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Comparative in vitro assessment of protein quality in selected animal- and plant-derived foods based on the digestible indispensable amino acid score (DIAAS)

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ABSTRACT

In many regions, nutritional claims regarding food protein quality rely solely on protein content, neglecting the digestible indispensable amino acid score (DIAAS), which is internationally recognized as a physiologically relevant measure of protein quality. DIAAS is preferably determined using in vivo methods, however, animal models raise time, cost, and ethical concerns that have restrained protein quality assessments of ingredients. Therefore, this study aimed to evaluate the protein quality of common animal- and plant-derived foods using the INFOGEST Quant protocol, a validated static in vitro method that mimics in vivo models. Nineteen foods were digested, and the absorbable and non-absorbable fractions were analyzed for total nitrogen, amino groups, and individual amino acids to determine total protein digestibility, individual amino acid digestibility, and DIAAS. Total amino acid-based digestibility exceeded 90% for all products except cooked potato (81.2%), soy flour (88.4%), raw pinto bean (61.8%), and egg white powder (76.3%). Cooked meat achieved a DIAAS above 100% using both the young-child and adult reference patterns, whereas casein, skim milk powder, and soy protein isolate exceeded 100% using the adult reference pattern. Some legumes reached a DIAAS of ≥75%, whereas cereals, nuts, and cooked potato mostly remained below this threshold, primarily because of their low lysine contents. The principal limiting amino acids in legumes were sulfur amino acids, leucine and valine, while raw pinto beans showed low DIAAR values for all amino acids. Overall, these findings highlight that protein concentration, amino acid profile and digestibility must be considered when evaluating dietary protein sources.

1. Introduction

The nutritional quality of dietary proteins depends on three crucial components: total protein content, the proportion of indispensable (also called essential) amino acids, and digestibility. Research has consistently shown that the quality of animal proteins is generally higher than that of plant-based foods (Herreman et al., 2020), and when protein content is corrected for digestibility, quality measures change and tend to decrease for certain plant-based proteins, such as legumes, nuts, and seeds (Drewnowski, 2025). Considerable variability in protein quality has also been observed among plant species (Herreman et al., 2020). In light of this, some countries such as Canada and the USA have incorporated protein quality parameters, specifically the protein digestibility-corrected amino acid score (PDCAAS), into the regulation of protein-related claims (Code of Federal Regulations, 2025; Government of Canada, 2024, 2025).

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The PDCAAS represents the ratio of the first-limiting amino acid *per* gram of protein in a given food relative to a reference amino acid requirement pattern. However, this score has been widely criticized for not accounting for the individual digestibility of indispensable amino acids and for capping all values at 100%, thereby not recognizing the superior quality of certain proteins. Previous research shows that amino acid composition and total protein digestibility alone are insufficient to accurately evaluate protein quality (FAO, 2013; Mathai et al., 2017). For this reason, the digestible indispensable amino acid score (DIAAS), which overcomes the limitations of PDCAAS by using ileal digestibility values for individual amino acids and by allowing scores above 100%, is recognized as a more accurate and physiologically relevant measure of protein quality (FAO, 2013).

Since its adoption as the preferred measure for protein quality in 2013 (FAO, 2013), some studies have determined the DIAAS of various protein foods. However, the most recommended methods to measure this score require an in vivo digestibility assessment (FAO, 2013). Requirements for in vivo methods have hampered protein quality assessment of traditional proteins and their ingredient derivatives due to cost, time, and ethical concerns, also presenting challenges for food innovation. INFOGEST Quant, named after the INFOGEST international network for digestion research, is a validated static in vitro digestion method developed to accurately determine total and individual indispensable amino acid digestibility of foods and, ultimately, assess protein quality. Given that microbial fermentation is not part of the method, digestibility coefficients represent protein and amino acid digestibility at the terminal ileum.

Even with available in vitro experimental alternatives, the range of protein sources for which DIAAS has been determined remains very limited, especially in the case of certain plant-based foods. Accordingly, the present study used the INFOGEST Quant in vitro protein digestibility method (Egger et al., 2026; Sousa, Recio, et al., 2023) to assess the protein quality, considering digestibility and DIAAS, of several food ingredients widely recognized as sources of protein and commonly used both in households and in the food industry. The aim was not only to contribute to the characterization of different dietary proteins in terms of nutritional quality, but also to study how the inclusion of digestibility and DIAAS in protein quality measures affects foods from different origins.

2. Materials and methods

2.1. Reagents and samples

Unless otherwise stated, all reagents for in vitro digestion and subsequent analyses were purchased from Merck (Darmstadt, Germany). Pepsin from porcine gastric mucosa (P7012), pancreatin from porcine pancreas (P7545), α-amylase from human saliva (A1031) and porcine bile extract (B8613) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The activities of all commercial enzymes were determined prior to the experiments using the corresponding methods described by Egger et al. (2026) and Brodkorb et al. (2019). Simulated salivary, gastric and intestinal fluids were prepared according to the same references.

The following food materials were included in the study: wheat flour (P&H Milling Group, Cambridge, ON, Canada), soy flour (Cargill, Plymouth, MN, USA), rice flour (Dawn Foods, Calgary, AB, Canada), pistachio flour, almond flour, chickpea flour, yellow pea, green lentil (Avena Foods, Rowatt, SK, Canada), pinto bean (Viterra, Carman, MB, Canada), Russet potato (Kroeker Farms, Winkler, MB, Canada), soy protein isolate (Bunge, Chesterfield, MO, USA), ground beef (local butcher, Switzerland), egg white powder (Burnbrae Farms, Winnipeg, MB, Canada), skim milk powder, casein (Dyets Inc., Bethlehem, PA, USA), and wheat gluten (IMCD Canada Ltd., Brampton, ON, Canada).

Purchased flours were produced from raw foods and were not thermally processed. Pulses (yellow peas, green lentil and pinto beans) were analyzed in raw and cooked formats. Raw pulses were ground to a particle size of 0.75 mm prior to digestion. Cooked pulses, i.e. whole yellow peas, green lentils and pinto beans were prepared by soaking in tap water (1:4, w/v) for 16 h, followed by draining and boiling in the same volume of water for 19 min (pinto beans), 26 min (green lentils) or 37 min (yellow peas) following the average cooking times in Nosworthy et al. (2017). In that study, the cooking time for pinto beans was determined using an automated Mattson cooker and was defined as the time required for 80% of the plungers to penetrate the seeds. Cooking times for green lentil and yellow pea were determined using a modified tactile method, where samples were boiled in deionized water for 8–10 min and squeezed between thumb and forefinger; the point at which 4 out of 5 seeds had little to no resistance was considered as the cooking time. Cooking was stopped by adding half the volume of cold tap water. The mixture was drained, freeze-dried, and ground to approximate particle size of 0.75 mm.

Russet potatoes were peeled, cut into cubes (2-cm-side-length), and cooked in a steam kettle (Groen D-10) for 12 min with sufficient water to cover the potatoes plus 7–8 cm. The cooked potatoes were removed, cooled to room temperature, freeze-dried, and milled to 0.75 mm. Ground beef was cooked in a non-stick skillet at medium-high heat until 71 °C were reached (8–10 min). Excess water was drained, and the cooked meat was freeze-dried, defatted for 12 h using the Soxhlet extraction, and ground to 0.75 mm.

Soy protein isolate, egg white powder, skim milk powder, casein and wheat gluten were used as supplied, without any further processing. In addition, a protein-free cookie was prepared according to specifications in Egger et al. (2026) and Moughan et al. (2005).

2.2. Static in vitro digestion

Prior to digestion, the total nitrogen content of the samples was analyzed using the Kjeldahl method (International Organization for Standardization, 2004; see section 2.3.1). In addition, pH-test experiments were performed for each food ingredient as described in Egger et al. (2026) and Brodkorb et al. (2019). Simulated salivary, gastric and intestinal fluids were prepared according to Egger et al. (2026).

