Sustainable Food Technology

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Sustainability statement

This work contributes to sustainable food development by assessment of pulse albumins—protein fractions typically recovered as by-products from pulse processing. Compared with conventional plant-derived proteins isolates, pulse albumins offer a renewable and low-carbon alternative with excellent solubility, functionality, and balanced amino acid composition. Utilizing these underexploited proteins supports resource efficiency and waste minimization. By systematically comparing albumins from ten pulse species, this study provides a scientific foundation for their targeted use in plant-based formulations, thereby promoting the circular utilization of pulse resources and contribute to the achievement of United Nations Sustainable Development Goals (SDGs).

ARTICLE

Comparative Assessment of Albumins from Ten Pulses: Techno-functional Properties and Amino Acid Profiles for Sustainable Food Applications

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Pulse albumins, often recovered as by-products during pulse processing, have attracted increasing interest due to their favorable solubility and nutritional properties. However, compared with extensively studied animal proteins such as whey protein, systematic information on the techno-functional properties and amino acid profiles of pulse albumins across different species remains limited, restricting their targeted application in food systems. This study addresses the gap by systematically investigating the techno-functional properties and amino acid profiles of albumin fractions extracted from ten major pulses: *Vigna radiata* (mung bean, MB), *Vigna angularis* (adzuki bean, RB), *Phaseolus vulgaris* (red kidney bean, RK; white kidney bean, WK), *Cicer arietinum* (kabuli chickpea, KCP; desi chickpea, DCP), *Pisum sativum* (green pea, GP; yellow pea, YP), *Vicia faba* (broad bean, BB), and *Lens culinaris* (lentil, LP). All albumins exhibited excellent pH-independent solubility (>70%). Notable variations were observed: *P. vulgaris* albumins showed superior solubility at high concentration and water-holding capacity, while *P. sativum* albumins had outstanding foaming properties. Most pulse albumins surpassed whey protein in foaming but were inferior in emulsification. Amino acid scores met FAO/WHO standards, with *P. vulgaris* albumins displaying the highest nutritional indices. These findings demonstrate pronounced interspecific differences in the techno-functional and nutritional characteristics of pulse albumins, providing evidence that species selection is critical for targeted food applications.

1. Introduction

As the global population continues to rise and the need for sustainable food sources intensifies, plant proteins have garnered significant interest for their environmental benefits. In addition to being nutritionally rich, plant proteins significantly reduce greenhouse gas emissions and represent a promising alternative to animal-based proteins.[1] According to the Food and Agriculture Organization (FAO) of the United Nations, pulses are defined as dry-harvested leguminous crops except high oil content seeds, such as soybeans and peanuts.[2] The global production of pulses in 2023 reached approximately 103 million tons, with kidney bean (*Phaseolus vulgaris*) as the dominant species.[3] This species is widely cultivated in countries such as India, Myanmar, Brazil, and China. Other major pulse crops included chickpeas (*Cicer arietinum*), dry peas (*Pisum sativum*), fava beans (*Vicia faba*), and lentils (*Lens culinaris*). China is one of the world's largest producers and consumers of pulses, with mung beans (*Vigna radiata*), kidney beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*), peas (*Pisum sativum*), broad beans (*Vicia faba*), and lentils (*Lens culinaris*) widely cultivated.

Pulses are high-yield crops with a substantial protein content of 20%–30%, which is two to three times greater than that of most cereals (8%–15%)[4], making them an important dietary source of plant-based protein. According to the Osborne classification, pulse proteins are categorized into albumins, globulins, prolamins, and glutelins. Among these, globulins are the main storage proteins, typically accounting for 50–70% of the total protein content.[5] However, their poor water solubility and tendency to aggregate at pH 4–5 limit their application in food systems that require high solubility. In contrast, although albumins are present in smaller amounts (10–25%),[5] they demonstrate distinct advantages in solubility across a wider pH range compared to globulins.[6] Solubility is a key property of pulse albumins, as it underpins their applicability in food systems and also a pre-requisite for other related functional properties such as foaming and emulsification.[7]

Existing research indicates that albumin derived from certain pulse exhibits significant functional properties. For instance, Djoullah et al. [8] demonstrated pea albumin retains high solubility (around 85%) even at pH 5, whereas pea globulin solubility drops sharply to ~20% under the same conditions. Yang et al.[9] reported that albumin fractions from mung bean and yellow pea exhibit foaming capacities comparable to or superior to whey and egg white proteins. Beyond their favourable processing properties, some variety of pulse albumins were investigated for their amino

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acid profiles. Chagas et al.[10] found that albumins in *Phaseolus vulgaris* varieties demonstrated a higher cysteine content than globulins, making them valuable for compensating the general deficiency of sulfur-containing amino acids in pulse proteins. Moreover, pulse albumins are rich in various bioactive components. Previous studies have demonstrated that white kidney bean extract rich in alpha-amylase inhibitors exhibits beneficial health effects in preventing hyperglycemic episodes and fat accumulation.[11] Pulse albumin contains trypsin inhibitor which can also inhibit proteases produced by pathogens; therefore, they hold potential for use as antibacterial agents.[12]

The current research on pulse albumins remains significantly underexplored compared to studies on water-soluble proteins from animal sources (e.g., cow's milk whey protein). While the techno-functional properties—such as solubility, emulsifying, and foaming capacities—of albumins from specific individual pulses have been studied to some extent[6,13], systematic comparative analyses across different pulse species are strikingly scarce. Concurrently, on the nutritional front, a clear contrast exists on the pulse globulins which are known to be deficient in essential sulfur-containing amino acids[14], whereas comprehensive comparative studies assessing and contrasting the amino acid profiles of albumins from diverse pulses are notably absent, leaving their nutritional potential inadequately understood. Therefore, a systematic comparative study of the techno-functional properties and amino acid profiles of different pulse albumins is of considerable scientific and practical significance.

In this study, we conducted a comprehensive investigation of the techno-functional properties and amino acid profiles of albumins isolated from ten major pulse species: *Vigna radiata* (mung bean, MB), *Vigna angularis* (adzuki bean, RB), *Phaseolus vulgaris* (red kidney bean, RK; white kidney bean, WK), *Cicer arietinum* (kabuli chickpea, KCP; desi chickpea, DCP), *Pisum sativum* (green pea, GP; yellow pea, YP), *Vicia faba* (broad bean, BB), and *Lens culinaris* (lentil, LP). To ensure the representativeness of the samples used in this study, the ten selected pulse varieties cover the dominant species commonly grown and consumed both in China and globally, and all were sourced from their typical major producing regions in China. Using a uniform extraction and analytical protocol, we evaluated the techno-functional properties against whey protein as a benchmark and analysed amino acid composition. This approach provides a more comprehensive understanding of pulsed albumin and offers a broader perspective on the potential of pulse albumins in food applications.

