1.3 | Glutelins

Glutelins, the third major protein fraction in cowpea seeds, account for 6.40%–15.60% of the total protein, depending on their solubility in acidic or alkaline solutions (Vasconcelos et al. 2010). Glutelins are predominantly alkali-soluble and have the lowest lysine content compared to other CP fractions, which may affect their nutritional value (Vasconcelos et al. 2010). Interestingly, the glutelin content in cowpea seeds is significantly higher than that of other legumes, such as black gram (2%) (Padhye and Salunkhe 1979) and soybean (approximately 0%) (Sánchez-Chino et al. 2015). Electrophoretic analysis of cowpeautelins shows four main protein bands with MWs of 101, 68, 31, and 29 kDa, along with a minor band ranging from 44 to 62 kDa (Chan and Phillips 1994).

2.1.4 | Prolamins

Prolamins, the least abundant protein fraction in cowpea seeds, range from 1.3% to 5.0% of the total protein content, similar to their limited presence in other legumes, such as soybean, dry bean, and pea (approximately 0%), and lupin (1%) (Sánchez-Chino et al. 2015). Prolamin is also characterized by high proline and glutamine contents (Vasconcelos et al. 2010). Unlike legumes, where prolamins are minimal, these proteins serve as a primary storage fraction in cereals and significantly influence the nutritional and functional properties of cereal proteins (Shewry and Halford 2002; Tekka et al. 2020). SDS-PAGE analysis of cowpea prolamins identifies four main protein bands with MW of 105, 62, 59, and 54 kDa (Chan and Phillips 1994).

2.2 | Amino Acid Profile

The amino acid profile of CPs is summarized in Table 1. The amino acid composition may vary as a result of cultural practices, cultivars, genetic changes, and environmental conditions (Giami 2005; Gupta et al. 2010; Rangel et al. 2004). Previous studies have indicated that CPs contain all the essential amino acids required by adults and most of those necessary for preschool children (Chan and Phillips 1994; Vasconcelos et al. 2010). However, except for histidine and isoleucine, the essential amino acids in CPs required for preschool children and adults fall below the FAO/WHO (2007) recommendations and USDA (2005) (Affrifah et al. 2022).

The most sulfur-containing amino acids are also in globulins and albumins fractions. Notably, the amino acid distribution within the CPs is dominated by glutamic acid and aspartic acid, with glutamic acid ranging from 16.2 g/100 g of protein to 18.9 g/100 g and aspartic acid ranging from 11.6 g/100 g of protein to 12.1. However, this protein lacks tryptophan and sulfur-containing amino acids, including methionine and cysteine like other legume proteins (Affrifah et al. 2022; Rangel et al. 2004; Vasconcelos et al. 2010). CPs contain significantly higher levels of lysine, with 6.8 g/100 g of protein, compared to those found in wheat (3.4 g/100 g), barley (2.98 g/100 g), rice bran (4.55 g/100 g), and other cereal proteins. Its lysine content is comparable to that of casein (8.15 g/100 g) and soy protein isolate (6.14 g/100 g), making it an attractive complement to cereals rich in sulfuric amino acids (Han et al. 2015; Matemtu et al. 2021; Rangel et al. 2004). Chan and Phillips (1994) also reported that all four fractions of cowpea seeds had high levels of aspartic (11.67%) and glutamic (16.51%) acids, leucine (8.65%), and lysine (8.16%), whereas glutelin and albumin fractions showed the lowest (7.58%) and the highest (9.17%) level of lysine, respectively. They also claimed that glutelin and prolamin have the highest essential amino acids among all four fractions. The difference in amino acid composition in different cowpea genotypes may also be related to their major protein fractions. Gupta et al. (2010) reported that those genotypes whose major fraction was globulin had the lowest sulfur-containing amino acids, whereas those varieties whose major fraction was prolamins were low in lysine. Therefore, there is a negative relationship between lysine and prolamins and between sulfur-containing amino acids and globulin.

2.3 | Structure

FTIR, fluorescence spectroscopy, and CD are widely used to investigate the molecular structure of proteins, including their secondary and tertiary structures. In contrast, SEM provides complementary insights by examining the surface morphology of various protein samples. Table 2 provides essential information about the structural and morphological characteristics of CPs based on these techniques.

2.3.1 | SEM

SEM was employed to examine the surface morphology of CPs. A previous study reported that the untreated CPs generally

TABLE 1 | Amino acid profile of cowpea proteins (CPs) (Gupta et al. 2010; Rangel et al. 2004).

Amino acid	CPs (g/100 g protein)	FAO/WHO (2007) recommendation (g/100 g protein) Adults preschool		USDA (2005) recommendation (g/100 g protein) Adults preschool	
Threonine	3.89–5.12	1.50	1.80	2.40	2.70
Leucine	6.45–8.50	3.90	4.40	5.20	5.50
Phenylalanine	6.6	2.50 ^a	—	4.10 ^a	4.70 ^a
Cystine	0.84–1.80	1.50 ^b	—	2.30 ^b	2.50 ^b
Valine	5.0	2.60	2.90	2.90	3.20
Isoleucine	4.17–5.46	2.0	2.30	2.30	2.50
Lysine	7.30–8.74	3.0	3.50	4.70	5.10
Histidine	1.85–3.70	1.0	1.20	1.70	1.80
Tryptophan	1.0–1.33	0.4	0.5	0.6	0.7
Aspartic acid	11.6				
Serine	5.3				
Glutamic acid	16.2				
Alanine	3.90				
Proline	4.50				
Arginine	7.50				
Tyrosine	3.90				
Methionine	1.28–2.06				
Glycine	3.80				

^aPhenylalanine + tyrosine.^bCystine + methionine.**TABLE 2** | Structural characteristics of cowpea proteins from different analytical techniques.

Structural properties		References
SEM	Open structure with sheet/flake-shape, crystalline, wrinkled, and uneven globular surfaces	Loushigam and Shanmugam (2023), Rudra et al. (2016)
FTIR	1700–1600 cm ⁻¹ : amide I (C=O stretching); 1600–1500 cm ⁻¹ : amide II (N–H bending vibrations and C–N stretching); 1228 cm ⁻¹ : amide III (C–N stretching and N–H bending vibrations); around 3637 cm ⁻¹ : O–H stretching; near 1406 cm ⁻¹ : free carboxylates stretching	Gómez et al. (2021), Mune and Sogi (2016), Loushigam and Shanmugam (2023)
Fluorescence spectroscopy	When fluorescence spectroscopy was performed over the range of 300–460 nm, the maximum fluorescence emission λ max was observed at 337.5 and 348 nm, depending on the pH	Gómez et al. (2021), F. Peyrano et al. (2016)
CD	Secondary structures (α -helix, β -turn, β -sheet, and random coil)	Aluko et al. (1997)

Abbreviations: CD, circular dichroism; FTIR, Fourier-transform infrared; SEM, scanning electron microscopy.

displayed an open, flaky, or sheet-like structure. In contrast, sonicated CPs showed rougher and more irregular surfaces (Loushigam and Shanmugam 2023).

The CPs obtained through vacuum or freeze-drying exhibited a highly crystalline surface (see Figure 5), attributed to protein aggregation. This aggregation likely results from protein adsorp-

tion at ice/solvent interfaces or the removal of water during the drying process. Conversely, spray-dried CPs displayed an uneven globular morphology, which is likely due to the denaturation of surface proteins caused by the mechanical forces during pumping and the exposure of droplets to high temperatures (Rudra et al. 2016).