Each sample was digested in triplicate, except for skim milk powder, that was digested using six replicates to confirm the results; the replicates were digested on different days. Simultaneously with each digestion batch, a separate digestion of 1 g of protein-free cookie (without sample) was performed and used as a blank to correct for the amino acids coming from the digestive enzymes. The protein-free cookie was used as a blank for all the samples from the same batch. The amount of sample used for in vitro digestion was adjusted to provide 40 mg of protein, based on total nitrogen and using a nitrogen-to-protein conversion factor of 6.25 for all ingredients, in accordance with the FAO recommendations for dietary protein quality evaluation (FAO, 2013). In addition, 250 mg of protein-free cookie were added to the casein, soy protein isolate, wheat gluten and egg white to mimic reference in vivo study designs. In such studies, isolated proteins are typically combined with a protein-free food matrix for administration to human participants or animals (Sousa, Recio, et al., 2023). All samples were adjusted to a final mass of 1 g with ultrapure water.

The in vitro digestion was performed following the INFOGEST Quant method (Egger et al., 2026; Sousa, Recio, et al., 2023), which allows the estimation of ileal protein and amino acid digestibility, as well as DIAAS. The oral phase was simulated by mixing the food samples and corresponding cookie blanks with salivary fluid (pH 7.0). For samples containing starch (and their corresponding cookie blanks), the simulated oral fluid also contained α-amylase (75 international units (IU)/mL final activity), and the oral phase was incubated for 2 min. For the gastric phase, the oral bolus was mixed with a simulated gastric fluid (pH 3.0) containing pepsin (2000 IU/mL final activity) and incubated for 2 h. Finally, a simulated intestinal fluid containing solubilized pancreatin (100 IU/mL final trypsin activity) and bile (10 mmol/L final concentration) was mixed with the gastric digesta and incubated for 2 h. For

solubilization of pancreatin, it was suspended in simulated intestinal fluid, vortexed for 10 s, ultrasonicated for 5 min at room temperature, centrifuged at 3000*g* for 5 min at room temperature, and the trypsin activity of the resulting supernatant was determined (Egger et al., 2026; Sousa, Recio, et al., 2023). All digestions were conducted at a constant temperature of 37 °C. For the gastric and intestinal phases, the pH was adjusted with hydrochloric acid (HCl; 2 mol/L) or sodium hydroxide (NaOH; 2 mol/L) using food-specific volumes predetermined in the preliminary pH-tests.

Immediately after completion of the intestinal digestion, enzymatic activity was stopped by adding ice-cold pure methanol (80% in water (v/v), final concentration), followed by incubation for 1 h at −20 °C. The absorbable (supernatant) and non-absorbable (pellet) fractions of the digesta were separated by centrifugation (4000*g*, 4 °C, 15 min; Heraeus Multifuge X3R, Thermo Fisher Scientific, Waltham, MA, USA). Supernatants were transferred to clean 50 mL plastic tubes with a pipette, and pellets were washed twice, each time by adding 5 mL of an aqueous methanol solution (80%, v/v) and centrifuging at 4000*g* and 4 °C for 10 min (Heraeus Multifuge X3R, Thermo Fisher Scientific). Supernatants obtained in the washing steps were discarded. The initial supernatants and washed pellets were immediately kept at −20 °C until analysis. Tube weights were monitored throughout the entire procedure as detailed by Egger et al. (2026). A detailed description of the in vitro digestion and methanol-based fractionation procedure is provided in Egger et al. (2026).

2.3. Analysis of the digested fractions

2.3.1. Total nitrogen

The total nitrogen content of the undigested foods, the digested pellets, and supernatants was determined in duplicate using the Kjeldahl method (International Organization for Standardization, 2004). This procedure involves the complete hydrolysis of organic matter with equal volumes of sulfuric acid (H_2SO_4; 96% v/v) and hydrogen peroxide (H_2O_2) followed by titration of the liberated ammonia.

2.3.2. Acid hydrolysis for total amino acid and alkaline hydrolysis for tryptophan quantification

Undigested samples, pellets and supernatants were subjected to acid hydrolysis according to Jaudzems et al. (2019). The reaction mixture contained ultrapure water, dithiodipropionic acid (1%, w/v, in NaOH 0.2 mol/L), HCl 0.2 mol/L, and concentrated HCl (37%, v/v), with L-norvaline (10 mmol/L final concentration) as the internal standard. The reaction was carried out for 15 h (digesta) or 24 h (food) at 110 °C. An L-cysteine standard (10 mmol/L final concentration) was hydrolyzed in parallel.

Additionally, undigested food ingredients were hydrolyzed under alkaline conditions according to ISO 13904:2016 (International Organization for Standardization, 2016) to quantify tryptophan. In brief, samples were reacted with NaOH 8.4 mol/L and ultrapure water in the presence of starch and of 1-methyl-L-tryptophan (320 μg/mL) as internal standard. An L-tryptophan standard (1.6 mg/mL) was also hydrolyzed in parallel. The reaction was incubated for 20 h at 110 °C. Immediately after, hydrolysates were mixed with 5 mL phosphate buffer (0.2 mol/L, pH 7.0) and stored at 4 °C until analysis.

2.3.3. Total amino groups

The amino groups released from acid hydrolysis of supernatants and pellets were analyzed using the o-phthalaldehyde (OPA) method (Kopf-Bolanz et al., 2012; Sousa, Recio, et al., 2023). Pellets and supernatants were diluted 10-fold and 5-fold, respectively, with perchloric acid (0.5 mol/L). Amino acids were derivatized with a solution of borate (0.05 mol/L), sodium dodecyl sulfate (10 g/L), OPA (0.8 g/L), 2-mercapto-ethanosulfonic acid (5 g/L), and Tergitol™ (5 g/L). Absorbance was measured spectrophotometrically at 340 nm (SpectraMax iD3, Molecular Devices, San Jose, CA, USA) using a glutamic acid calibration curve (0.25–8.00 mmol/L).

2.3.4. Individual amino acids

All samples subjected to acid hydrolysis (foods, supernatants and pellets) were analyzed to determine their profiles of proteinogenic amino acids (Jaudzems et al., 2019). Hydrolysates were neutralized with one part of NaOH 6 mol/L and two parts of HCl 0.1 mol/L, followed by filtration through cellulose filter plates (Eppendorf, Hamburg, Germany) using centrifugation at 370*g* and 15 °C for 5 min. Then, 10 μL of each filtered solution were derivatized with 20 μL of saturated 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in acetonitrile (AccQ-Tag Ultra reagent, Waters, Milford, MA, USA), and 70 μL borate buffer (5%, *w/v*). After the reaction at 55 °C for 10 min under agitation (1000 rpm, ThermoMixer C, Eppendorf), amino acids were quantified by ultra-high performance liquid chromatography (UHPLC) coupled to an ultraviolet detector (Vanquish system, Thermo Fisher Scientific). The column used was an Acquity UPLC BEH C18 (2.1 × 150 mm, 1.7 μm; Waters). Analytical conditions are described in the reference method (Jaudzems et al., 2019).

For tryptophan quantification of undigested foods, 100 μL of the alkaline hydrolysates were neutralized with 50 μL of ice-cold HCl 6 mol/L and 2.35 mL phosphate buffer (0.2 mol/L, pH 7.0). The mixtures were filtered through cellulose filter plates (Eppendorf) by centrifugation (370*g*, 15 °C, 5 min), and the filtrates were injected into a UHPLC system equipped with a fluorescence detector (Vanquish, Thermo Fisher Scientific). Analytical parameters followed ISO 13904:2016 (International Organization for Standardization, 2016).

2.3.5. Calculations

Crude protein content in the substrates was estimated by measuring total nitrogen and applying a nitrogen-to-protein conversion factor of 6.25 for all samples, as recommended by FAO (2013), to ensure comparability with in vivo data. Total protein digestibility (based on total nitrogen, amino groups and amino acids), individual amino acid digestibility, digestible indispensable amino acid ratios (DIAAR) and DIAAS values were calculated according to Egger et al. (2026).