2. Materials and methods

2.1 Materials

Ten varieties of pulses were used in this study, including *Vigna radiata* (mung bean, MB), *Vigna angularis* (adzuki bean, RB), *Phaseolus vulgaris* (red kidney bean, RK; white kidney bean, WK), *Cicer arietinum* (kabuli chickpea, KCP; desi chickpea, DCP), *Pisum sativum* (green pea, GP; yellow pea, YP), *Vicia faba* (broad bean, BB), and *Lens culinaris* (lentil, LP). To ensure representativeness,

all pulse seeds were purchased from major producing areas in China during the year 2024, and each batch was confirmed to be uniform in variety. MB and RB were obtained from Heilongjiang Province, China; RK, WK, and BB from Yunnan Province, China; KCP and DCP from Xinjiang, China; GP and YP from Qinghai Province, China; and LP was sourced from Shanxi Province, China. All seeds were packed in airtight and stored in a dry environment at 4°C until use. All the reagents and chemicals utilized for the research were of analytical reagent grade.

2.2 Methods

2.2.1 Extraction of Pulses Albumins. The protein fractions were extracted using the procedure by Yang et al.[9] with minor adjustments. Pulses were milled using a high-speed blender (Model MX-H2201, Panasonic Appliances (China) Co., Ltd.). Then, the flour was sieved through a standard 60-mesh sieve to obtain uniform flour (Fig. 1). The flour was defatted by mixing with n-hexane at a 1:3 (w/v) ratio with continuous stirring for 60 min. The mixture was then centrifuged, and the upper organic solvent layer was discarded. This defatting procedure was repeated three times. The defatted flour was subsequently collected by filtration and air-dried at room temperature in a fume hood overnight to ensure complete evaporation of residual n-hexane.
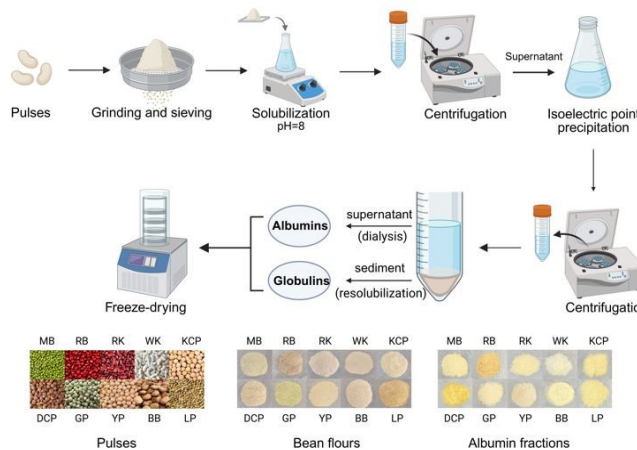

Fig.1 A schematic overview of pulse protein extraction process.

After defatting, the flour was dispersed with deionized water to prepare a 10% (w/v) suspension, the initial pH of the suspension was approximately 6.52-6.67. The pH of the suspension was adjusted to 8.0 using 1 M NaOH, and the mixture was stirred for 2 h, then centrifuged at $8,000 \times g$ for 30 min at 4 °C using a high-speed refrigerated centrifuge (64RL, Beckman Coulter, USA), after which the supernatant was collected and filtered. To isolate the globulin fraction, the pH of the supernatant was adjusted to 4.5 with 1 M HCl stirred continuously for 1 h, followed by another centrifugation under the same conditions. The resulting supernatant and precipitate were collected separately. The supernatant was dialyzed using a 5 kDa membrane for 48 hours to obtain albumin. The sediment was redispersed in ultrapure water and stirred for 2 h while gradually adjusting the pH to 7.0. Finally, both albumin and globulin fractions were freeze-dried using a freeze dryer (Christ, Osterode am Harz, Germany). The extraction yields, mass yield

and purities of the albumin and globulin fractions were calculated as described in Equations (1), (2) and (3), respectively. Each extraction process was performed in triplicate.

$$\text{Mass yield}(\%) = \frac{\textbf{Mass of protein powder}}{\text{Mass of raw material used}} \times 100 \quad (1)$$

$$\text{Extraction yields}(\%) = \frac{\text{Mass of extracted protein}}{\text{Total protein content in raw material}} \times 100 \quad (2)$$

$$\text{Purity}(\%) = \frac{\text{Mass of protein in the powder}}{\text{Total mass of protein powder}} \times 100 \quad (3)$$

2.2.2 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The protein composition of albumin and globulin fractions extracted from ten pulses was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Following the protein quantification method described by Xing et al.[15] with slight modifications. Samples were homogenized with loading buffer (Solarbio, P1015) at a 3:1 (v/v) ratio, heated in a boiling water bath for 5 min, and centrifuged (5,000 × g, 4°C, 10 min) to obtain electrophoretic preparations. Each sample mixture (10 µL) was then applied to precast gels. A 10–250 kDa molecular weight pre-stained protein markers (Sangon Biotech, C520010-0001) was used as the standard. Electrophoresis was performed at a constant voltage (80 V) until the dye front reached the bottom of the gel. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 for 1 h and destained using a 20% methanol and 10% acetic acid solution until clear background was obtained. The stained gels were scanned, and protein molecular weights were estimated using a ChemiDoc™ MP Imaging System (Bio-Rad Laboratories Inc., CA, USA). Each SDS-PAGE analysis was performed in triplicate.

2.3 Determination of the techno-functional properties of pulses albumins

2.3.1 Protein solubility. Protein solubility of albumin was determined according to the method of Yang et al.[16] with slight modifications. Briefly, a protein solution of 3% (w/v) concentration was prepared and stirred for 30 min under different pH conditions (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) and concentration gradients (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10%, w/v). Subsequently, the solution was centrifuged at 8000×g for 20 min. The protein concentration in the supernatant was measured using the Coomassie Brilliant Blue method, with three replicates per experimental group. Solubility was defined as the ratio of protein content in the supernatant to the total protein content. Each experiment was performed in triplicate.

2.3.2 Water-holding capacity (WHC) and oil-holding capacity (OHC). Water-holding capacity (WHC) and oil-holding capacity (OHC) was performed according to the method described by Habib et al.[17] with slight modifications. In brief, 1.0 g of the sample was weighed into a centrifuge tube, followed by the addition of 5 mL distilled water (or rapeseed oil for OHC analysis). The mixture was vortexed and allowed to stand for 30 min. Subsequently, centrifugation was conducted at 6,000 ×g for 20 min at room temperature. The centrifuge tube with the protein residue was subsequently weighed, and the WHC/OHC value was calculated using eqn(3). Each experiment was performed in triplicate.

$$\text{WHC/OHC (g/g)} = \frac{W_2 - W_1 - W_0}{W_0} \quad (3)$$

where W_0: initial sample weight, W_1: tube weight, W_2: tube weight with residue after centrifugation.

2.3.3 Foam formation and stability. The foaming capacity was determined following the method of Narale et al.[18] with minor modifications. Briefly, a 3% (w/v) protein solution was prepared, and its pH was adjusted to various values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0). After that, the solution was homogenized for 1 min at 13,500 rpm using the Polytron PT 3100 homogenizer. The total volume of foam and liquid phase was recorded immediately after homogenization. After 30 min of quiescent standing, the remaining foam volume was measured again. The foaming capacity (FC) was calculated using eqn (4), foam stability (FS) was determined using eqn (5). Each experiment was performed in triplicate.

$$\text{FC (\%)} = \frac{V_2 - V_1}{V_1} \times 100 \quad (4)$$

$$\text{FS(\%)} = \frac{V}{V_2 - V_1} \times 100 \quad (5)$$

where V: foam volume after 30 min standing (mL), V_1: volume of the protein solution before homogenization (mL), V_2: total volume of foam and liquid phase after homogenization (mL).