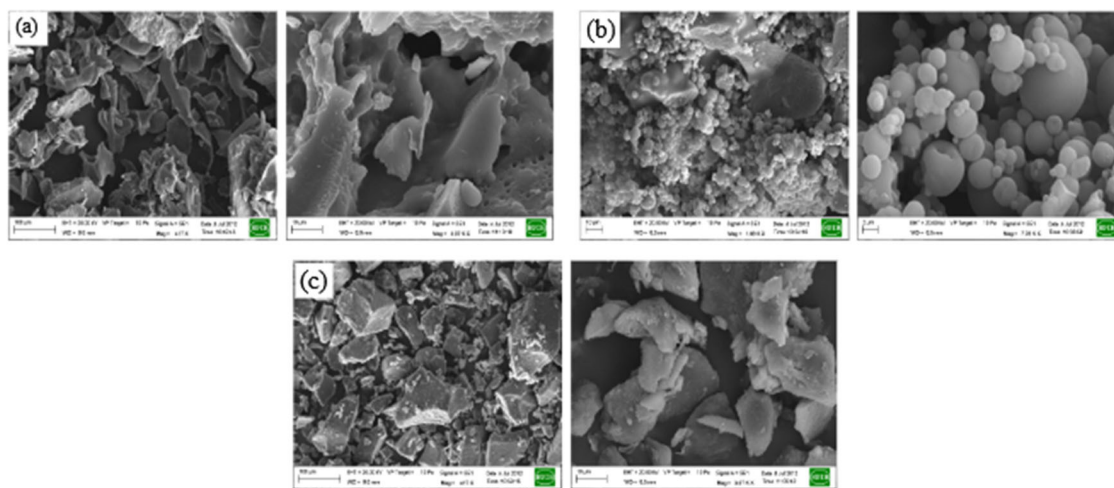


FIGURE 5 | Surface electron micrographs of (a) freeze dried (b) spray dried, and (c) vacuum dried cowpea protein isolate. *Source:* From Rudra et al. (2016).

2.3.2 | FTIR, Fluorescence Spectroscopy, CD

FTIR spectroscopy, along with fluorescence spectroscopy and CD, was employed to investigate the secondary and tertiary structures of CPs. The FTIR analysis revealed five notable peaks: 1700–1600 cm^{-1} (Amide I), 1600–1500 cm^{-1} (Amide II), 1228 cm^{-1} (Amide III), and peaks around 3637 and 1406 cm^{-1} . The peak in the 1700–1600 cm^{-1} range is associated with C=O stretching vibrations. The 1600–1500 cm^{-1} region is indicative of N–H bending vibrations and C–N stretching. The peak at 1228 cm^{-1} corresponds to C–N stretching and N–H bending vibrations. Peaks around 3637 and 1406 cm^{-1} are attributed to O–H stretching and the stretching of free carboxylates, respectively (Gómez et al. 2021; Loushigam and Shanmugam 2023; Mune and Sogi 2016). Fluorescence spectroscopy provides insights into the conformational changes of proteins. Analysis conducted in the range of 300–460 nm revealed maximum fluorescence emission (λ_{max}) at 337.5 nm at pH 8.0 and 348.5 nm at pH 10.0 (F. Peyrano et al. 2016). Furthermore, CD analysis revealed that CPs primarily exhibit a β -sheet structure. The CD data also indicated the presence of α -helix, β -turn, β -sheet, and random coil structures (Aluko et al. 1997).

The structural and morphological characteristics of CPs revealed by SEM, FTIR, fluorescence spectroscopy, and CD have direct implications for their functional performance in food systems. For example, the β -sheet-rich secondary structure observed via CD is generally associated with higher gel strength. A more flexible structure may enhance foaming and emulsifying capacities (Tan et al. 2019). Surface morphology from SEM, such as increased roughness or globularity, can influence hydration and oil absorption capacities (Yang et al. 2020). Similarly, changes in tertiary structure, as shown by shifts in fluorescence λ_{max} , indicate protein unfolding or rearrangement, which can enhance interfacial activity, benefiting emulsification and foaming (Li Zhai et al. 2013). These structure–function relationships are further discussed in Section 4 in the context of processing and applications.

3 | CP Extraction Methods

Protein extraction is a crucial step in plant protein processing, as it significantly impacts the protein's yield, purity, functionality, and structural characteristics. The choice of extraction technique can affect essential properties such as solubility, emulsification, gelation, and thermal stability (Table 3), which are important for various food applications (Yuliarti et al. 2021).

3.1 | Wet Extraction

Alkali extraction is widely recognized as the most efficient method for obtaining high-purity CPs, as demonstrated in recent experiments comparing various wet extraction techniques. A study focusing on protein extraction from Indian pulses, including cowpea, evaluated alkaline extraction, acid extraction, water extraction precipitation, and micellization precipitation. Among these, alkaline extraction consistently outperformed the others with the highest protein yield (13.5%–18%), recovery (58%–67%), and purity (~79% for CP concentrate). Although the extraction method significantly impacted yield and recovery, protein purity remained unaffected across techniques. The study highlighted that the higher solubility of proteins at pH 9 and minimal coprecipitation of nonprotein components make alkali extraction the preferred method for CP purification (Penchalaraju and Bosco 2022). The alkali extraction method typically involves extracting proteins using an alkaline solution, often sodium hydroxide (NaOH) at pH 9.0, followed by isoelectric precipitation with hydrochloric acid (HCl) at pH 4.5 to isolate the proteins. After extraction, the protein is processed into a powdered form using drying techniques such as freeze-, spray-, or drum-drying. Among these methods, spray drying is often preferred for its efficiency, cost-effectiveness, and suitability for large-scale production. In contrast, freeze-drying, although highly effective at preserving protein structure and functionality, is both time-intensive and expensive, making it less practical for large-scale industrial applications (Boye et al. 2010; Brishti et al. 2020; Preethi et al.

TABLE 3 | Summary of cowpea protein extraction methods: comparative overview of advantages, disadvantages, and protein recovery and functional characteristics.

Extraction method	Conditions	Advantages	Disadvantages	Yield and purity	Functional characteristics	References
Alkaline extraction	Flour: NaCl (0.25 M) solution = 1:10, pH = 8.43, stirred for 2 h at 35°C Flour: water = 1:10, pH = 9.0, 120 min	–High protein yield and recovery –High purity (especially for isolates) –Well-established and scalable	–High water and chemical usage –Environmental concerns –May alter protein functionality	Yield: 13.5–18% Recovery: 58–67% Purity: ~79% (concentrate); > 84% (isolate)	WSI, WAC, OHC, EA, ES, FA Solubility, WHC, FC, FS, EA, ES, digestibility	Mune Mune et al. (2014) Loushigam and Shanmugam (2023)
Salt extraction	Flour: water = 1:10, stir for 1 h, 1.0 M NaCl, 70% ethanol or 0.2% NaOH	–Exploits salt-in/salt-out effects for selective precipitation –Good viscosity, WHC, and dispersibility	–Functionality varies with pH/salt –Moderate purity	Yield: 26.8% Purity: 95.7%	Solubility, WHC, OHC, bulk density, dispersability	Ragab et al. (2004)
Water extraction	Flour: water = 1:10, 4°C for 16 h, centrifugation (4°C, 2000 rpm, 15 min), spray drying	–Simple and mild –No chemical reagents	–Long extraction time –Lower purity and yield	Yield: 14.4% Purity: 74%	—	Penchalaraju and Bosco (2022)
Acid extraction	Flour/Water = 1:10, pH = 2.0, 1 M HCl, 8 h at 25°C	–Similar to alkali in process –Reasonable protein yield and purity	–Less commonly used –High water and chemical use	Yield: 11.1% Purity: 72%	—	Penchalaraju and Bosco (2022)
Air classification	Classifier wheel speed of 8000, 10,000, and 12,000 rpm, feed rate = ~250 g/h, airflow = 47 m ³ /h	–Environment friendly –Preserves native functionality –Minimal water/Chemical use	–Lower protein purity –Requires optimization for functionality	Protein content: Up to 51.3% Max ~55% (fine fraction, 8000 rpm)	Gelation, WHC	Schlangen et al. (2022)

Abbreviations: EA, emulsifying activity; ES, emulsion stability; FA, foaming ability; FC, foaming capacity; FS, foaming stability; OHC, oil-holding capacity; WAC, water absorption capacity; WHC, water-holding capacity; WSI, water solubility index.

2021). In alkaline extraction, pH and ionic strength are key factors that influence protein yield and content, leading to alterations in the physicochemical and functional properties of the proteins. The CP isolate extracted using this method achieved the highest protein content (greater than 84%) and yield (greater than 87%) at a pH of 9.1 and NaCl concentration of 0.15 M (Mune Mune et al. 2008).