3. Results and discussion

3.1. Total protein content and amino acid composition

To assess protein digestibility and DIAAS, the first step is to quantify the total protein content and amino acid composition of the undigested food products. The total crude protein content of the foods is summarized in Table 1. All protein isolates or concentrates (wheat gluten, soy protein isolate, casein) contained more than 80% protein. Excluding these, egg white had the highest protein content (83%; comparable to the protein isolates), followed by nut (pistachio and almond) and soy flours (47–59%), and by skim milk powder (35%). Similar protein contents were observed in pulses (raw and cooked), chickpea flour and cooked meat (21–26%), whereas cereal flours and cooked potato contained the lowest protein concentrations (< 20%). These results align well with previously reported values (FAO, 2001).

The amino acid profile is a key characteristic that defines the quality of a protein source and depends on the proportion of indispensable amino acids. In legumes and animal proteins, between 40% and 50% of all amino acids were indispensable, while in cereals (except rice flour, with 42%), potatoes and nuts, they only accounted for between 30 and 40% of total amino acids (Fig. 1). Consequently, legumes, dairy products, eggs, and meat have a more favorable amino acid profile, whereas cereals and potatoes generally supply lower amounts of both total and indispensable amino acids. This confirms common dietary guidelines where soy and pulses are considered to be protein sources. When the summed indispensable amino acid content was expressed relative to the crude protein content, animal-derived foods also contain more indispensable amino acids (41 ± 6%) than plant-based foods (31 ± 4%),

Table 1
Protein content of the studied substrates before (crude protein) and after correction with total protein digestibility (in vitro ileal digestibility-corrected protein), expressed as percentage (%, w/w) ± standard deviations, and the corresponding decrease between both values.

	Crude protein (%)	In vitro ileal digestibility-corrected protein (%)	Decrease (%)
Cereals, potatoes and nuts			
Rice flour	8.12 ± 0.00	8.05 ± 0.00	0.841
Wheat gluten	81.9 ± 0.4	75.8 ± 0.4	7.44
Wheat flour	15.8 ± 0.2	15.7 ± 0.2	0.601
Cooked potato	6.93 ± 0.24	5.63 ± 0.20	18.8
Pistachio flour	46.7 ± 0.1	46.5 ± 0.1	0.450
Almond flour	59.4 ± 0.0	59.0 ± 0.0	0.657
Legumes			
Chickpea flour	21.2 ± 0.1	21.0 ± 0.1	1.23
Soy flour	49.8 ± 0.2	44.0 ± 0.1	11.6
Soy protein isolate	86.8 ± 0.1	86.3 ± 0.1	0.599
Raw yellow pea	23.2 ± 0.1	23.2 ± 0.1	0.062
Cooked yellow pea	24.3 ± 0.1	24.2 ± 0.1	0.641
Raw green lentil	25.1 ± 0.0	25.0 ± 0.0	0.317
Cooked green lentil	25.5 ± 0.1	25.5 ± 0.1	0.273
Raw pinto bean	24.1 ± 0.0	14.9 ± 0.0	38.2
Cooked pinto bean	26.0 ± 0.2	25.7 ± 0.2	1.00
Animal-derived foods			
Skim milk powder	34.7 ± 0.2	33.1 ± 0.2	4.53
Casein	86.9 ± 0.5	82.7 ± 0.5	4.79
Egg white	82.5 ± 0.7	62.9 ± 0.5	23.7
Cooked meat	24.1 ± 1.6	24.0 ± 1.6	0.356

Crude protein was based on total nitrogen measured with the Kjeldahl method (International Organization for Standardization, 2004) and a nitrogen-to-protein conversion factor of 6.25. Digestibility-corrected protein was calculated by multiplying the crude protein values with the corresponding total in vitro protein digestibility measured based on total amino acids.

which aligns with previously reported data on the same foods (Gorissen et al., 2018; van Vliet et al., 2015).

When expressed as percentages of the total amino acid content, tryptophan, histidine, methionine and cysteine were the least abundant amino acids among the selected foods, except for the relatively high cysteine contents observed in wheat products and in egg white, as also observed by Gorissen et al. (2018). Glutamic acid was the most abundant amino acid in all the foods except for cooked potato, in which aspartic acid was the most abundant, as has been reported in previous studies as well (Zhu et al., 2010). Aspartic acid was also among the most abundant amino acids in all foods except for dairy products and wheat products; similarly, Mathai et al. (2017) reported a lower proportion of aspartic acid in milk and wheat. During acid hydrolysis before chromatographic amino acid quantification, asparagine is converted to aspartic acid and glutamine to glutamic acid (Jaudzems et al., 2019). Accordingly, the reported aspartic acid values represent the combined contents of aspartic acid and asparagine, whereas the glutamic acid values represent the combined contents of glutamic acid and glutamine. Legumes, rice and nuts contained relatively high proportions of arginine, while dairy and wheat products contained relatively high proportions of proline. Nuts, cooked potato and wheat were characterized by comparatively low proportions of lysine (Gorissen et al., 2018). Overall, the amino acid profiles observed in this study were consistent with previously reported compositional data (FAO, 1970).

3.2. Protein quality

3.2.1. Ileal digestibility: total protein and individual amino acids

Total in vitro protein digestibility was estimated from three different measurements: total amino acids, total amino groups and total nitrogen. All three methods have been validated, although total amino acid determination is recommended due to its greater analytical accuracy and its utility for calculating DIAAS values (Sousa, Recio, et al., 2023).

As shown in Fig. 2, cooked potatoes, raw pinto beans, and egg white exhibited the lowest ileal digestibility, with values below or close to 80% (based on total amino acids). The digestibility of most plant proteins was close to 100%, except for wheat gluten and soy flour, which showed slightly lower values. In contrast, the digestibility of milk proteins exceeded 90% and cooked ground beef was the most digestible protein source (~ 100% based on the three in vitro analytical methods, i.e. total amino acids, amino groups and nitrogen. These results are closely consistent with data from literature using both in vitro and in vivo approaches, although direct quantitative comparisons are limited by differences in food materials and processing conditions among studies (Bailey et al., 2020; Hodgkinson et al., 2025; Sousa, Portmann, et al., 2023).

Interestingly, most raw and cooked pulse proteins were highly digestible, with only raw pinto beans showing limited digestibility, which increased to almost 100% after cooking. This high digestibility, even among raw pulses, may be partially caused by the small particle size of the tested substrates, as has been shown for navy bean flour, where smaller particles correlated with higher protein digestibility (Byars et al., 2021). This occurs most likely because grinding breaks down cell walls and releases intracellular compounds to the extracellular space, thereby facilitating interactions between enzymes and substrates (Brummer et al., 2015). However, legumes, in general, are known to contain antinutritional factors with trypsin inhibition activity. These trypsin inhibitor proteins can be partially removed by soaking or effectively inactivated by thermal processing (including conventional cooking) among other treatments (Avilés-Gaxiola et al., 2018; Patterson et al., 2017). In fact, previous studies have reported substantial reductions in trypsin inhibitor activity after cooking in chickpeas (Tavano et al., 2008; Wang et al., 2010), lentils (Wang et al., 2009), beans (Wang et al., 2010), and peas (Wang et al., 2008). Trypsin inhibitor activity is highest in beans (5.9 mg/g dry matter for pinto beans, 5.9–10.9 mg/g dry matter for other varieties; Wang et al., 2010), moderate in lentils (1.9–2.8 mg/g dry matter; Wang et al., 2009) and lowest in peas (1.3–2.0 mg/g dry matter; Wang et al., 2008). Therefore, the results from the present study suggest that the high trypsin inhibition activity in raw beans is strong enough to interact with intestinal trypsin and limit protein digestibility. In addition to the effect of antinutritional factors, pinto beans are richer in resistant starch and show a more ordered structure of the cell walls than green lentils. They also contain soluble fibers that are larger (in terms of molecular weight) and more viscous compared to those in yellow peas and green lentils, which creates a stronger physical barrier to the enzymes reaching the substrate, potentially affecting digestibility (Brummer et al., 2015). However, it is worthwhile to note that legumes (soy and pulses) are generally thermally treated before consumption, either through traditional cooking methods or other heat treatments in processed foods, which would substantially reduce the effect of some antinutritional factors affecting digestibility (Avilés-Gaxiola et al., 2018; Patterson et al., 2017). In contraposition, certain heat treatments may cause the formation of Maillard reaction products and protein conformational changes that can decrease protein or amino acid digestibility (Carbonaro et al., 2012; Deng et al., 2017). This would certainly be worthwhile to explore in studies using whole foods rather than freeze-dried substrates. This study was part of a larger project comparing in vitro protein digestibility methods (Goldberg et al., 2026; House et al., 2019); therefore, the substrates were prepared to ensure stability during transport among labs. Nevertheless, freeze-drying and milling alter the physical food