2.3.4 Emulsion formation and stability. The emulsifying property was determined according to the method of Zheng et al.[19] with slight modifications. Briefly, a 3% (w/v) protein solution was prepared and adjusted to different pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0), followed by the addition of 1 mL of soybean oil. The mixture was homogenized at 10,000 rpm for 2 min, using a Polytron PT 3100 homogenizer. Subsequently, 50 µL aliquots were withdrawn from the bottom of the emulsion and mixed with 5 mL of 0.1% (w/v) SDS solution. The absorbance (A_0) was immediately measured at 500 nm using a UV spectrophotometer, followed by a second measurement (A_{30}) after 30 min. All experiments were performed in triplicate. The emulsifying capacity (EC) was calculated using eqn (6), emulsion stability (ES) was calculated using eqn(7). Each experiment was performed in triplicate.

$$\text{EC}\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_0 \times D}{C \times I \times \varphi \times 10000} \quad (6)$$

$$\text{ES(min)} = \frac{A_0}{A_0 - A_{30}} \times 30 \quad (7)$$

where A_0: absorbance at 500 nm measured immediately, A_{30}: absorbance at 500 nm after 30 min, D: dilution factor, C: sample concentration (g/mL), φ: volume fraction of soybean oil in the emulsion (%), I: optical path length (cm).

2.3.5 Amino Acid Analysis. The amino acid composition of pulse albumins was determined using an L-8900 Amino Acid Analyzer (Hitachi High-Tech Science Co., Japan), following the method described by Bidlingmeyer et al.[20] with slight modifications. Briefly, approximately 30 mg of each freeze-dried albumin sample was hydrolyzed in 6 M hydrochloric acid (HCl) under vacuum at 110 °C for 24 hours in sealed glass tubes to release protein-bound amino acids. After hydrolysis, the hydrolysates were evaporated to dryness under reduced pressure at 40 °C, reconstituted in citrate buffer (pH 2.2), and filtered through a 0.22 µm membrane prior to injection. The measurement was performed in triplicate.

246 **2.3.6 Statistical analysis.** All experiments were performed in
247 triplicate. The results are presented as the mean ± standard
248 deviation, with error bars indicating the standard deviation.
249 Statistical differences were considered significant when $p < 0.05$.
250 Statistical analyses were conducted using SPSS Statistics 25
251 followed by one-way ANOVA with the Duncan post-hoc test.

3. Results and discussion

252

253 ### 3.1 Fractionation of albumin and globulin

254 Figure 2A shows the mass yield of albumin and globulin fractions
255 from these pulses. The albumin mass yield ranged from 5.27% (LP)
256 to 7.55% (WK), while the globulin mass yield ranged from 13.52%
257 (LP) to 17.88% (WK). Figure 2B shows the extraction yields of
258 albumin and globulin fractions from these pulses. Overall,
259 albumin extraction yields ranging from 18.62% (YP) to 25.72%
260 (WK), while globulin extraction yields showed higher value
261 ranging from 55.40% (LP) to 61.15% (WK). The extraction ratio of
262 albumin to globulin was approximately 1:3, consistent with the
263 previously reported ratio range of these proteins in pulses (1:3 to
264 1:6.3).[21] Ye et al.[22] reported albumin and globulin extraction
265 yields of 36.30% and 52.51%, respectively, in chickpeas through
266 fractional extraction, aligning with the trends observed in this
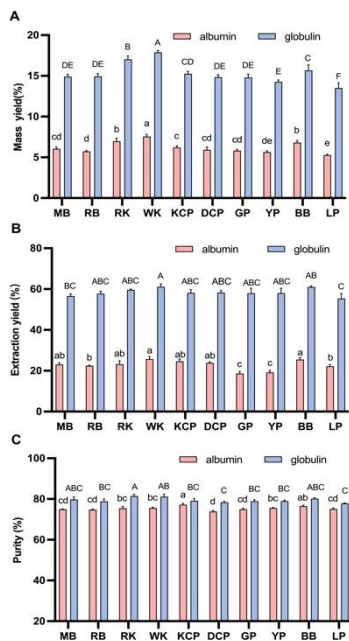

Fig.2 The extraction yield(A) ,mass yiele(B) and purity(C) of ten types of pulse
albumin and globulin. Data are presented as mean ± SD (n = 3). Different lowercase
letters (a–e) indicate statistically significant differences among the albumin
samples, while different uppercase letters (A–F) indicate significant differences
among the globulin samples (p < 0.05)

267 study. However, the numerical differences may arise from
268 species-specific characteristics. Fig. 2C further analyses the purity
269 of albumin and globulin from the ten pulses. The results show
270 that the purity of albumin ranged from 73.88% to 77.19% wet
271 basis [w.b.], while that of globulin ranged from 77.87% to 81.47%
272 [w.b.], indicating that the purity of the two types of proteins
273 varied less among different bean species and were above 70%.

274 The lower purity of albumin is due to its extraction from the
275 water‑soluble fraction, which may retain traces of non‑protein
276 components like carbohydrates and phenolics**.**

277

278 ### 3.2 SDS-PAGE

279 Figure 3 presents the SDS-PAGE profiles of albumin (A) and
280 globulin (G) fractions from ten pulses varieties. The results
281 indicated that the albumin fractions exhibited fewer
282 electrophoretic bands and predominantly lower molecular
283 weights. Most albumin fractions exhibited electrophoretic bands
284 corresponding to molecular weights between 15 and 98 kDa,
285 primarily consisting of low-molecular-weight components. These
286 proteins mainly corresponded to PA1 albumin (4–18 kDa) and
287 PA2 albumin (23–26 kDa).[9] Albumin fractions also contained 30–
288 35 kDa bands, which were considered to belong to
289 phytohemagglutinin.[23] A high molecular weight band at 98 kDa
290 was observed, which might correspond to the lipoxygenase
291 based on previous studies.[24] Distinct variations were observed in
292 the electrophoresis patterns of the albumin samples. Lanes RK-A
293 and WK-A showed bands at around 40 kDa, which might be
294 associated to α-amylase inhibitor with subunit molecular weights
295 of approximately 40–45 kDa.[25] The band observed at 20–25 kDa
296 is likely attributed to low-molecular-weight subunits or partial
297 degradation products. A 60 kDa band observed in lane KCP-A and
298 lane DCP-A probably from the water-soluble subunit of vicilins.[26]
299 Lane LP-A exhibited faint electrophoretic bands, possibly
300 reflecting its inherently low albumin content.
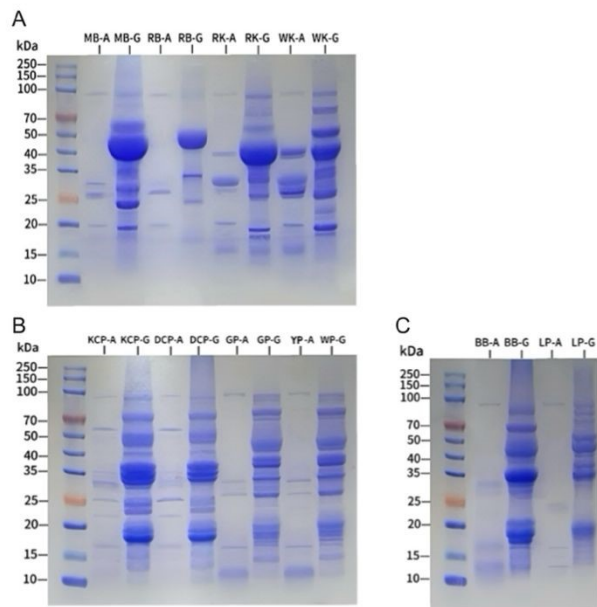
Fig.3 SDS-PAGE of ten types of pulse albumin and globulin. A–albumin, G–globulin.
A representative gel image from three independent experiments is shown.