Water extraction is another method of isolating proteins by dissolving them in water, followed by homogenization, separation, protein precipitation, and drying to obtain a protein concentrate or isolate (Boye et al. 2010). Penchalaraju and Bosco (2022) extracted CPs using water at 4–8°C for 16 h, resulting in a protein yield of 14.4% and a purity level of 74%.

The principle of salt extraction is based on the “salt-in” and “salt-out” effects. Salt is used to alter protein solubility, inducing precipitation. This is followed by separating insoluble materials and purifying proteins, followed by drying the protein isolate (Boye et al. 2010). The functional properties of CPs are significantly affected by pH and NaCl concentration, as reported by Ragab et al. (2004). Lower functional property values were noted under slightly acidic conditions and at higher salt concentrations. Protein solubility reached its lowest around pH 5.0, whereas the highest solubility occurred at pH 10. Furthermore, the minimum gelation concentration was identified as 6% when the protein was dissolved in either 0.5 or 1.0 M NaCl. CPs exhibited high viscosity and dispersibility (72%), with a water-holding capacity (WHC) of 2.20 mL/g, an oil-holding capacity (OHC) of 1.10 mL/g, and a bulk density of 0.82 g/mL.

Acid extraction of CPs follows a process similar to alkaline extraction but uses an acidic pH during the initial phase. Although less commonly used, this method can still achieve good protein yield and purity, with reported values of 11.1% and 72%, respectively (Penchalaraju and Bosco 2022). However, like other wet extraction methods, it requires substantial water and chemical usage, raising concerns about environmental sustainability (Schutyser et al. 2015).

3.2 | Dry Fractionation

Dry fractionation is a sustainable alternative to traditional wet extraction for isolating protein-rich fractions from cowpeas. Studies have shown that dry fractionation of cowpeas can achieve a protein content of up to 51.3%, demonstrating its potential for producing high-protein ingredients (Tyler et al. 1981). This process involves milling to break down cotyledon cells, releasing starch granules and protein particles, followed by air classification to separate them based on differences in density and particle size (Möller et al. 2021). Unlike wet methods, dry fractionation uses minimal water and chemicals, making it environment friendly. In addition, air classification preserves the natural functionality of proteins by avoiding the use of chemical reagents (Schutyser et al. 2015). Although dry separation of CPs results in lower protein purity than wet methods, high purity is generally not expected to be high for most food applications (Schutyser et al. 2015). However, the functional properties of these fractions require further exploration. A study by Schlangen et al. (2022) investigated the functional properties of dry-fractionated legume proteins

(cowpea, mung bean, and yellow pea), revealing that protein content was influenced by classifier wheel speed, with the highest protein content of 55% for CPs achieved at an optimal speed of 8000 rpm, a feed rate of approximately 250 g/h, and airflow of 47 m³/h. The fine fractions showed strong gelation abilities, producing gels with the highest *G'* value after heat treatment. In contrast, the flour and coarse fractions formed firmer, more solid-like gels that could endure greater deformation. The functional differences observed in fine fractions from different legumes were linked to variations in protein properties rather than the amount of protein.

Overall, although wet extraction methods like alkali and salt extraction remain the most effective for achieving high protein yield and purity from cowpeas, their environmental impact due to substantial water and chemical use cannot be overlooked. Emerging alternatives like dry fractionation, which avoids the use of water and chemicals, are gaining attention despite their lower protein purity, as they maintain native protein functionality and offer a more eco-friendly solution. These developments highlight the need for innovative methods that balance efficiency, functionality, and sustainability in CP extraction.

4 | Techno-Functional Characteristics of CPs

The functional properties of CPs are influenced by various processing factors. A comprehensive understanding of these factors is essential for developing novel food products as they significantly influence texture, flavor, and overall product quality. This section overviews the techno-functional properties of CPs, including solubility, foaming, OHC, WHC, emulsification, and gelation, and factors influencing these properties (Table 4).

4.1 | Solubility

Solubility is a crucial functional property of proteins, significantly impacting their behavior in food systems. It affects other functional characteristics such as gelation, emulsification, and foaming, which are essential for effective food formulation and processing (Wang and Kinsella 1976). High protein solubility is generally desirable, as it contributes to reduced sedimentation during storage and ensures the rapid achievement of stable, homogeneous solutions (Hall 1996). Factors influencing protein solubility include salt concentration, pH, surface charge (zeta potential), and surface hydrophobicity (K. Shevkani et al. 2019). According to Sefa-Dedeh and Stanley (1979), CP fractions undergo association-dissociation behavior, which significantly influences their solubility. They reported that increasing ionic strength from 0.05 to 1.0 M can lead to the dissociation of large protein fractions, resulting in enhanced solubility. Conversely, thermal treatment of water-soluble fractions from 25°C to 100°C can have a minimal impact on solubility due to the denaturation of proteins and the formation of soluble aggregates.

CPs exhibited a U-shaped pH-dependent pattern with the lowest solubility observed around the isoelectric point (pH

TABLE 4 | Functional properties of cowpea proteins.

Functionality	Characteristics	Impacting elements	References
Solubility	–U-shaped pH-dependent solubility –The lowest solubility at pH 3.5–5.0 (isoelectric point) (5.0%)–The highest solubility at pH 9.0 (80%)–Alkaline extraction = 60%–100% solubility, below 40% at the isoelectric point–Salt extraction (0.5 M NaCl) or limited enzyme hydrolysis can enhance solubility at the isoelectric point	pH, solvent type, ionic strength, temperature	Gómez et al. (2021), Ragab et al. (2004)
WHC/OHC	–Highest at pH 7.0 = WHC: 3.55 g/g and OHC 6.59 g/g –Enzymatic hydrolysis improves the OHC of CPs to 4.6 g/g	Amino acid composition, structure, hydrophobic to hydrophilic amino acid ratio, particle size, pH, and temperature	Ge et al. (2021), Mune Mune (2015)
Emulsifying properties	–Emulsifying ability index (EAI) at pH 3.0 = 8.70–9.62 m ² /g; at pH 7.0 = 9.10–10.33 m ² /g; at pH 9.0 = 9.59–10.16 m ² /g –Emulsifying stability index (ESI) at pH 3.0 = 110–460 min; at pH 7.0 = 250–465 min; at pH 9.0 = 500–2800 min	Protein properties (resource, hydrophilicity, and hydrophobicity of surface, solubility, etc.), environmental factors (pH, temperature, etc.), processing conditions, and the composition of the emulsion system	Ge et al. (2021), McClements (2004b)
Foaming capacity	–The highest foaming properties at alkaline pH –The lowest foaming properties at the isoelectric point –The extraction method affects the foaming ability of CPs	Protein structure and flexibility, surface hydrophobicity, environmental conditions (such as pH and ionic strength), protein molecular weight and concentration, and rheological properties	Damodaran (1994), Rangel et al. (2003)
Gelling ability	–Improved gel firmness at pH 10 (LGC of 16% after treatment at 70°C)	Protein concentration, pH, ionic strength, and heating rate	Nagano et al. (1992)

3.5–5.0) (Gómez et al. 2021; Ragab et al. 2004). At the isoelectric point, proteins possess minimal net charge, leading to decreased electrostatic repulsion between molecules. This reduced repulsion results in increased aggregation and precipitation (Gómez et al. 2021). The solubility of CPs varies in different extraction solutions due to the diverse interactions that stabilize protein structures (F. Peyrano et al. 2021). Gómez et al. (2021) reported that the solubility of alkaline-extracted CPs ranged from 60% to 100%, whereas it was below 40% at the isoelectric point. However, methods such as salt addition (0.5 M NaCl) (Ragab et al. 2004) or limited enzyme hydrolysis (Gómez et al. 2021) can enhance solubility at the isoelectric point. Protein hydrolysis decreases peptide size and exposes hydrophilic groups, improving their interaction with water and consequently increasing solubility near the isoelectric pH (Gómez et al. 2021).