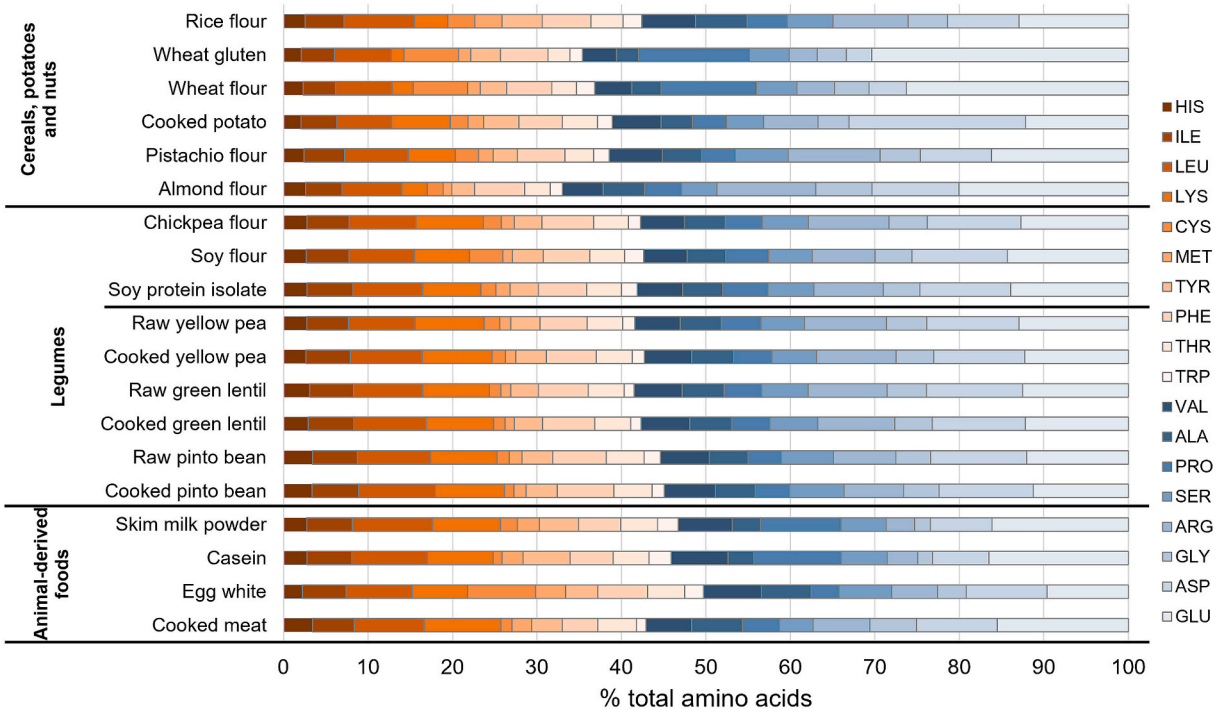


Fig. 1. Amino acid profile of the studied protein sources, expressed as percentage (%) of total amino acids. The orange spectrum represents indispensable amino acids, whereas the blue spectrum represents dispensable amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

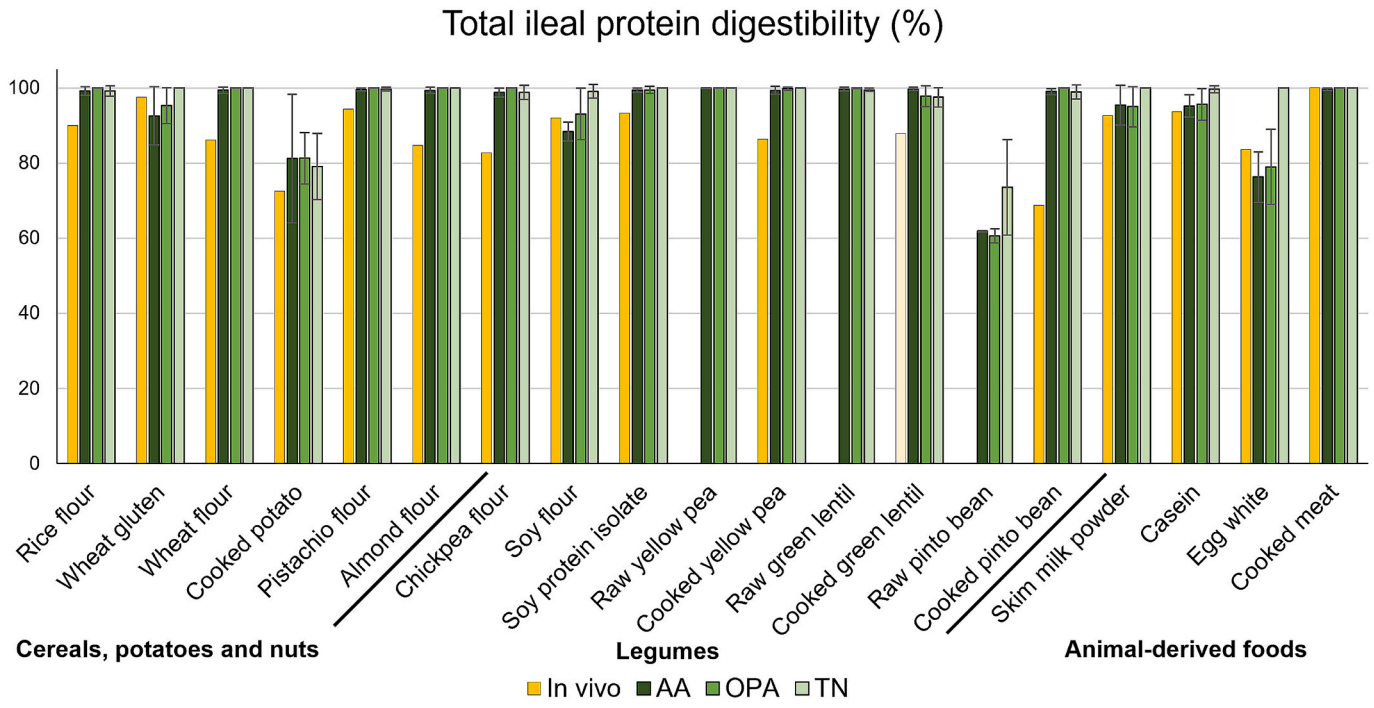


Fig. 2. Total protein digestibility of the studied protein sources expressed as percentage (%). Green bars represent in vitro ileal digestibility calculations based on total amino acids (AA), total amino groups (OPA) or total nitrogen (TN). Error bars indicate the standard deviation of triplicate digestions. Yellow bars represent in vivo data from literature*, determined in humans or pigs (dark yellow) or, when not available, in rats (light yellow). *Rice (white), wheat flour, (freshly) cooked potato, almond (raw, milled), chickpea (boiled and slightly mashed), soy protein isolate, cooked yellow pea (not flour), cooked pinto bean, skim milk powder, casein and egg white (powder): Hodgkinson et al. (2025); rice (white): Cervantes-Pahm et al. (2014); wheat gluten (seitan): Reynaud et al. (2021); wheat flour: Bos et al. (2005), Cervantes-Pahm et al. (2014); pistachio flour (raw, ground): Bailey and Stein (2020); soy flour, soy protein isolate and skim milk powder: Mathai et al. (2017); casein: Guillin et al. (2022); egg white (powder): Kashyap et al. (2018); cooked ground beef: Bailey et al. (2020). No data were found for raw pinto bean. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

matrix and therefore limit the extent to which the results can be extrapolated to foods consumed in their intact form.