301 Globulins exhibited more electrophoretic bands and higher
302 molecular weights, and included a variety of protein subunits,
303 primarily 7S and 11S subunits, with bands distributed across a
304 broad molecular weight range (20–70 kDa).[27] Specifically, lanes
305 MB-G, RB-G, RK-G and WK-G displayed concentrated bands at
306 40–50 kDa, confirming 7S globulins as their primary subunits.[9]

307 Lane KCP-G and lane DCP-G showed prominent bands near 35
308 kDa (lanes KCP-G, DCP-G), corresponding to 11S legumin
309 subunits.[28] Lanes GP-G, YP-G and BB-G shared similar banding
310 patterns, with subunits clustered at 20, 35, 40, 50, and 70 kDa.
311 Notably, the 70 kDa subunit was absent in lane RB-G and lane LP-
312 G.

313 **3.3 Protein solubility**

314 **3.3.1. Pulse albumin solubility at different pH.** The solubility
315 of albumin fractions from ten pulse varieties across varying pH
316 levels is illustrated in Fig. 4A. All pulse albumin (3%, w/v)
317 exhibited high solubility (>76%) throughout this pH range. In
318 contrast, the solubility of whey protein (WP) ranged from 59.18%
319 to 82.27%, with a notable decline at pH 5 (59.18 ± 1.79%). At pH
320 5, the solubility of assayed albumin fractions remained higher
321 than WP, with WK showing the highest value (85.15 ± 2.06%) and
322 RB the lowest (76.73 ± 2.51%). Although the isoelectric points of
323 most pulse proteins typically fall between pH 4 and 5,[29] the
324 solubility of pulse albumin remained relatively stable across pH
325 3-9, demonstrating pH-independent behaviour. These
326 differences may be linked to variations in protein subunit
327 composition. Albumin enriched with hydrophilic amino acids
328 (e.g., glutamic acid and aspartic acid) may possess enhanced
329 solubility.[30] The findings align with established trends in protein
330 solubility reported in the literature. Mundi et al.[13] observed
331 minimal solubility of kidney bean globulin within pH 4–6,
332 whereas albumin maintained high solubility across a broad pH

333 range. Similarly, Nwachukwu et al.[31] reported that flaxseed
334 albumin exhibited significantly higher solubility than globulin
335 within pH 3–9. This study further confirms that pulse albumin,
336 with its good solubility across a wide pH spectrum, holds
337 potential for expanding its applications in food and beverage
338 formulations.

339 **3.3.2 Pulse albumin Solubility at different Concentration.** The
340 solubility profiles of albumin fractions from ten pulses under
341 varying concentration conditions are presented in Figure 4B. As
342 shown, all pulse albumins, along with WP, exhibited a decreasing
343 trend in solubility with increasing protein concentration. At low
344 concentrations (1–3%, w/v), solubility remained relatively high
345 across all samples, exceeding 80%, with RK (89.21 ± 2.23%)
346 showing the highest solubility at 3% (w/v) concentration.
347 However, with increasing concentration to moderate and high
348 levels (5–10%, w/v), solubility progressively declined to a range
349 of 67.81–76.40% at 10% (w/v). This trend aligns with the
350 phenomenon of enhanced intermolecular interactions and
351 aggregate formation in proteins at higher concentrations.[32]
352 Notably, pulse albumins exhibited higher solubility than WP at all
353 concentrations, with more pronounced differences observed
354 above 5% (w/v). For example, at a concentration of 10% (w/v),
355 the solubility of WK remained high at 76.40 ± 2.60%, compared
356 with that of WP, which decreased to 65.61 ± 1.27%. Pulse
357 albumins demonstrated a smaller reduction in solubility with
358 increasing concentration indicating their superior ability to
359 remain soluble at higher protein levels. Among the ten pulses, RK
360 and WK showed the highest solubility performance, maintaining
361 values above 76% even at 10% (w/v) concentration. In contrast,
362 RB and BB displayed the lowest solubility at this concentration,
363 decreasing to 67.81 ± 2.03% and 68.86 ± 1.99%, respectively.

364 **3.4. Water-holding capacity (WHC) and oil-holding capacity**
365 **(OHC)**

366 Figure 5 shows the water-holding capacity (WHC) and oil-holding
367 capacity (OHC) of albumins from ten pulse sources. The results
368 revealed significant differences in both WHC and OHC among the
369 different pulse albumins. In terms of WHC (Fig.5A), WK
370 (3.99 ± 0.13 mL/g), RK (3.67 ± 0.10 mL/g), and YP
371 (3.51 ± 0.06 mL/g) exhibited higher values than WP
372 (3.38 ± 0.15 mL/g), while the other pulse albumins showed lower
373 WHC with MB recording the lowest value (2.04 ± 0.02 mL/g).
374 Nevertheless, all pulse albumins displayed significantly higher
375 WHC than commercial soy protein isolate (1.26 mL/g).[33]

376 However, pulse albumins demonstrated lower OHC than WP
377 (2.44 ± 0.11 mL/g) (Fig 5B). Among them, KCP (2.22 ± 0.12 mL/g),
378 RB (2.01 ± 0.14 mL/g), and YP (1.95 ± 0.08 mL/g) had relatively
379 higher OHC, whereas LP had the lowest value (1.11 ± 0.09 mL/g).
380 The high WHC of pulse albumins may result from their high levels
381 of polar or charged amino acids, which can form hydrogen bonds
382 with water molecules, enhancing the protein's water-binding
383 ability[34]. Conversely, the lower proportion of hydrophobic amino
384 acids may reduce their interaction with lipids, resulting in
385 relatively lower OHC.[35] In addition, a comparison with the
386 solubility data (Fig. 4) suggests that pulse albumins with higher
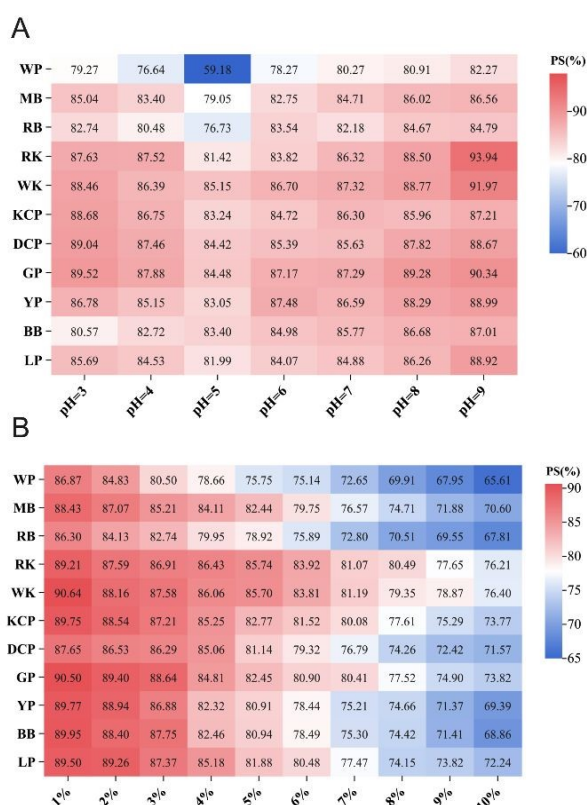387 solubility generally exhibited higher WHC values. This finding