Despite these strategies, CPs low solubility (5.0% at pH 5.0 and 80% at pH 9.0) (Ragab et al. 2004) may limit its application in food products. Therefore, exploring methods to improve solubility, such as altering particle sizes and surface charge, is essential. Enhanced solubility can lead to better texture, stability, and overall quality in food products, making CPs more effective as functional ingredients.

4.2 | OHC and WHC

WHC and OHC, also referred to as water and oil absorption abilities, are key functional properties in food systems, representing the ability of ingredients to retain water and oil, respectively, which impact texture, mouthfeel, flavor retention, stability, and quality. Factors such as amino acid composition, structure, hydrophobic to hydrophilic amino acid ratio, particle size, pH, and temperature influence these properties (Khan et al. 2011; Shen, Hong, Li 2022). The WHC of proteins plays a key role in enhancing food products' juiciness, thickness, and overall viscosity (Rahman and Lamsal 2021), whereas the OHC influences sensory attributes like mouthfeel and flavor retention, particularly in products like meat (Loushigam and Shanmugam 2023).

CPs demonstrate high WHC and OHC, comparable to soy protein isolates and pea protein isolates, particularly at neutral pH. At pH 7.0, CP's WHC and OHC reach values of 3.55 and 6.59 g/g, respectively, which makes it a suitable ingredient for food products that require both moisture retention and enhanced mouthfeel, such as breads and muffins (Ge et al. 2021). According to research, enzymatic hydrolysis can improve the OHC of CPs to 4.6 g/g at a degree of hydrolysis (DH) of 25%–30%. This increase is likely due to the exposure of hydrophobic groups,

which have a greater ability to trap oil (Mune Mune 2015). It is also reported that the balance between polar and nonpolar amino acids in the structure of unfolded CPs allows them to interact well with water and other proteins. It contributes to holding water more effectively and increases WHC (F. Peyrano et al. 2021). The high WHC of CPs is also due to their strong affinity for water, despite having a gel structure that differs from the more typically ordered and compact matrix commonly linked to high WHC. Instead of relying on capillarity, CPs retain water through strong intermolecular interactions, which allow them to hold water effectively despite variations in pore size and filament thickness in their gel structure (Heremans et al. 2017; F. Peyrano et al. 2021). WHC ranging from 1.49 to 4.72 g/g is essential for achieving a suitable thickness and texture in viscous foods like soups and sauces (Aletor et al. 2002). Research indicates that the good WHC of CPs allows them to serve as highly effective functional ingredients in various food formulations.

4.3 | Emulsifying Properties

Proteins are commonly utilized as emulsifiers because they can adsorb at the boundary between oil and water, creating stable layers around the dispersed droplets. During emulsion formation, proteins or protein aggregates attach to the droplet surfaces and rearrange themselves at the interface. The hydrophobic parts of the proteins orient toward the oil phase, whereas their hydrophilic regions face the water phase. This rearrangement reduces interfacial tension, preventing the droplets from merging or clumping together, which stabilizes the emulsion and controls the texture and quality of many food products (Karaca et al. 2011).

The emulsifying ability of proteins depends on various factors, such as protein properties (resource, hydrophilicity and hydrophobicity of surface, solubility, etc.), environmental factors (pH, temperature, etc.), processing conditions, and the composition of the emulsion system (McClements 2004b). To measure the emulsifying properties of food proteins, the emulsion stability index (ESI) and emulsifying activity index (EAI) are commonly employed (K. Shevkani et al. 2015). CPs demonstrate higher emulsifying properties than legumes, such as mung bean and kidney bean proteins, across pH levels of 3.0, 7.0, and 9.0. The EAI of all proteins ranges from 8.70 to 9.62 m²/g at pH 3.0, 9.10 to 10.33 m²/g at pH 7.0, and 9.59 to 10.16 m²/g at pH 9.0. In terms of ESI, they exhibit values of 110–460 min at pH 3.0, 250–465 min at pH 7.0, and 500–2800 min at pH 9.0 (Ge et al. 2021).

4.4 | Foaming Properties

The foaming ability of proteins extends their use in various food products, such as ice cream, whipped cream, and cakes. For a stable foam to form, proteins must quickly migrate to the air–water interface and exhibit sufficient flexibility to attach to it. The conformational changes allow proteins to reorient at the surface and create a viscoelastic film around air bubbles, which prevents bubble coalescence, lowers surface tension, and effectively traps air to form foam (Amagliani et al. 2017; Cano-Medina et al. 2011). Foaming ability is often assessed by two key parameters: foaming capacity (FC), which describes the protein's ability to generate stable foams when whipped or aerated, and foaming stability

(FS), which indicates the foam's stability over time (K. Shevkani et al. 2019). FC and FS are influenced by several factors, including protein structure and flexibility, surface hydrophobicity, environmental conditions (such as pH and ionic strength), protein MW and concentration, and rheological properties (Damodaran 1994).

In a study, vicilin (the major storage globulin) of cowpea and pea was purified by ammonium sulfate precipitation, and its foaming ability was compared to semipurified CPs. The foaming ability of CPs and vicilins improved at alkaline pH (pH = 8.0), likely due to an increase in net charge. This weakens hydrophobic interactions, enhances protein flexibility, and facilitates partial unfolding at the air–water interface. However, purified vicilins showed lower FC and FS compared to CPs, which may be attributed to their limited unfolding capacity at the interface (Rangel et al. 2003). Proteins have no net charge at the isoelectric point. Without a net charge, the proteins cannot generate the repulsive forces needed to keep oil droplets apart. For effective emulsion stability, proteins must have a sufficient surface charge to create these repulsive forces, which prevent the droplets from merging and help maintain a stable emulsion (McClements 2004a). In addition, higher protein solubility generally correlates with improved foaming properties (K. Shevkani et al. 2015). An increased surface charge on proteins enhances FC by weakening hydrophobic interactions, which in turn improves protein solubility and flexibility. This allows proteins to spread quickly at the air–water interface, efficiently trapping air bubbles and promoting stable foam formation (K. Shevkani et al. 2019). The extraction method significantly affects the solubility of CPs, which in turn influences their foaming ability. Proteins extracted with urea demonstrated the highest solubility (approximately 94%) compared to those extracted with saline or SDS buffers, corresponding to greater foaming ability (F. Peyrano et al. 2021).

4.5 | Gelling Properties

Many proteins gel upon heating. Heat causes proteins to denature, meaning that they unfold and lose their natural structure. Once unfolded, these proteins can interact more easily with each other, forming bonds that create a stable gel network. Heat-induced gels are widely used in cooked products like custards, soups, and puddings (Grasso et al. 2022; Joshi et al. 2017).

The gelling properties of proteins are influenced by various factors, such as protein concentration, pH, ionic strength, and heating rate, each impacting gel texture, strength, and stability (Nagano et al. 1992). Controlling these variables is essential to achieve desired textures in food products.