On the other hand, potatoes also contain protease inhibitors in a high, although variable concentration, depending on the variety. These form a heterogeneous group of proteins that can mainly inhibit trypsin and chymotrypsin (Pouvreau et al., 2001), thereby interfering with intestinal digestion. Specifically, potatoes are rich in thermally stable protease inhibitors (Ryan et al., 1976), which might be responsible for the limited protein digestibility even after cooking (Bailey et al., 2023). Egg white is also rich in ovomucoid, a highly glycosylated trypsin inhibitor (Abeyrathne et al., 2013). Similarly to raw pinto beans, potato starch forms solutions with very high viscosity (Zaidul et al., 2007), which might have slowed down the protein-protease interactions during digestion.

Taking this into account, crude protein values were corrected for total digestibility (Table 1). For most of the substrates, the content of bioaccessible protein, i.e. protein hydrolyzed during digestion and made potentially absorbable (proxy for bioavailable protein), was ≤1.23% lower than the crude protein calculated from total nitrogen. Larger decreases were observed for raw pinto bean (38.2%), cooked potato (18.8%), soy flour (11.6%) and wheat gluten (7.44%). Among animal sources, the largest decrease corresponded to egg white (23.7%), while skim milk powder and casein proteins were less affected (4.53% and 4.79%, respectively). Altogether, these results underscore the importance of considering not only protein concentration but also compounds that may affect its bioaccessibility, particularly protease inhibitors.

The in vitro results generally aligned with in vivo data reported in literature (Fig. 2), confirming the validity of the method for assessing protein digestibility. However, agreement was the strongest for animal proteins, wheat gluten and soy flour, while other foods showed more pronounced differences between in vivo and in vitro data. On one hand, this could be related to the great variability in protein quantity and amino acid profile among plant species (e.g. Köse et al., 2024; Pepó & Győri, 2007; Stone et al., 2019; Tomičić et al., 2022). On the other hand, samples were differently processed among in vitro and in vivo studies. For instance, pulses in vivo were typically cooked and slightly mashed before feeding pigs (Hodgkinson et al., 2025), whereas in the present study, the foods were freeze-dried and powdered. Almonds were also tested as raw, hammer-milled seeds in vivo (Hodgkinson et al., 2025) but as flours in vitro, which may have influenced digestibility outcomes. In contrast, animal-based foods underwent similar preparation in both in vivo (Bailey et al., 2020; Guillin et al., 2022; Hodgkinson et al., 2025; Kashyap et al., 2018) and in the present study, facilitating comparison. Unfortunately, the actual food substrates studied here have not been tested in vivo to date. Thus, Fig. 2 provides only an overview, and direct comparisons remain limited. Altogether, there is value in assessing in vitro digestibility using the INFOGEST Quant method on intact cooked and masticated/mashed pulses and other plant proteins with subsequent comparisons to in vivo digestion.

Beyond total protein digestibility, assessing the digestibility of individual proteinogenic amino acids is crucial to assess protein quality. In fact, food processing can modify the side chain of certain amino acids, thereby decreasing their bioavailability. Factors like extreme pH, high temperatures, or contact with water can induce oxidation, deamidation, glycation, or non-native amino acid cross-linking, negatively affecting digestibility. Histidine, lysine, methionine, threonine, and tryptophan are particularly reactive (Loveday, 2023).

In this study (Figs. 3–6, Table 2), nuts, raw and cooked legumes (except raw pinto bean), and cooked meat showed nearly complete digestibility (~100%) for most amino acids, with slight reductions in arginine, tyrosine, lysine and sulfur amino acids (cysteine and methionine), and in tyrosine and methionine for cooked meat. Most amino acids were also digested at over 90% in rice, wheat, chickpea flour, and in soy protein isolate, except for tryptophan digestibility in wheat flour (80.9%), and methionine in chickpea flour (82.2%) and soy protein isolate (88.0%). In wheat gluten and soy flour, only histidine and glycine (and aspartic acid in wheat gluten) were 100% digestible, and other amino acids ranged from 83.0% to 95.1% in wheat gluten, and from 59.7% to 93.5% in soy flour. Digestibility of most amino acids in cooked potato was limited (lowest for lysine: 53.3%) with only histidine, methionine, leucine, phenylalanine, and tryptophan exceeding 90%. The least digestible vegetable protein was that of raw pinto beans, with values as low as 22.4% for tryptophan, and only sulfur amino acids exceeded 80% digestibility (cysteine, 89.2%; methionine, 85.3%). Among animal-derived foods other than meat, milk proteins (casein and skim milk powder) exhibited a high digestibility for all amino acids

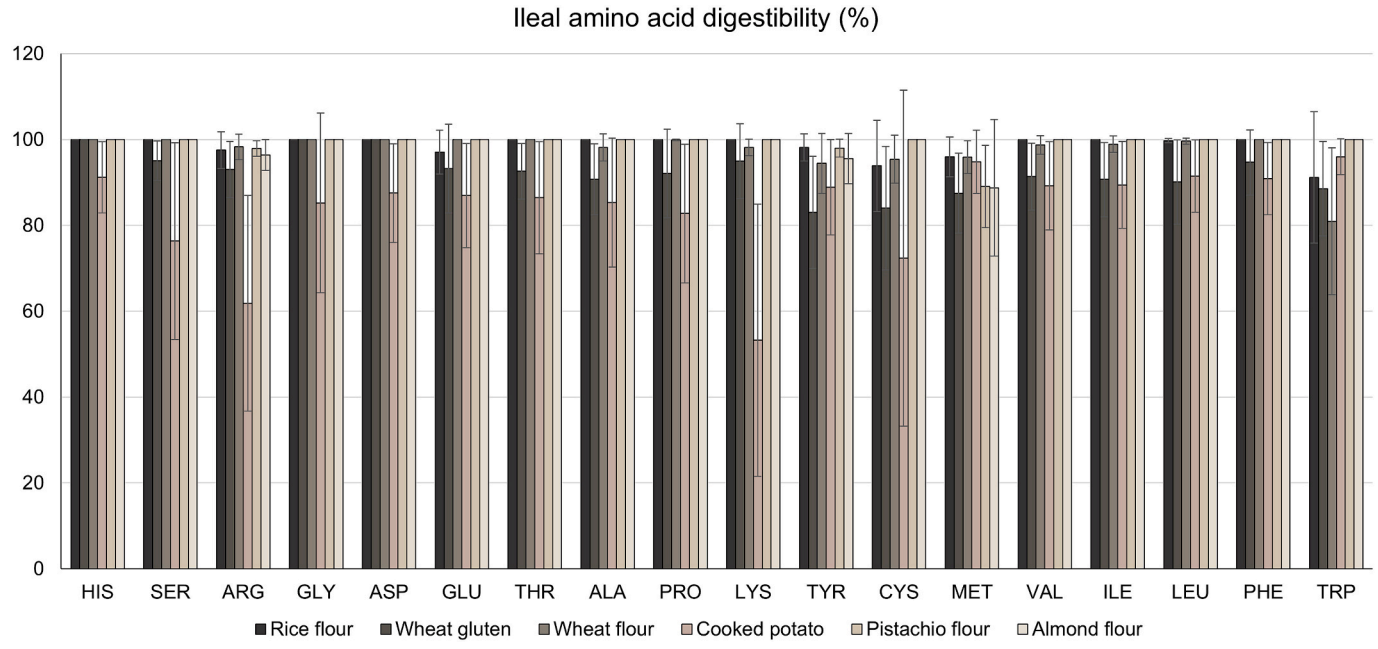


Fig. 3. In vitro ileal digestibility of individual amino acids in cereals, potatoes and nuts, expressed as percentage (%). Error bars represent the standard deviation of triplicate digestions. HIS: histidine; SER: serine; ARG: arginine; GLY: glycine; ASP: aspartic acid; GLU: glutamic acid; THR: threonine; ALA: alanine; PRO: proline; LYS: lysine, TYR: tyrosine; CYS: cysteine; MET: methionine; VAL: valine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TRP: tryptophan.