Fig.4 Effect of pH (A) and concentration (B) on the solubility of ten types of pulse albumin. Values shown in the heatmap represent mean values of three replicates (n = 3). Statistical analysis was performed using mean ± SD, as described in the Methods section.

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aligns with the underlying mechanism that albumin fractions, rich in polar and charged amino acids, readily interact with water.[34] Proteins with high solubility possess a greater number of hydrated molecules and an expanded structure in solution, which subsequently facilitates increased water entrapment and retention within the protein matrix, thereby leading to a higher WHC. Therefore, the superior WHC of certain pulses like WK and RK can be partially attributed to their excellent solubility, which synergistically enhances their potential application in aqueous food systems.
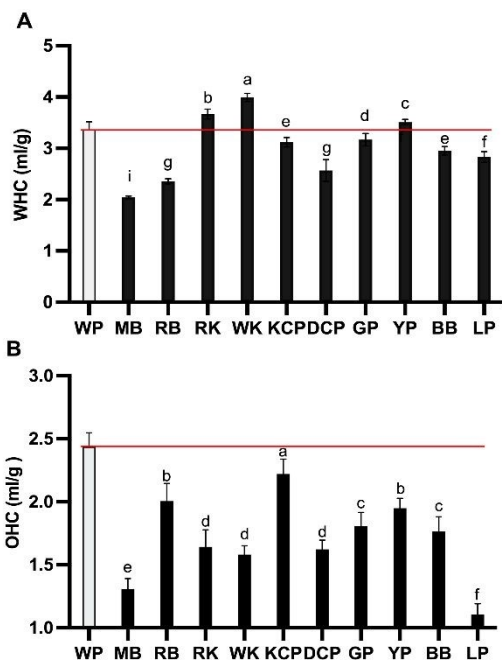
Fig.5 WHC (A) and OHC (B) of 10 types of pulse albumin. Data are presented as mean ± SD (n=3). Different lowercase letters indicate statistically significant difference (P < 0.05).

3.5. Foaming capacity (FC) and foam stability (FS)

Foaming capacity (FC) refers to the ability of proteins to incorporate air and generate foam during whipping, while foam stability (FS) reflects the ability of the formed foam to maintain its structure over time. These two techno-functional properties are crucial in the formulation of aerated beverages, protein drinks, and bakery products. FC and FS are primarily influenced by the molecular structure of proteins, their interfacial behaviour, and environmental factors such as pH. [36] As shown in Figure 6, both the FC and FS of ten pulse albumins exhibited significant pH dependence within the pH range of 3–9. In general, the lowest FC and FS values were observed near pH 5, and both increased markedly as the pH increased. This trend is consistent with findings by Lawal et al.[37] who reported enhanced foaming properties of African locust bean albumin under alkaline conditions. In alkaline environments, increased net surface charge and reduced hydrophobic interactions among protein molecules enhance protein flexibility. This facilitates their migration and unfolding at the air–water interface, promoting foam formation and stabilization.[38] Our previous experiments also confirmed that the solubility of pulse albumins increases

under alkaline conditions, which is a prerequisite for achieving good foaming properties. Compared with WP, pulse albumins exhibited superior FC and FS across the entire pH range. WP showed the highest FC (34.57 ± 1.19%) at pH 9, while all tested pulse albumins exhibited higher FC values at the same pH, ranging from 57.07% (DCP) to 75.67% (GP), which were approximately 1.6 to 2.2 times greater than that of WP. Similarly, the FS of WP peaked at 26.72% at pH 9, whereas the FS of pulse albumins ranged from 57.13% (LP) to 76.22% (GP), which is about 2.14 to 2.85 times higher than that of WP. These results indicate that pulse albumins outperform WP in both foaming capacity and foam stability, demonstrating strong potential as techno-functional properties protein alternatives. Foaming performance also varied among the different pulse albumins. Pea albumins, particularly GP, consistently demonstrated superior and stable foaming capacity (FC) across all pH levels, with values ranging from 62.76% to 75.67%. These were followed by YP, which also exhibited excellent FC, ranging from 53.81% to 71.84%. In contrast, RB (48.31%–61.89%) and DCP (47.99%–57.04%) showed comparatively lower values. In terms of FS, GP achieved the highest stability at pH 9 (76.24 ± 1.17%) but dropped significantly to 49.72 ± 1.22% at pH 5. In contrast, KCP demonstrated more stable FS across pH levels, with values of 73.92 ± 2.23% at pH 9 and 62.24 ± 0.25% at pH 5. On the other
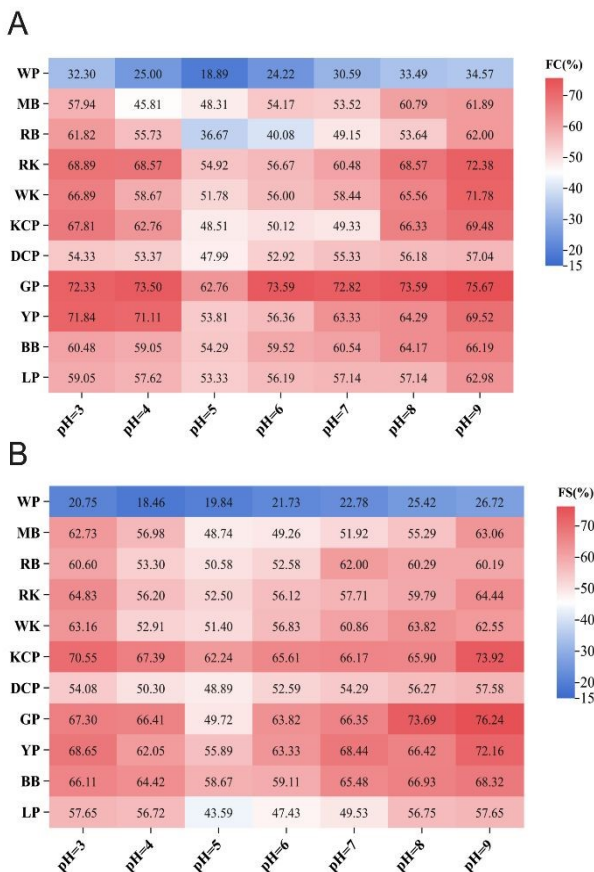
Fig.6 Effect of pH on the foaming properties and foaming stability of ten types of pulse albumin. Values shown in the heatmap represent mean values of three replicates (n = 3). Statistical analysis was performed using mean ± SD, as described in the Methods section.

hand, DCP (48.89%–57.78%) and LP (43.49%–57.75%) exhibited relatively weak foam stability across the tested pH range.