The extraction process significantly influences the gelling properties of CPs. During extraction, adjusting pH may modify protein structure and increase surface hydrophobicity, thereby enhancing gelling ability (F. Peyrano et al. 2021). The least gelation concentration (LGC) method is a widely used approach to evaluate the gelling properties of proteins, which identifies the minimum protein concentration needed for gel formation; a high LGC value indicates lower gelation efficiency, requiring more protein to form a stable gel (Boye et al. 2010). CPs showed a lower LGC value (12%) (F. Peyrano et al. 2016) than chickpea protein (14%–18% w/v) (Kaur and Singh 2007) and soy protein isolate (18%) (Branch

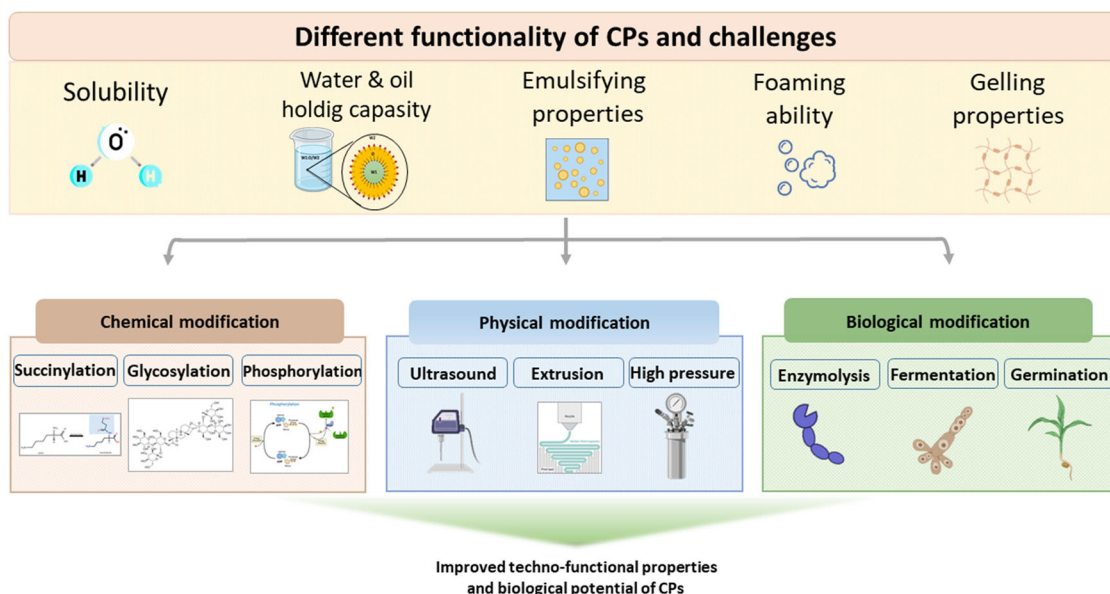


FIGURE 6 | Various modification methods of cowpea proteins (CPs).

and Maria 2017), resulting in better gelling ability than other legume proteins. The CP gel extracted at pH 10 showed enhanced firmness with an LGC of 16% after treatment at 70°C, likely due to strengthened hydrophobic interactions within the gel structure (F. Peyrano et al. 2016).

5 | Modifying CPs

As mentioned earlier, the limited functional properties of CPs, such as their emulsifying, gelling, and foaming abilities, present challenges when incorporating them into food matrices. To overcome these limitations, various chemical, physical, and biological methods have been developed to modify CP properties. The summary of these methods is displayed in Figure 6.

5.1 | Chemical Modification

Chemical modification of proteins is a targeted approach to enhance or alter their native properties by inducing specific chemical reactions. These reactions often involve covalent modifications of functional groups on amino acid side chains, such as amino, carboxyl, and hydroxyl groups, allowing controlled alteration of protein structure and function. By strategically modifying these groups, researchers can adjust properties like solubility, charge, and thermal stability, which, in turn, impact the protein's techno-functional and biophysical characteristics (Akharume et al. 2021; Shen, Du et al. 2022; Shieh et al. 2021).

Common modification methods applied to plant proteins include succinylation, glycosylation, and phosphorylation. Succinylation and glycosylation can improve solubility and emulsification, making the proteins more versatile in food formulations (Basak and Singhal 2022; Shen, Hong, Li 2022; Shen and Li 2021; Y. Wang et al. 2024). Phosphorylation, another widely studied modification, has been linked to calcium absorption, offering added nutritional benefits (Li et al. 2010). These chemical modifications

enable the optimization of CPs for a wide range of functional applications.

5.1.1 | Succinylation

Succinylation is a widely used form of acylation, where succinic anhydride introduces succinyl groups to the hydroxyl or amino groups on a protein. This process typically targets the ϵ -amino group of lysine or other accessible amino groups, modifying specific amino acid residues and altering the protein's functional properties (Qazi et al. 2019).

Succinylation of CP concentrate significantly improved protein solubility, water solubility index, and fat absorption capacity, with the highest fat absorption observed at 0.75 g succinic anhydride/g concentrate. Emulsifying activity also increased, particularly at higher concentrations, with improved emulsifying stability at 1.0 g anhydride/g concentrate. However, at pH 3.5, protein solubility remained low (<8%), and foam stability decreased following acylation (Mune Mune et al. 2011).

5.1.2 | Glycosylation

Glycosylation is a key posttranslational modification in which carbohydrate molecules, such as single sugars or complex polysaccharides, are covalently bonded to specific amino acid residues within a protein, commonly at lysine or asparagine. This modification can occur naturally, often through the Maillard reaction, a nonenzymatic browning process that attaches sugars to proteins without the need for additional chemicals, making it a green modification method (Akillioğlu and Gökmen 2016; Zha et al. 2021). By altering surface properties and enhancing the stability of proteins, glycosylation enhances their functional properties, making glycosylated proteins especially valuable in food applications.

Research shows that glycosylation significantly improves the functional properties of CPs. Denatured CP isolate (DCPI) and glycosylated CP isolate (GCPI), prepared by heating at 85°C, exhibited reduced free amino groups compared to untreated CP isolate, indicating glycation degrees of 17.12% and 49.31%, respectively. This reduction results from Maillard reactions with residual reducing sugars in cowpea flour, leading to structural modifications such as polymerization and crosslinking, which contribute to improved functional properties (Ahmed 2013). These structural changes are instrumental in enhancing CP's suitability for food applications, demonstrating glycosylation's potential to optimize plant proteins for industry use. For instance, glycosylated CP yielded softer bread dough and maintained high sensory acceptability, enabling up to 20% egg replacement in cake formulations without compromising quality (Campbell et al. 2016).

5.1.3 | Phosphorylation

Phosphorylation is a chemical modification in which phosphate groups are covalently attached to specific amino acid residues in proteins, such as serine, threonine, or tyrosine. Chemical phosphorylation is commonly performed using reagents like phosphorus oxychloride (POCl₃), sodium tripolyphosphate, and sodium trimetaphosphate (STMP), which introduce additional phosphate groups to protein molecules, increasing their polarity and surface charge (Li et al. 2010). These modifications improve proteins' emulsifying, foaming, and gelling properties, which are important in formulating food systems.

When applied to CPs, phosphorylation has been shown to enhance their solubility, emulsification, and stability properties under various pH conditions. For example, cowpea globulin treated with protein kinase exhibited increased solubility in the pH range 4–6, which is particularly useful for applications requiring stability in acidic environments (Aluko and Yada 1995). Additionally, although the EAI slightly decreased after phosphorylation, the stability of emulsions increased significantly. This modification also resulted in increased FC and foam stability at the pH ranges 4–6 and 4–5, respectively, further broadening the potential applications of CPs in foamed and aerated food products, as well as in formulations where stable emulsions are critical (Aluko and Yada 1995).

In a related study, the phosphorylation of cowpea globulin increased the phosphate content from 5.81 to 10.55 µg/mg, resulting in significantly enhanced solubility and fluorescence intensity across pH 3–8 and reduced heat-induced coagulation susceptibility. This modification enables the phosphorylated protein to be more versatile in food applications requiring high solubility, whereas deglycosylated proteins showed increased coagulation potential for applications needing heat stability.

5.2 | Physical Modification

Research on the effects of physical modification methods on CPs has been relatively limited but is gaining attention due to their potential to improve functionality and nutritional quality.

Techniques such as ultrasonication, heat treatments, extrusion, and high-pressure processing (HPP) have been studied for their ability to influence the structural, functional, and biological attributes of these proteins. These methods can significantly affect protein folding, denaturation, aggregation, and solubility, ultimately improving their applicability in various food systems (Mirmoghtadaie et al. 2016). This section discusses the effects of specific physical modification techniques, including ultrasound, extrusion, and HPP, on the techno-functional and biological characteristics of CPs and their potential applications in food product development.

5.2.1 | Ultrasound Technique

Ultrasound technology is an innovative, nonthermal method that has been extensively used for modifying the structural and functional properties of plant proteins, including CP isolates. The process relies on acoustic cavitation, which generates localized high temperatures, shear forces, and turbulence, effectively altering protein structures to enhance physicochemical (hydrophobicity, particle size, free SH groups), biological, and functional properties (Rahman and Lamsal 2021).