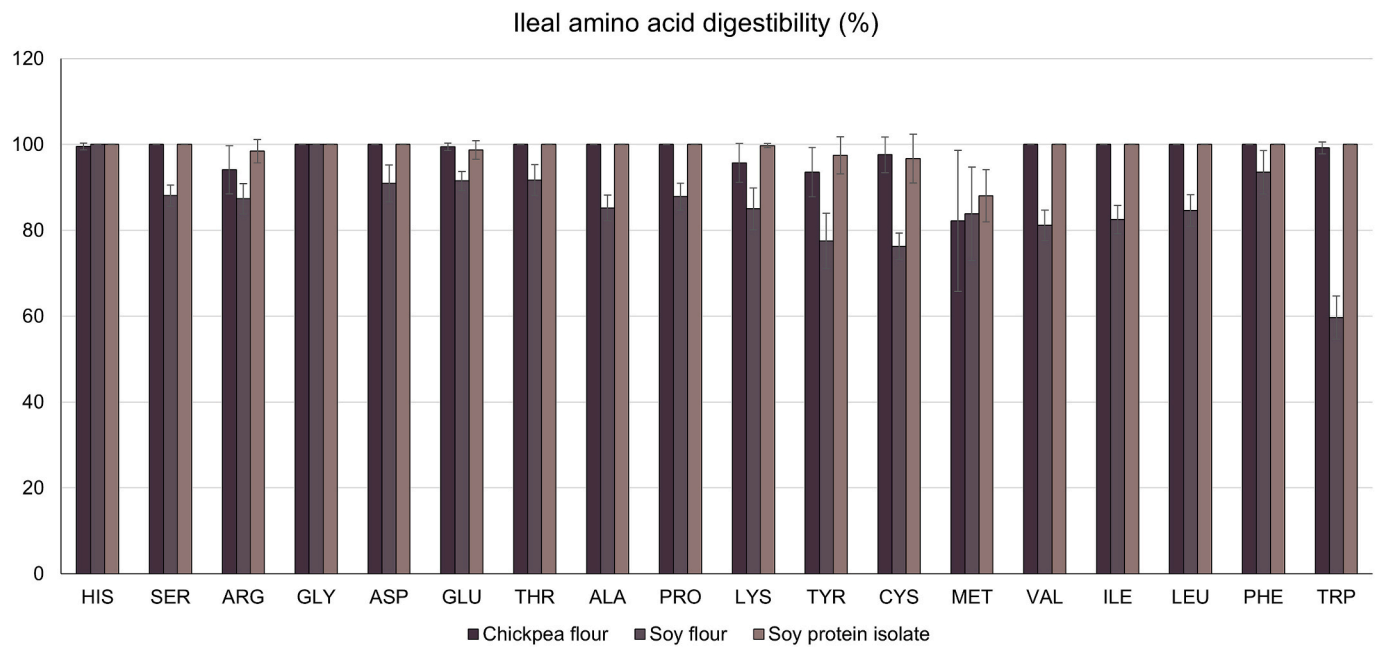


Fig. 4. In vitro ileal digestibility of individual amino acids in processed legumes, expressed as percentage (%). Error bars represent the standard deviation of triplicate digestions. HIS: histidine; SER: serine; ARG: arginine; GLY: glycine; ASP: aspartic acid; GLU: glutamic acid; THR: threonine; ALA: alanine; PRO: proline; LYS: lysine, TYR: tyrosine; CYS: cysteine; MET: methionine; VAL: valine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TRP: tryptophan.

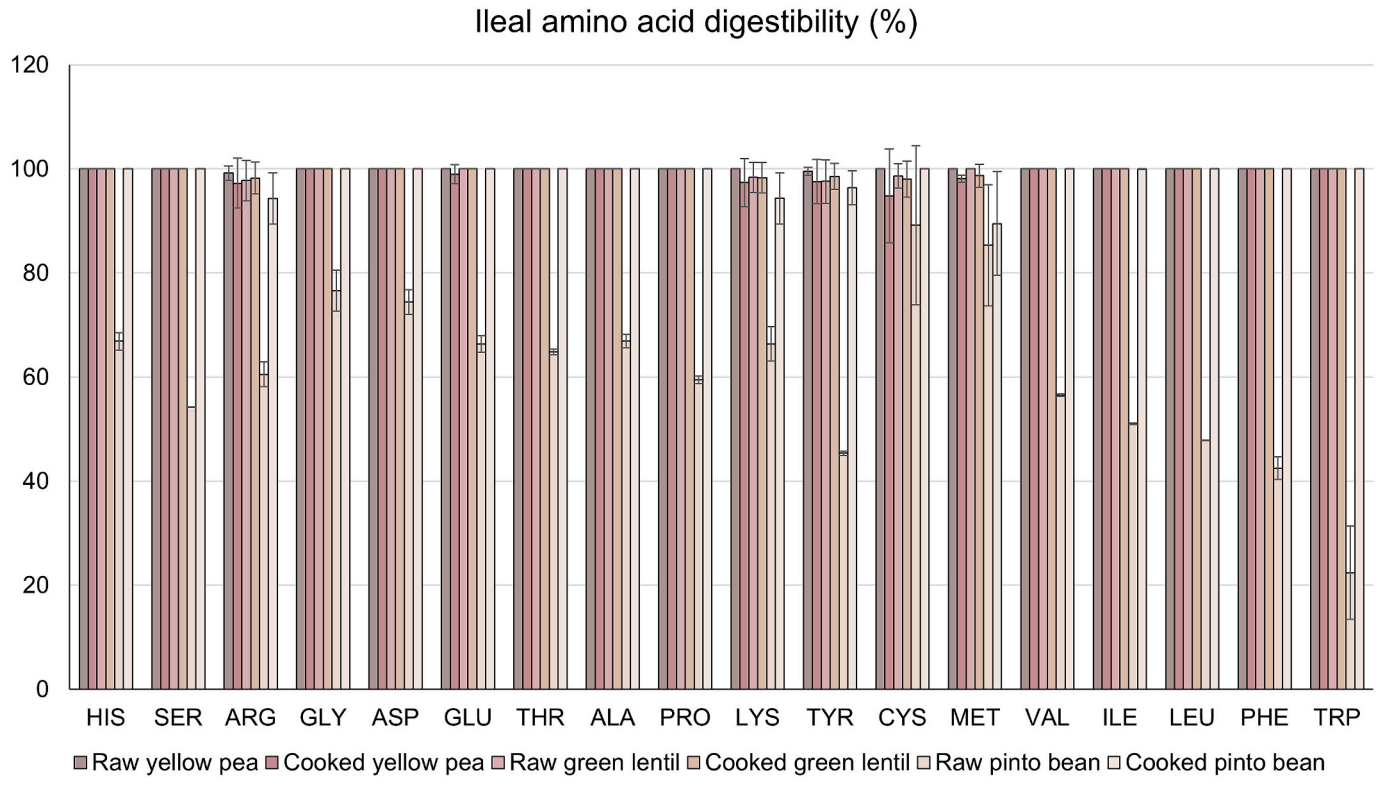


Fig. 5. In vitro ileal digestibility of individual amino acids in raw and cooked legumes, expressed as percentage (%). Error bars represent the standard deviation of triplicate digestions. HIS: histidine; SER: serine; ARG: arginine; GLY: glycine; ASP: aspartic acid; GLU: glutamic acid; THR: threonine; ALA: alanine; PRO: proline; LYS: lysine, TYR: tyrosine; CYS: cysteine; MET: methionine; VAL: valine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TRP: tryptophan.