3.6. Emulsifying properties

Emulsifying activity (EAI) indicates a protein's capacity to adsorb at the oil–water interface, while emulsifying stability (ESI) measures its effectiveness in forming and maintaining stable emulsions. As shown in Figure 7, the EAI and ESI values of all ten pulse albumins across the pH range of 3–9 were generally lower than those of whey protein (WP). This may be attributed to the highly hydrophilic nature and relatively low content of hydrophobic groups in pulse albumins. Such strong hydrophilicity may limit their interaction with the oil phase, thereby weakening their emulsifying capacity. The EAI of pulse albumins exhibited clear pH dependence. The lowest EAI values were observed around pH 5–6, near their isoelectric point, whereas significantly higher values were recorded under alkaline conditions (pH 8–9).
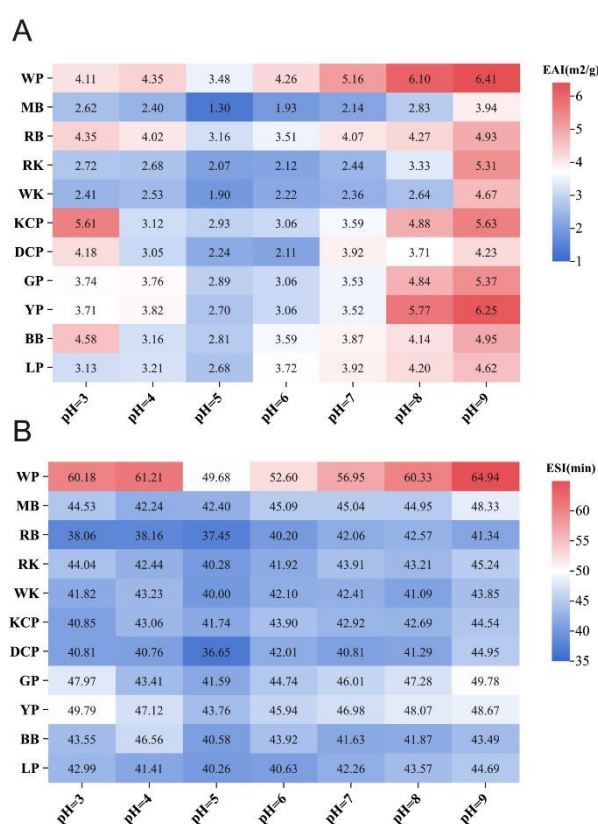

Fig.7 Effect of pH on the emulsifying activity (A) and emulsion stability(B) of ten types of pulse albumin. Values shown in the heatmap represent mean values of three replicates (n = 3). Statistical analysis was performed using mean ± SD, as described in the Methods section.

This trend is likely due to increased negative surface charges and partial unfolding of protein molecules under alkaline pH, which expose more hydrophobic regions, promoting faster migration and adsorption at the oil-water interface.[23] Among the tested samples, YP and KCP exhibited superior emulsifying activity, with YP showing the highest EAI of 6.25 ± 0.02 m²/g at pH 9, significantly outperforming other pulse albumins. In contrast, MB showed lower emulsifying ability, with an EAI of 3.94 ± 0.03m²/g at pH 9, decreasing to 1.30 ± 0.02m²/g at pH 5. The trend

observed for ESI was similar to that for EAI, with the lowest stability observed near pH 5 and significant improvements under acidic or alkaline conditions. GP and YP exhibited the highest ESI values. These observations align with previous reports. For instance, Shevkani et al.[39] found that both the EAI and ESI of kidney bean proteins dropped significantly at pH 4.5, close to their isoelectric point. Similarly, Oluwajuyitan et al.[40] reported that pea albumin formed smaller emulsion droplet sizes at pH 9 compared to pH 5, further supporting the enhanced emulsification performance under alkaline conditions.

3.7. Amino Acid Composition Profile

3.7.1 Amino acid composition and content. The amino acid composition and content of albumins from ten pulse types are summarized in Table 1. Pulse albumins were found to be rich in both variety and total content of amino acids. Among them, glutamic acid + glutamine was the most abundant (12.69–18.84%), followed by aspartic acid + asparagine (8.26–12.71%), indicating that acidic amino acids are the primary components of pulse albumins. This finding is consistent with Stałanowska et al.[41], who also identified acidic amino acids as predominant constituents in plant proteins. Pulse albumins contained a substantially higher proportion of negatively charged amino acids (NCAA: 23.79–30.98%) compared to positively charged amino acids (PCAA: 11.08–16.74%). The abundance of negatively charged residues, particularly carboxyl groups, enhances hydrophilicity and solubility by strengthening protein–water interactions[30]. In addition, lysine content was relatively high, ranging from 5.51% (RB) to 9.04% (WK), this aligns with the results of Mundi et al.[13] who observed a lysine content of 7.50% in kidney bean albumin. The sulfur-containing amino acids ranged from 0.65% (BB) to 1.59% (MB) for methionine and from 1.16% (MB) to 2.76% (GP) for cysteine. These values are consistent with those reported by Ye et al.22 for chickpea albumin, which contained 0.72% methionine and 2.53% cysteine. The ratio of essential amino acids to total amino acids (E/T) ranged from 36.26% (RB) to 45.06% (WK), all exceeding the FAO/WHO recommended ideal value of 36%. Notably, WK (43.50 ± 0.07%), RK (43.50 ± 0.07%), GP (41.66 ± 0.21%), and YP (41.46 ± 0.12%) exhibited higher essential amino acid contents. Branched-chain amino acid (BCAA) levels varied from 11.02% (LP) to 17.78% (RK). RK (17.78 ± 0.20%) and WK (17.67 ± 0.10%) showed significantly higher BCAA contents compared to other pulses, such as lentil (11.02 ± 0.12%). The BCAA levels in RK, WK, GP, and YP exceeded the 15.44% reported for flaxseed globulin31, suggesting a superior potential for promoting muscle protein metabolism.

3.7.2 Amino acid scores (AAS). The amino acid score (AAS) is defined as the ratio of the content of essential amino acids in the test protein to that in the FAO/WHO reference protein. A value close to or greater than 100 indicates higher nutritional quality of the protein. As shown in Table 2, the AAS values for lysine in all pulse albumins were above 100 (ranging from 100.22 ± 0.61 to 164.45 ± 1.46), confirming their potential to compensate for the lysine deficiency typically found in cereal proteins.[42] In addition, the scores for threonine, isoleucine, and phenylalanine + tyrosine

523

524 Table 1Table 1 Amino acid composition of 10 types of pulse albumin. Values are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate statistically
525 significant difference within each row (P < 0.05).