The ultrasound-assisted extraction (UAE) of CPs has shown significant improvements across multiple functional metrics. For example, using an ultrasonic intensity of 200 W for 10 min enhanced protein yield by 85.53%, solubility by 20%, and WHC by approximately 21% compared to untreated samples. Such intensities also led to a notable decrease in particle size (from 763 to 559 nm), which correlates with improved emulsification properties as well as increased surface hydrophobicity, likely due to the unfolding of protein structures and exposure of hydrophobic groups. The result of SDS-PAGE indicated that ultrasound decreased the MW of CPs compared to untreated samples (Loushigam and Shanmugam 2023).

In addition to its effects on extraction yield and particle size, ultrasound also improves the antioxidant potential of CPI hydrolysates, as evidenced by increased oxygen radical absorbance capacity (ORAC) values in sonicated samples. However, the ferric-reducing antioxidant power (FRAP) and superoxide radical scavenging activity (SRSA) of hydrolysates from ultrasound-treated CPs were lower than those from untreated samples, likely due to a reduction in cysteine levels after sonication. Cysteine is positively correlated with FRAP and SRSA, suggesting that ultrasound-induced structural changes may impact certain amino acids and thus influence antioxidant properties (Quansah et al. 2013). Increasing sonication time and intensity (200 W, 10 min) boosted extraction yield by 24% due to the rapid collapse of sonic bubbles in the solvent. The resulting acoustic cavitation enhanced solvent penetration into the cell matrix, disrupting molecular bonds and accelerating mass transfer and extraction (Loushigam and Shanmugam 2023). Overall, given its eco-friendly nature, ultrasound-assisted modification of CP isolates and optimizing the parameters represent a valuable approach for enhancing their functional and bioactive properties, making them a valuable ingredient for the food industry.

5.2.2 | Extrusion Technique

Extrusion, a process combining high heat, shear force, and pressure, is another physical method to modify the structure and function of proteins in multiple ways. It enhances their techno-functional properties (e.g., solubility, emulsification, and texture) and digestibility and reduces anti-nutritional factors. During extrusion, proteins undergo structural changes like denaturation, aggregation, and degradation which improve their suitability for food applications (Xiao et al. 2025; J. Zhang et al. 2019). Extrusion modifies native proteins by altering parameters such as moisture content, temperature, and screw speed. These changes expose hidden amino acids and hydrophobic groups, increasing their interactions with food components but reducing solubility. This occurs due to the formation of disulfide and hydrophobic bonds. Lower moisture, speed, and temperature intensify denaturation and thermal damage, resulting in greater structural changes compared to higher parameter settings (Camire 1991).

Various research articles have explored how extrusion parameters such as temperature, moisture content, and ingredient composition impact the nutritional, functional, and sensory qualities of cowpea-based products. Several studies have reported that extrusion enhances the protein digestibility of cowpea isolates. For instance, Pelembe et al. (2002) found that extrusion at 130–165°C increased the protein content and solubility, although higher temperatures reduced nitrogen solubility and protein digestibility. Similarly, the work by Gbenyi et al. (2016) showed that increasing barrel temperatures from 120°C to 160°C significantly improved the *in vitro* protein digestibility of cowpea-based extrudates, despite a reduction in protein solubility and residual polyphenols. This suggests that heat treatment during extrusion not only improves protein digestibility but also reduces anti-nutritional factors, such as phytic acid and polyphenols, which can inhibit nutrient absorption. Although extrusion enhances protein digestibility, it may also cause significant changes in protein structure, resulting in the denaturation of certain proteins and a reduction in solubility. Studies have shown that both high and low moisture conditions during extrusion may lead to unfavorable protein modifications (Hossain Brishti et al. 2021). This suggests a need for further optimization of extrusion parameters, such as moisture content and temperature, to balance the benefits of improved digestibility with minimal protein loss.

The incorporation of cowpea flour into extruded products also influenced their functional properties. In a study by Hewa Nadun-godage et al. (2022), the addition of cowpea flour and whey protein concentrate (WPC) to rice flour led to increased bulk density and hardness and reduced expansion, which were linked to changes in the extrusion parameters. Notably, increasing cowpea content decreased the expansion ratio and water absorption index (WAI) in extrudates, with the optimal conditions being found at 130°C for cowpea: sorghum blends (Pelembe et al. 2002).

Sensory analysis has been a crucial component in determining the acceptability of extruded products. Studies consistently found that cowpea fortification impacted the sensory attributes of the products. For example, in a study by Dilrukshi et al. (2021), cowpea and WPC fortification resulted in a more acceptable texture and overall liking compared to the control samples. The sensory evaluation also indicated that the most favorable

sensory attributes were associated with cowpea levels between 10% and 15% and WPC concentrations around 5%. These results were corroborated by principal component analysis (PCA), which revealed positive associations between crispiness and lightness (L^* value) and between hardness and color (b^* and a^* values). Overall, extrusion effectively modifies CP isolates' nutritional and functional properties. Temperature, moisture, and ingredient composition impact protein content, digestibility, and sensory qualities. However, optimizing extrusion conditions and preserving bioactive compounds while minimizing protein denaturation requires further research.

5.2.3 | High Hydrostatic Pressure

HPP has been widely studied to enhance the physicochemical and functional properties of CPs. Thermal (70–90°C) and HPP treatments (200–600 MPa) were compared for their effects on CPs extracted at pH 8.0 (A8) and pH 10.0 (A10). HPP enhanced gelation and WHC more efficiently while maintaining high solubility (72%–97%). The effects are more pronounced in CPs extracted at pH 8.0 compared to pH 10.0. This disparity is attributed to variations in protein structure induced by extraction pH, where pH 8.0 leads to higher surface hydrophobicity and improved gelation properties. Notably, HPP-treated CPs form soluble aggregates stabilized by disulfide bonds, with significant unfolding and denaturation enhancing gelation at moderate conditions (e.g., 200 MPa) (F. Peyrano et al. 2016). Additionally, HPP facilitates the dissociation and exposure of hydrophobic residues, promoting non-covalent interactions that are partially reversible upon depressurization. Gels produced at lower protein concentrations (7.5%–10.5% w/w) display rheological behaviors consistent with entangled solutions, whereas higher concentrations (12%–13.5% w/w) yield firmer gels, albeit softer than those created via thermal treatments (F. Peyrano et al. 2021).

Existing studies provide limited insights into the sensory attributes, long-term storage stability, and interactions of HPP-treated CPs in complex food systems. The protective effect of calcium on protein stability under moderate HPP (e.g., 400 MPa) highlights potential avenues for enhancing functional properties, though its efficacy diminishes at higher pressures (600 MPa). At 400 MPa, calcium stabilized CPs by increasing the denaturation temperature proportionally to calcium concentration (0.3°C/mM). However, at 600 MPa, calcium's protective effect diminished (F. Peyrano et al. 2017). Future research should prioritize optimizing HPP conditions to balance functionality and stability, exploring synergies with other food components, and addressing consumer preferences.

5.3 | Biological Modification

The use of chemical reagents and materials in food processing has raised growing concerns among consumers who increasingly prefer safe and healthy food products. Consequently, researchers have been exploring strategies to reduce the reliance on questionable chemicals in protein modification processes and replace them with nonchemical alternatives. These alternatives, often referred to as “green” or “clean-label” approaches, include both thermal and non-thermal techniques widely employed to mod-

ify plant proteins. In this context, biological methods such as enzymatic hydrolysis, fermentation, and germination have been effectively utilized to modify CPs.

5.3.1 | Enzymolysis

The enzymatic hydrolysis of proteins is an effective approach to enhance their techno-functional, nutritional, and antioxidant properties by modifying their structure. Various commercial proteases, including Alcalase, pancreatin, trypsin, papain, and pepsin, have been utilized to generate protein hydrolysates and peptides with improved functionality from CPs. These peptides exhibit bioactive properties such as antimicrobial, antidiabetic, antihypertensive, and immunomodulatory activities, which are influenced by their structural characteristics and amino acid composition (Sarmadi and Ismail 2010).