(over 80%) except for tryptophan (87.2% and 73.2%, respectively). Amino acid digestibility in egg white ranged from 50.5% (tryptophan) to 92.7% (glycine).

3.2.2. *Digestible indispensable amino acid scores (DIAAS)*

DIAAS values of the substrates were calculated based on their amino acid composition and individual amino acid digestibility, using the reference values provided by FAO (2013). DIAAS are shown in Fig. 7, and all DIAAR values (calculated for each indispensable amino acid as a

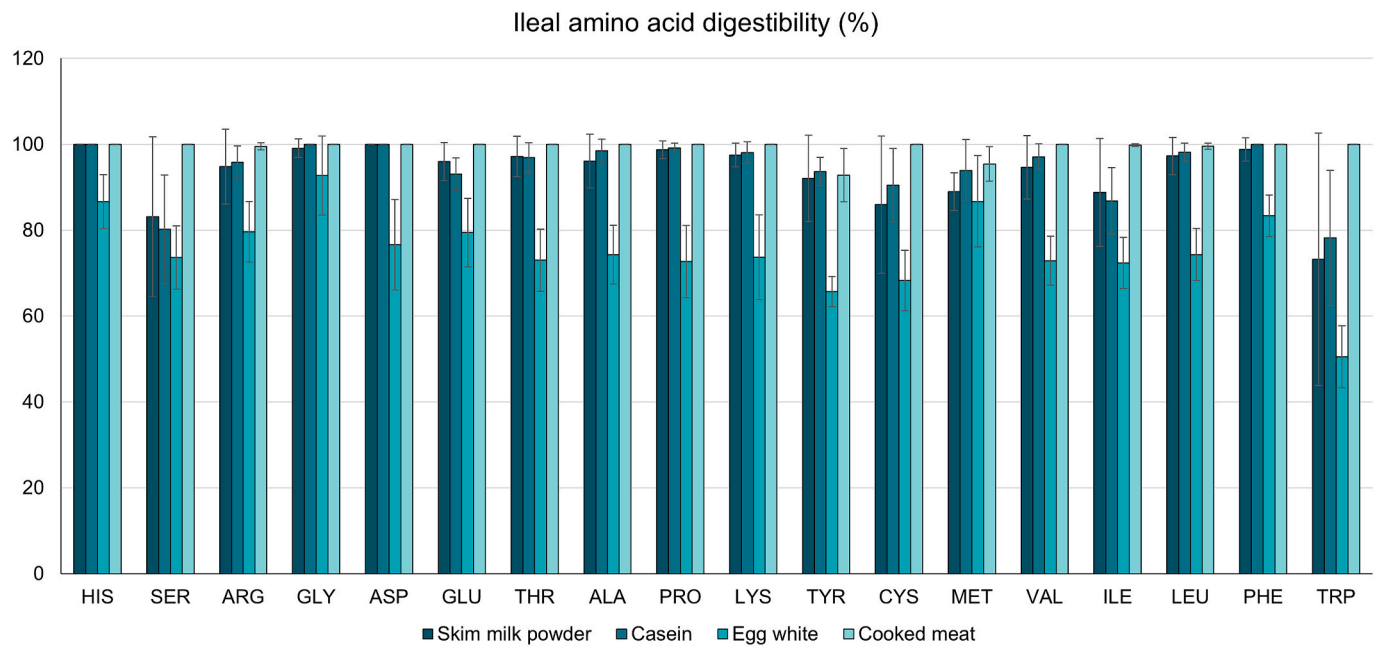


Fig. 6. In vitro ileal digestibility of individual amino acids in animal-derived foods, expressed as percentage (%). Error bars represent the standard deviation of triplicate digestions. HIS: histidine; SER: serine; ARG: arginine; GLY: glycine; ASP: aspartic acid; GLU: glutamic acid; THR: threonine; ALA: alanine; PRO: proline; LYS: lysine, TYR: tyrosine; CYS: cysteine; MET: methionine; VAL: valine; ILE: isoleucine; LEU: leucine; PHE: phenylalanine; TRP: tryptophan.

ratio compared to the reference values) for infants (birth to 6 months), children (6 months to 3 years), and older children, adolescents and adults (over 3 years) are provided in Supplementary material Tables S1–S3. DIAAS (which is the limiting DIAAR value) for cooked meat exceeded 100% for both children (6 months to 3 years) and adults (children above 3 years, adolescents and adults). For soy protein isolate, skim milk powder and casein, in vitro DIAAS were above 100% using the adults' reference pattern. These results confirm that cooked beef and other animal protein sources have high protein quality and can complement limiting amino acids in foods with lower bioaccessibility. Other substrates, including chickpea flour and raw yellow pea, showed DIAAS values above 75% for both children and adults, whereas pistachio flour, cooked yellow pea, and cooked pinto bean exceeded this threshold for adults only.

An important finding was that high total protein digestibility did not necessarily translate into a high DIAAS. Rice flour, wheat flour, almond flour, and raw and cooked green lentils, for example, showed high overall protein digestibility but were limited by the content of individual indispensable amino acids. Similarly, cooked potato, soy flour, raw pinto beans and egg white presented low DIAAS values mainly due to limited protein digestibility. A reduction in the digestibility of the first-limiting amino acid can have a disproportionate effect on DIAAS, even when overall protein digestibility remains high. This distinction illustrates why measurements based only on total nitrogen, amino groups, or total amino acids cannot fully describe protein quality. Determination of individual amino acid digestibility is therefore essential for identifying the specific nutritional limitation of a protein source.

A similar DIAAS distribution was observed among plant and animal proteins by Herreman et al. (2020), except for potato and egg showing higher scores, probably due to an effect of sample processing, as discussed in section 3.2.1 of this article. Mathai et al. (2017) also found higher DIAAS values in animal products compared to plant-based foods. The FAO (2013) has suggested that a DIAAS $\geq$75% using the amino acid reference pattern for children can be used to qualify a food as a "good protein source". However, the discretionary 75% threshold has been questioned as it often excludes plant protein foods that, while having a lower protein quality, can still provide substantial levels of bioaccessible amino acids to diets, and complement other plant and animal foods. In addition, the suggested reference requirements of indispensable amino acids for DIAAS calculation (for non-infant foods) are those for children from 6 months to 3 years, which are higher than those for adults (FAO, 2013). Given this, protein quality values tend to be lower when reference patterns for children are used compared to those for older children and adult populations. The collection of data on protein quality, calculated using various reference standards, can provide important and nuanced insights into protein and amino acid intake when analyzing dietary patterns.

In general terms, the main limiting amino acids differed among food groups: lysine in cereals, potatoes and nuts; leucine, valine, sulfur amino acids and tryptophan in legumes; isoleucine, sulfur amino acids and threonine in dairy proteins; and leucin and lysine in egg. As cooked meat showed DIAAS values above 100% for both children (6 months-3 years) and adults (>3 years), no limiting amino acid was identified for this food. Interestingly, in raw pinto beans, the limiting amino acid was tryptophan instead of sulfur amino acids, although these became limiting after cooking, confirming in vivo data (Calderón de la Barca et al., 2021) and reflecting the very low tryptophan digestibility in the raw product. Tryptophan was also the limiting amino acid for infants in raw and cooked green lentils. Legumes are known for being inherently lower in sulfur amino acids, although substantial variability among pulse species and varieties has been described (Herreman et al., 2020; Nosworthy et al., 2017; Stone et al., 2019). Cereals, on the other hand, are typically limited in lysine (Cervantes-Pahm et al., 2014; Herreman et al., 2020; Stone et al., 2019). Lysine has also been previously identified as the first limiting amino acid in pistachios (Bailey & Stein, 2020) and almonds (House et al., 2019).

It is worthwhile to note that whole legumes (soy and pulses), cereals, and plant-derived flours are not consumed raw and, as previously discussed, they are generally thermally treated before consumption, either through traditional cooking methods or other heat treatments in processed foods. Thermal processing can eliminate most heat labile antinutrients that affect protein digestibility (Patterson et al., 2017). Thus, some of the raw ingredients used in this study would undergo some form of thermal treatment when incorporated into a food product and could demonstrate higher digestibility values.