Amino acid（%）	Vigna		Phaseolus		Cicer		Pisum		Vicia	Lens
	MB	RB	RK	WK	KCP	DCP	GP	YP	BB	LP
Asx	8.26±0.17h	10.08±0.05d	12.14±0.19b	12.71±0.04a	9.67±0.04f	9.31±0.02g	11.69±0.08c	12.00±0.07b	9.85±0.06e	9.89±0.02e
Thr	4.03±0.06ef	3.95±0.03f	5.95±0.02b	6.58±0.11a	4.44±0.26d	4.19±0.07e	5.49±0.08c	5.80±0.08b	3.98±0.05f	4.03±0.11ef
Ser	5.14±0.09a	5.13±0.04a	4.74±0.03b	4.11±0.08e	3.65±0.02g	4.66±0.02b	4.39±0.06d	4.01±0.02f	4.48±0.05c	3.73±0.06g
Glx	17.29±0.05b	15.30±0.02e	18.84±0.03a	17.32±0.04b	15.96±0.05d	12.69±0.05h	16.76±0.03c	16.74±0.02c	15.04±0.09f	13.90±0.11g
Gly	5.78±0.03a	5.26±0.05b	4.07±0.04f	4.04±0.02g	4.44±0.02c	3.54±0.03g	4.31±0.04d	4.25±0.05e	5.29±0.06b	4.44±0.02c
Ala	4.49±0.03bc	4.00±0.07c	4.02±0.01c	3.84±0.06c	4.34±0.06bc	4.51±0.03bc	4.08±0.05b	5.42±0.05a	5.58±0.07a	4.95±0.03b
Cys	1.16±0.07g	1.42±0.09f	2.29±0.03bc	2.18±0.05c	2.17±0.09c	1.99±0.02d	2.76±0.10a	2.37±0.02b	2.74±0.09a	1.60±0.23e
Val	3.50±0.07e	3.37±0.08f	5.14±0.06a	5.18±0.04a	3.55±0.09e	3.32±0.08f	4.88±0.03b	4.39±0.06c	4.07±0.06d	3.50±0.01e
Met	1.59±0.09a	1.24±0.02c	1.49±0.02b	1.47±0.05b	1.16±0.04d	0.94±0.02e	1.01±0.06e	0.95±0.06e	0.65±0.05g	0.85±0.04f
Ile	3.58±0.06f	3.48±0.06f	5.67±0.11a	5.69±0.02a	4.70±0.07c	4.01±0.03e	5.37±0.1b	5.65±0.15a	4.35±0.08d	3.34±0.05g
Leu	5.20±0.05e	5.05±0.10f	6.98±0.04a	6.80±0.05b	6.17±0.07d	5.11±0.09ef	6.79±0.12b	6.32±0.04c	6.28±0.05cd	4.19±0.09g
Tyr	2.04±0.03f	1.6±0.01g	2.30±0.04e	2.58±0.02c	3.28±0.03a	2.47±0.05d	3.00±0.05b	3.01±0.05b	2.60±0.04c	2.51±0.01d
Phe	2.97±0.05f	3.27±0.05de	4.53±0.05b	5.20±0.03a	4.47±0.24b	3.37±0.21de	3.19±0.11e	3.38±0.14d	3.67±0.02c	3.42±0.10d
Lys	5.81±0.03f	5.51±0.03h	8.95±0.04a	9.04±0.08a	7.80±0.09c	6.74±0.08d	7.87±0.10c	8.10±0.07b	6.38±0.10e	5.65±0.15g
His	2.76±0.05e	2.49±0.02g	3.44±0.05b	3.63±0.02a	2.98±0.09c	1.75±0.03h	3.60±0.02a	3.60±0.04a	2.86±0.04d	2.61±0.02f
Arg	2.50±0.13h	4.89±0.03b	4.06±0.04c	4.07±0.05c	3.88±0.05d	3.03±0.01g	3.46±0.02e	3.12±0.04f	4.06±0.01c	5.09±0.01a
Pro	1.93±0.06f	2.17±0.04e	2.29±0.05d	2.31±0.15d	3.43±0.05a	2.21±0.07de	3.06±0.07b	3.00±0.03b	2.64±0.02c	2.21±0.02de
E/T	37.74±0.25g	36.26±0.32h	43.50±0.07b	45.06±0.23a	40.97±0.25d	39.85±0.41e	41.66±0.21c	41.46±0.12c	38.16±0.14f	36.33±0.14h
BCAA	12.27±0.09f	11.90±0.11g	17.78±0.2a	17.67±0.10a	14.42±0.21e	12.44±0.19f	17.04±0.20b	16.36±0.17c	14.71±0.16d	11.02±0.12h
PCAA	11.08±0.11g	12.89±0.03e	16.45±0.09b	16.74±0.02a	14.66±0.22c	11.52±0.05f	14.92±0.12c	14.82±0.04c	13.30±0.08d	13.34±0.16d
NCAA	25.55±0.23e	25.38±0.03e	30.98±0.18a	30.03±0.08b	25.63±0.09e	22.00±0.05h	28.45±0.09d	28.74±0.09c	24.89±0.12f	23.79±0.13g

Asx: aspartic acid + asparagine.

Glx: glutamic acid + glutamine.

528 E/T: ratio of essential to total amino acids.

529 BCAA: Branched chain amino acids (valine, isoleucine, leucine).

530 PCAA: positively charged amino acids (histidine, lysine).

531 NCAA: negatively charged amino acids (Asx, asparagine + aspartic acid; Glx, glutamine + glutamic acid).

532

533 were generally close to or exceeded 100. Notably, the AAS values
534 of methionine + cysteine in RK (108.16 ± 0.44), WK
535 (104.43 ± 2.69), and GP (107.62 ± 4.14) were above the reference
536 value of 100, indicating an improved sulfur-containing amino acid
537 profile that compensates for a common nutritional limitation of
538 pulse proteins. In contrast, the AAS values of leucine (Leu) and
539 valine (Val) were generally lower.

540 **3.7.3 Essential Amino Acid Index (EAAI).** Table 3 presents the
541 Essential Amino Acid Index (EAAI) of the albumin fractions from

542 ten pulses. The EAAI is an indicator of food protein quality,
543 reflecting the ratio of the essential amino acid content in the
544 protein to human requirements. The closer the EAAI is to 100, the
545 more similar the essential amino acid profile is to that of a
546 reference protein, indicating a higher nutritional value. As
547 presented in Table 3, EAAI indices for WK (126.70 ± 0.95), RK
548 (123.30 ± 0.54), GP (115.65 ± 0.70), YP (113.44 ± 0.52), and KCP
549 (105.13 ± 1.08) exceeded the reference value of 100, signifying
550 superior nutritional value. In contrast, BB (98.96 ± 0.32), DCP
551 (87.23 ± 1.29), MB (81.11 ± 0.52), RB (78.11 ± 0.31), and LP
552 (77.66 ± 1.20) exhibited comparatively lower EAAI values. Among

Table 2 Amino acid scores (AAS) of 10 types of pulses albumin. Values are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate statistically significant difference within each row (P < 0.05).