Hydrolysis using Alcalase (up to 4 h) significantly increased the antioxidant capacity of CPs by approximately 70%, from 293.4 to 993.7 $\mu\text{mol TE/g}$ of SP. Additionally, hydrolysates subjected to 1 h of Alcalase treatment displayed the strongest dipeptidyl peptidase IV (DPP-IV) inhibitory activity ($\text{IC}_{50} = 0.58 \text{ mg SP/mL}$) while maintaining high antioxidant potential, after in vitro simulated gastrointestinal digestion (de Souza Rocha et al. 2014). Another study investigated the impact of various enzymes, including Alcalase, Flavourzyme, and pepsin-pancreatin, on the angiotensin-I converting enzyme (ACE-I) inhibitory and antioxidant activities of CP hydrolysates fractionated using ultrafiltration. All peptide fractions exhibited enhanced ACE-I inhibitory and antioxidant properties. Among them, the <1 kDa fraction derived from the Flavourzyme hydrolysate demonstrated the highest overall biological activity, with an IC_{50} range of 0.04–170.6 (M. R. Segura Campos et al. 2010). In addition, CP hydrolysates produced using pepsin demonstrated enhanced functional properties (Mune Mune 2015). The hydrolysates exhibited high water solubility indices at DH of 20% and 30%, exceeding 95% at pH 7 and 55% at pH 4.5. Enzymatic hydrolysis also improved OHC, emulsifying activity, and FC. However, proteolysis negatively affected foam stability and did not significantly enhance emulsion stability within a 10%–30% DH range. M. R. Segura-Campos et al. (2012) reported that CP hydrolysates produced using Flavourzyme (pH = 8.0, 50°C) displayed greater solubility and surface hydrophobicity compared to those obtained with Alcalase. This difference is likely attributed to the extensive hydrolysis achieved with Alcalase (DH = 23.6%) and the presence of different molecular mass peptides. Despite these variations, both hydrolysates demonstrated digestibility and intestinal absorption levels similar to those of a casein control. Gómez et al. (2021) also reported that low MW peptides, ranging from 1.8 to 6.5 kDa, generated through Alcalase hydrolysis displayed the highest peroxyl radical scavenging activity among the fractions tested.

5.3.2 | Fermentation

Fermentation is a biological process widely used to enhance the nutritional, structural, physicochemical, and techno-functional

properties of plant proteins. During fermentation, enzymes produced by microorganisms break down proteins into hydrolysates and peptides. These low MW peptides often exhibit improved bioactive properties, including enhanced antibacterial, antihypertensive, and antioxidant activities (Torino et al. 2013). Notably, the effects of fermentation on CPs can vary depending on the specific microorganisms involved.

A study by Kapravelou et al. (2015) reported that fermentation with *Lactobacillus* and thermal processing significantly enhanced the antioxidant capacity and hypolipidemic effects of cowpea (*V. unguiculata*). Fermentation increased phenolic content, reducing capacity, and plasma antioxidant activity, while improving hepatic antioxidant enzyme activity and reducing cholesterol and triglyceride levels in animal models.

Natural fermentation of CPs at 37°C for 48 h significantly enhanced the bioavailability of essential dietary minerals, particularly phosphorus, in growing rats (Kapravelou et al. 2020). This improvement is likely due to the reduction in phytic acid content and the release of phosphorus in a more bioavailable form. Fermentation also increased the true digestibility of most essential amino acids and the apparent digestibility of tyrosine, valine, methionine, lysine, phenylalanine, leucine, and arginine. These effects can be attributed to the reduction in protease inhibitors, as fermentation has been shown to nearly eliminate trypsin inhibitor activity and decrease phytic acid and polyphenol levels, the primary antinutritional factors in cowpeas. In contrast, heat treatment of cowpea flour did not further enhance amino acid digestibility and instead reduced the apparent and true digestibility of sulfur-containing amino acids. This decline may be explained by methionine degradation through Maillard reactions during heat processing. Kiers et al. (2000) reported a 3% increase in the total digestibility of cowpeas subjected to fungal fermentation compared to those processed by cooking (40.9%). The digestibility was further influenced by the specific fungal strain used and the duration of fermentation. Additionally, the level of water-soluble dry matter in the food samples increased significantly during fermentation with *Rhizopus oryzae*, rising from 4.3% to 24.1%.

Fermentation also increased protein content by nearly 27% after 48 h on cowpea flours and reduced phytate and tannin content (Nnam 1995). Madodé et al. (2013) investigated the effects of traditional processing methods (dehulling, boiling, and soaking) and various fermentation techniques (using bacterial, fungal, or yeast cultures such as *Weissella beninensis*, *Bacillus subtilis*, *Rhizopus microsporus*, and *Saccharomyces cerevisiae*) on cowpeas. The in vitro fermentability index was consistently high across all processed cowpeas, except for those fermented with *Rhizopus* and *Bacillus*. However, traditional processing methods had a higher effect on the in vivo fermentability index of fermented cowpeas than the fermentation method. The shelf stability of cowpea bean flours fermented with either single-culture fermentation using *Lactobacillus plantarum* or mixed-culture fermentation involving a combination of lactic acid bacteria, acetic acid bacteria, and *S. cerevisiae* was significantly reduced. This reduction was attributed to a decrease in pH values during fermentation (Ferreira et al. 2019).

5.3.3 | Germination

Germination is an economical biological method used to modify the structural and biochemical properties of seeds while reducing anti-nutritional compounds (ANCs) such as lectins, phytic acid, protease inhibitors, and tannins (Aguilera et al. 2013). The process can also improve the nutritional, techno-functional, antioxidant, and physicochemical ability of proteins (Gu et al. 2023; Uppal and Bains 2012). Short-term germination (at 25°C for up to 48 h) has been shown to significantly increase the antioxidant activity and DPP-IV inhibitory potential of CPs (de Souza Rocha et al. 2014). Nevertheless, non-germinated samples and hydrolysates treated with Alcalase for 1 h demonstrated the strongest DPP-IV inhibition. Imbart et al. (2016) compared the effects of germination (24–72 h) and fermentation (48–96 h) on the emulsifying properties of CPs. The results showed that protein aggregates formed during 72 h of germination were more effective at stabilizing oil-in-water (O/W) interfaces compared to fermentation. In contrast, fermentation was found to destabilize emulsions due to protein degradation caused by microbial activity. Another study found that germination significantly improved the *in vitro* digestibility of CPs (Malomo et al. 2013). This was mainly linked to a decrease in total protein nitrogen due to the increased protease activity during germination. The researchers also noted that the longer the germination period, the greater the enhancement in protein digestibility. The germinated cowpea exhibited the highest flavonoid content (211.06 ± 8.17 mg/100 g) and antioxidant activity (% DPPH inhibition) ($49.837\% \pm 0.61\%$) compared to other germinated pulses, including black gram, chickpea, and yellow mustard (Khyade and Jagtap 2016). A study by De Benedetti et al. (2022) showed that structural changes occur during seed germination, with proteolytic degradation leading to the formation of a 27 kDa intermediate polypeptide from the cowpea seed protein β -vignin. Although the study suggests that these changes could affect the protein's functional properties, more research is required to fully understand the impact.

6 | Novel Food Applications

Cowpea seeds are commonly prepared by cooking and are used in stews and curries or processed into flour or paste (Duraipandian et al. 2022). Recently, they have been utilized as texture enhancers in fish and meat by changing protein conformation and flour's physicochemical properties (Adjei-Fremah et al. 2019). The anti-nutritional factors in cowpea, such as hemagglutinins and trypsin inhibitors, are heat-sensitive and easily deactivated through cooking, enhancing protein digestibility and absorption. This makes CP suitable for infant and baby food formulations (Sarwar Gilani et al. 2012). CPs are regarded as complete foods because of their high protein content and rich supply of essential amino acids. Therefore, they are a high-quality protein source for communities facing malnutrition and aim to improve their daily dietary intake (Jimenez-Lopez 2021). The functional properties of CPs, including its foaming, emulsifying, gelation, and thickening abilities, make it highly suitable for use as emulsifiers, supplements, and foaming agents in a variety of traditional food products such as mayonnaise, dressings, and beverages (HewaNadungodage et al. 2022; Rudra et al. 2016). It can also be effectively utilized in baked products due to its excellent water absorption capacity and acceptable organoleptic properties

Food applications of CPs

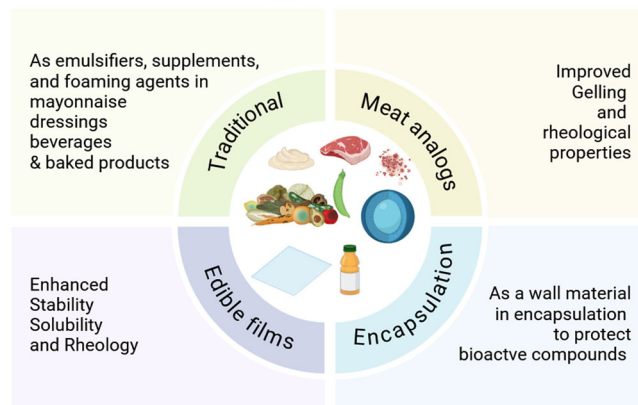


FIGURE 7 | Food applications of cowpea proteins (CPs).

(Campbell et al. 2016). This section aims to review the novel food applications of CPs, including meat analogs, edible films, and encapsulation (Figure 7).

6.1 | Meat Analogues

Plant-based meat analogs, widely produced as alternatives to animal meat, are designed to mimic the fibrous structure and sensory properties of traditional meat. Soybean protein, due to its exceptional functional properties, has been extensively studied and used as a key ingredient in meat alternative formulations. However, its allergenic potential highlights the need for exploring other nonallergenic protein sources (Schreuders et al. 2021; T. Zhang et al. 2021). CP isolate (20%–30%) combined with cocoyam-modified starch (20%–30%) was used to produce meat analogs through autoclaving and cooling processes. The results indicated that incorporating 30% protein and modified starch enhanced sensory properties, particularly color and taste. Improvements in the texture of the meat analogs were attributed to a slight increase in WHC and OHC. The optimal formulation resulted in meat analogs with 50.92% moisture content and 18.97% protein content (Rosida et al. 2021). Singh et al. (2008) reported that adding 10% cowpea flour did not significantly influence the texture of the meat analogs.

6.2 | Edible Films

Protein-based edible films are consumable, biodegradable, and environment-friendly layers of proteins, designed to serve as a barrier or protective coating for food products (Lionetto and Esposito Corcione 2021). They are known as a sustainable alternative to synthetic packaging. Hewage and Vithanarachchi (2009) developed a composite film using CP isolate with polyethylene glycol and glycerol as plasticizers. Films were produced across a pH range of 8–11, with varying polyethylene glycol concentrations from 2.5% to 10% (w/v) for each pH level. The results indicated that a pH of 10 and plasticizer concentrations of 2.5% (w/v) polyethylene glycol and 2% glycerol optimized the film's physicochemical properties, including improved flexibility, uniform structure, and transparency. Furthermore, the composite film exhibited notable antifungal activity. In another study,

biodegradable films were produced by incorporating varying concentrations (0%–20%) of maqui berry extract (MBE) into cowpea starch (CS). The addition of MBE significantly enhanced the antioxidant properties of the films, increasing their activity to approximately 88%. Moreover, CS films containing 20% MBE effectively delayed lipid oxidation in salmon during storage and completely blocked incident UV light (Baek et al. 2019). However, the application of CP in the development of edible films requires further investigation to explore its full potential.

6.3 | Encapsulation of Bioactive Compounds

Using plant proteins as wall materials in encapsulation systems is an innovative technique that effectively protects bioactive compounds, ensuring their stability and controlled release. The success of this technique relies on the techno-functional properties of proteins, such as their ability to form gels, emulsify, and create stable structures. CP, with its excellent functional properties, shows potential as a wall material in encapsulation, helping to protect bioactive compounds from degradation during digestion and enhancing their bioavailability. A recent study by Traffano-Schiffo et al. (2024) demonstrated that CPs, when incorporated into Ca(II)-alginate beads supplemented with guar gum, can enhance the protection and delivery of bioactive compounds during digestion and fermentation. These beads preserved the antioxidant capacity of cowpea-derived phenolic compounds and peptides while promoting short-chain fatty acid (SCFA) production, which supports gut microbiota modulation. The encapsulation system showed structural and functional stability and can be a promising approach for developing sustainable functional ingredients with potential health benefits. Despite these promising findings, research on the use of CP in encapsulation systems remains limited. Further studies are needed to optimize its properties and improve encapsulation efficiency.

7 | Conclusion and Future Prospective

Cowpea (*V. unguiculata*), a highly nutritious legume, has garnered considerable attention for its potential in the food industry, particularly as a valuable source of plant-based protein. The rising consumer demand for plant-based foods highlights the importance of CPs, which offers an excellent protein content and a well-balanced amino acid profile, making them a viable alternative to animal-derived proteins. However, CPs face challenges in their functional properties, such as solubility, emulsification, and gelling, when compared to animal proteins, which limits their widespread use in various food products.

To overcome these challenges, significant advancements have been made in protein modification techniques. Physical, chemical, and biological methods are being explored to enhance the functional properties of CPs, including improving their solubility, water, and oil retention capacity, foaming, emulsifying, and gelling abilities. Despite these advancements, there is still a lack of comparative research among different modification techniques, as well as limited data on the stability and performance of modified CPs during long-term storage and in real-world applications. This gap highlights the need for systematic studies that not

only compare methods under standardized conditions but also assess their scalability and impact on nutritional and sensory attributes.

The extraction of CP primarily involves wet methods such as water, salt, alkali, and acid extraction to produce high-purity protein isolates. However, these methods raise environmental concerns due to their high consumption of water and chemicals. As a result, future research must emphasize the development of eco-friendly extraction methods, such as dry fractionation, which eliminates the need for water and chemicals. Although dry extraction methods may yield lower protein purity, they better preserve the native functionality of proteins and offer a more sustainable solution. Combining dry methods with mild post-treatments (e.g., ultrasound or enzymatic treatment) could balance sustainability and functionality.

For the food industry, CP offers opportunities in meat analogs, baked goods, beverages, and nutraceutical carriers. However, adoption will require not only functional optimization but also sensory improvements. In addition to functional challenges, CPs may exhibit beany or grassy off-flavor, primarily attributed to volatile aldehydes and alcohols. Addressing these off-flavors remains important for consumer acceptance. Future research should explore effective deodorization strategies, including fermentation, enzymatic treatment, and adsorption techniques, to improve sensory quality without compromising functionality.

Moving forward, researchers should prioritize the development of scalable, green extraction methods, such as dry fractionation and UAE, to reduce environmental impact while preserving native functionality. Research should also focus on the design of combined modification approaches, especially those leverage biological–physical synergies to enhance performance across diverse food systems. Additionally, systematic evaluation of sensory, nutritional, and allergenic effects following modification is essential to ensure safety and consumer satisfaction. Finally, application-driven research into novel uses, such as edible films and 3D-printed foods, could be pursued to align with current food innovation trends. By focusing on these strategic areas, future work can bridge the gap between promising laboratory findings and real-world industrial applications, which unlocks cowpea's full potential as a next-generation plant protein.

Author Contributions

Roshanak Zolqadri: conceptualization, methodology, investigation, formal analysis, visualization, writing – original draft, writing – review and editing, validation. **Yonghui Li:** conceptualization, methodology, investigation, validation, formal analysis, supervision, funding acquisition, project administration, resources, writing – review and editing.

Acknowledgments

This is Contribution No. 25-166-J from the Kansas Agricultural Experiment Station. This work was in part supported by the USDA Agricultural Research Service Non-Assistance Cooperative Project (Grant Accession No. 443993, 439200) and USDA National Institute of Food and Agriculture Hatch Project (Grant Accession No. 7003330).

Conflicts of Interest

The authors declare no conflicts of interest.

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