Essential amino acid	Vigna		Phaseolus		Cicer		Pisum		Vicia	Lens	FAO/WHO
	MB	RB	RK	WK	KCP	DCP	GP	YP	BB	LP	
Leu	74.22±0.68e	72.14±1.43f	99.64±0.64a	97.18±0.78b	88.2±0.93d	73.05±1.24ef	96.98±1.69b	90.31±0.58c	102.16±1.30cd	59.82±1.27g	70
Ile	89.43±1.56f	86.97±1.56f	141.71±2.79a	142.18±0.57a	117.46±1.64c	100.15±0.72e	134.28±2.56b	141.28±3.69a	126.64±2.34d	83.38±1.28g	40
Lys	105.7±0.47f	100.22±0.61h	162.75±0.8a	164.45±1.46a	141.83±1.65c	122.51±1.40d	143±1.86c	147.24±1.33b	134.27±0.21e	102.65±2.65g	55
Met+Cys	78.46±2.51d	75.90±2.10d	108.16±0.44a	104.43±2.69a	95.26±3.62b	83.74±0.47c	107.62±4.14a	94.92±2.24b	96.83±4.01b	69.85±5.87e	35
Phe+Tyr	83.53±0.90f	81.18±0.70f	113.73±1.35b	129.82±0.85a	129.14±3.46a	97.26±2.90e	103.08±1.67d	106.64±2.91c	104.47±0.76cd	98.83±1.82e	60
Thr	100.83±1.40e	98.79±0.63e	148.79±0.41b	164.38±2.82a	110.93±6.54d	104.68±1.84e	137.27±2.07c	144.99±2.01b	113.77±2.66e	100.78±2.65e	40
Val	70.02±1.39e	67.37±1.60f	102.83±1.29a	103.53±0.89a	70.94±1.89e	66.46±1.53e	97.65±0.56b	87.73±1.17c	85.41±0.92d	70.04±0.24f	50

Table 3 Essential Amino Acid Index (EAAI) ang Biological Value (BV) of 10 types of pulse albumin. Values are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate statistically significant difference within each row (P < 0.05).

	Vigna		Phaseolus		Cicer		Pisum		Vicia	Lens
	MB	RB	RK	WK	KCP	DCP	GP	YP	BB	LP
EAAI	85.14±0.47h	82.39±0.28i	123.3±0.54b	126.7±0.95a	105.13±1.08e	90.77±1.19g	115.65±0.70c	113.44±0.52d	98.96±0.32f	81.98±1.10i
BV	81.11±0.52h	78.11±0.31i	122.7±0.58b	126.41±1.04a	102.9±1.18e	87.24±1.29g	114.36±0.76c	111.95±0.57d	96.16±0.35f	77.66±1.2i

all samples, WK and RK showed significantly higher EAAI scores than the other samples, indicating a more balanced essential amino acid composition and superior nutritional quality.

3.7.4 Biological Value (BV). Biological value (BV) is defined as the percentage of absorbed nitrogen from dietary protein that is retained and utilized by the body, reflecting the efficiency of protein utilization after digestion and absorption. A higher BV indicates a more efficiently utilized protein. As shown in Table 3, WK (126.41 ± 1.04) and RK (122.70 ± 0.58) had the highest BV values among all samples, followed by GP (114.36 ± 0.76), YP (111.95 ± 0.57), and KCP (102.90 ± 1.18), while LP exhibited the lowest BV (77.66 ± 1.20). The variation in BV among different pulse albumins highlights significant differences in protein quality and utilization efficiency. WK and RK, possessing the highest BV among all samples, demonstrate high efficiency of protein absorption and utilization, identifying them as high-quality protein sources. Proteins with high BV offer a distinct advantage in practical diets, as they provide essential amino acids more effectively, thereby enhancing overall nutritional utilization.

3.8. Integrated Comparison of techno-functional properties and Amino Acid Profiles of Ten Pulse Albumins

Figure 8 provides a comprehensive overview of the functional and compositional profiles of the ten pulse albumins, revealing distinct differences in processing characteristics and amino acid composition across the pulse varieties. In terms of functional properties, *Phaseolus vulgaris* albumins (WK and RK) consistently ranked among the top samples for water-holding capacity (WHC) and protein solubility (PS). *Pisum sativum* albumins (GP and YP) demonstrated excellent foaming and emulsifying capacities, whereas *Vigna* albumins (MB and RB) exhibited relatively low WHC and foaming ability. Within *Cicer arietinum*, KCP showed higher oil-holding capacity (OHC), while DCP recorded the lowest foaming performance. Amino acid analysis revealed that all albumins had multiple essential amino acid score exceeding FAO/WHO reference values. Among them, *P. vulgaris* albumins (WK and RK) achieved the highest values for the essential-to-total amino acid ratio (E/T), essential amino acid index (EAAI), and biological value (BV), highlighting their superior nutritional
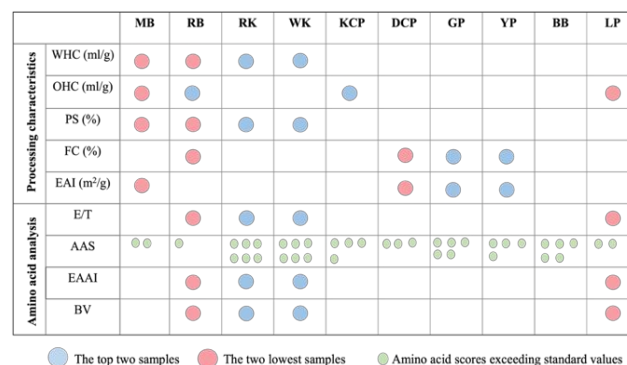

Fig.8 Comparison of techno-functional properties and amino acid analysis of ten pulse albumin.

quality. By contrast, MB and LP albumins presented the lowest E/T, EAAI, and BV, reflecting weaker protein quality. Collectively, these results underline the outstanding overall performance of WK and RK albumins, the specialized foaming and emulsifying advantages of GP and YP, and the limitations of MB, RB, and LP. MB and RB ranked among the lowest for WHC and PS, while RB and LP showed the lowest E/T, EAAI, and BV, indicating relatively lower in amino acids quality.

4. Conclusions

This study provides a systematic comparison of the techno-functional properties and amino acid composition of albumins from ten pulse species. Overall, all pulse albumins exhibited excellent solubility (>70%) across a broad pH range, confirming their potential as highly soluble protein fractions for diverse food applications. Nevertheless, notable interspecific differences were observed, reflecting inherent variations in amino acid composition, subunit structure, and molecular characteristics. Among the tested species, *Phaseolus vulgaris* albumins (WK and RK) showed high solubility even at high protein concentrations, possessed excellent water-holding capacity, and demonstrated the highest E/T, EAAI, BV, and BCAA content. These features make them promising candidates for high-protein beverages and foods. *Pisum sativum* albumins (GP and YP) demonstrated outstanding foaming capacity and stability, along with excellent emulsifying properties, suggesting their applications in aerated and emulsion-based formulations. Within *Cicer arietinum*, KCP exhibited excellent emulsifying activity and oil-holding capacity, whereas DCP showed comparatively lower functionality, particularly foaming capacity. Albumins from *Vigna* species (MB, RB) and *Lens culinaris* (LP) displayed lower nutritional scores and less favorable techno-functional properties (WHC, OHC, EAI), which may limit their use in direct applications.

In summary, these findings highlight the differences in the techno-functional properties and amino acid composition of albumins among different pulse species. These findings deepen the understanding of pulse albumin techno-functional properties and offer practical insights for the targeted use of different pulse species in food applications.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data would be available on request.

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Data availability

The data would be available on request.