Milk proteins have previously been described as an excellent protein

Table 2
Digestibility of individual amino acids, expressed as percentage (%, w/w). Large numbers refer to the average value of the triplicate digestions, whereas the small values in parenthesis represent the standard deviations.

	HIS	SER	ARG	GLY	ASP	GLU	THR	ALA	PRO	LYS	TYR	CYS	MET	VAL	ILE	LEU	PHE	TRP
Cereals, potatoes and nuts																		
Rice flour	100	100	97.5	100	100	97.1	100	100	100	100	98.2	93.9	95.9	100	100	99.7	100	91.2
	(0)	(0)	(4.3)	(0)	(0)	(5.1)	(0)	(0)	(0)	(0)	(3.2)	(10.6)	(4.6)	(0)	(0)	(0.5)	(0)	(15.3)
Wheat gluten	100	95.1	93.0	100	100	93.2	92.6	90.7	92.1	95.0	83.0	84.0	87.5	91.3	90.7	90.1	94.7	88.5
	(0)	(4.6)	(6.5)	(0)	(0)	(10.3)	(6.5)	(8.3)	(10.3)	(8.7)	(13.1)	(14.4)	(9.3)	(7.8)	(8.7)	(9.8)	(7.6)	(11.0)
Wheat flour	100	100	98.3	100	100	100	100	98.2	99.9	98.1	94.4	95.4	95.9	98.8	98.9	99.6	100	80.9
	(0)	(0)	(3.0)	(0)	(0)	(0)	(0)	(3.2)	(0.2)	(1.9)	(7.0)	(5.6)	(3.9)	(2.2)	(1.9)	(0.7)	(0)	(17.1)
Cooked potato	91.2	76.3	61.8	85.2	87.5	86.9	86.4	85.3	82.8	53.3	88.9	72.3	94.8	89.2	89.4	91.5	90.9	96.0
	(8.3)	(22.9)	(25.1)	(20.9)	(11.5)	(12.2)	(13.0)	(15.0)	(16.1)	(31.7)	(11.1)	(39.1)	(7.3)	(10.3)	(10.1)	(8.4)	(8.4)	(4.2)
Pistachio flour	100	100	97.9	100	100	100	100	100	100	100	98.0	100	89.0	100	100	100	100	100
	(0)	(0)	(1.8)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(2.1)	(0)	(9.6)	(0)	(0)	(0)	(0)	(0)
Almond flour	100	100	96.4	100	100	100	100	100	100	100	95.5	100	88.7	100	100	100	100	100
	(0)	(0)	(3.6)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(5.8)	(0)	(15.9)	(0)	(0)	(0)	(0)	(0)
Legumes																		
Chickpea flour	99.5	100	94.1	100	100	99.5	100	100	100	95.7	93.5	97.6	82.2	100	100	100	100	99.2
	(0.8)	(0)	(5.6)	(0)	(0)	(0.9)	(0)	(0)	(0)	(4.5)	(5.8)	(4.2)	(16.5)	(0)	(0)	(0)	(0)	(1.4)
Soy flour	100	88.1	87.3	100	90.9	91.5	91.7	85.2	87.8	85.0	77.5	76.3	83.8	81.2	82.5	84.6	93.5	59.7
	(0)	(2.4)	(3.6)	(0)	(4.3)	(2.2)	(3.6)	(3.0)	(3.2)	(4.9)	(6.4)	(3.1)	(10.9)	(3.5)	(3.3)	(3.7)	(5.0)	(5.0)
Soy protein isolate	100	100	98.4	100	100	98.7	100	100	100	99.7	97.5	96.7	88.0	100	100	100	100	100
	(0)	(0)	(2.7)	(0)	(0)	(2.2)	(0)	(0)	(0)	(0.5)	(4.4)	(5.7)	(6.1)	(0)	(0)	(0)	(0)	(0)
Raw yellow pea	100	100	99.2	100	100	100	100	100	100	100	99.6	100	100	100	100	100	100	100
	(0)	(0)	(1.4)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0.7)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Cooked yellow pea	100	100	97.2	100	100	98.9	100	100	100	97.3	97.6	94.8	98.1	100	100	100	100	100
	(0)	(0)	(4.8)	(0)	(0)	(1.8)	(0)	(0)	(0)	(4.6)	(4.2)	(9.0)	(0.7)	(0)	(0)	(0)	(0)	(0)
Raw green lentil	100	100	97.8	100	100	100	100	100	100	98.3	97.6	98.6	100	100	100	100	100	100
	(0)	(0)	(3.9)	(0)	(0)	(0)	(0)	(0)	(0)	(2.9)	(4.2)	(2.3)	(0)	(0)	(0)	(0)	(0)	(0)
Cooked green lentil	100	100	98.2	100	100	100	100	100	100	98.3	98.6	98.0	98.7	100	100	100	100	100
	(0)	(0)	(3.0)	(0)	(0)	(0)	(0)	(0)	(0)	(2.9)	(2.5)	(3.4)	(2.2)	(0)	(0)	(0)	(0)	(0)
Raw pinto bean	66.9	54.2	60.5	76.6	74.4	66.3	64.8	66.9	59.5	66.4	45.4	89.2	85.3	56.5	51.0	47.8	42.5	22.4
	(1.7)	(0.1)	(2.4)	(3.9)	(2.4)	(1.6)	(0.6)	(1.3)	(0.7)	(3.3)	(0.4)	(15.3)	(11.6)	(0.3)	(0.1)	(0.1)	(2.2)	(9.0)
Cooked pinto bean	100	100	94.3	100	100	100	100	100	100	94.3	96.4	100	89.5	100	100	100	100	100
	(0)	(0)	(4.9)	(0)	(0)	(0)	(0)	(0)	(0)	(4.9)	(3.3)	(0)	(10.0)	(0)	(0)	(0)	(0)	(0)
Animal-derived foods																		
Skim milk powder	100	83.1	94.8	99.1	100	96.0	97.1	96.1	98.7	97.5	92.0	85.9	88.9	94.6	88.8	97.3	98.8	73.2
	(0)	(18.7)	(8.7)	(2.2)	(0)	(4.5)	(4.7)	(6.2)	(2.0)	(2.8)	(10.1)	(16.0)	(4.4)	(7.4)	(12.5)	(4.3)	(2.7)	(29.4)
Casein	100	80.2	95.9	100	100	93.0	96.9	98.5	99.1	98.1	93.6	90.5	93.9	97.1	86.8	98.2	100	78.2
	(0)	(12.6)	(3.8)	(0)	(0)	(3.8)	(3.4)	(2.8)	(1.2)	(2.5)	(3.3)	(8.5)	(7.2)	(3.0)	(7.8)	(2.1)	(0)	(15.7)
Egg white	86.7	73.6	79.6	92.7	76.6	79.4	73.0	74.3	72.7	73.7	65.7	68.3	86.7	72.9	72.4	74.3	83.4	50.5
	(6.3)	(7.4)	(7.1)	(9.2)	(10.6)	(8.0)	(7.3)	(6.9)	(8.4)	(9.9)	(3.5)	(7.1)	(10.6)	(5.7)	(6.0)	(6.1)	(4.8)	(7.2)
Cooked meat	100	100	99.5	100	100	100	100	100	100	100	92.8	100	95.4	100	99.8	99.6	100	100
	(0)	(0)	(0.8)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(6.3)	(0)	(4.0)	(0)	(0.3)	(0.7)	(0)	(0)

ALA: alanine; ARG: arginine; ASP: aspartic acid; CYS: cysteine; GLU: glutamic acid; GLY: glycine; HIS: histidine; ILE: isoleucine; LEU: leucine; LYS: lysine; MET: methionine; PHE: phenylalanine; PRO: proline; SER: serine; THR; threonine; TRP: tryptophan; TYR: tyrosine; VAL: valine.

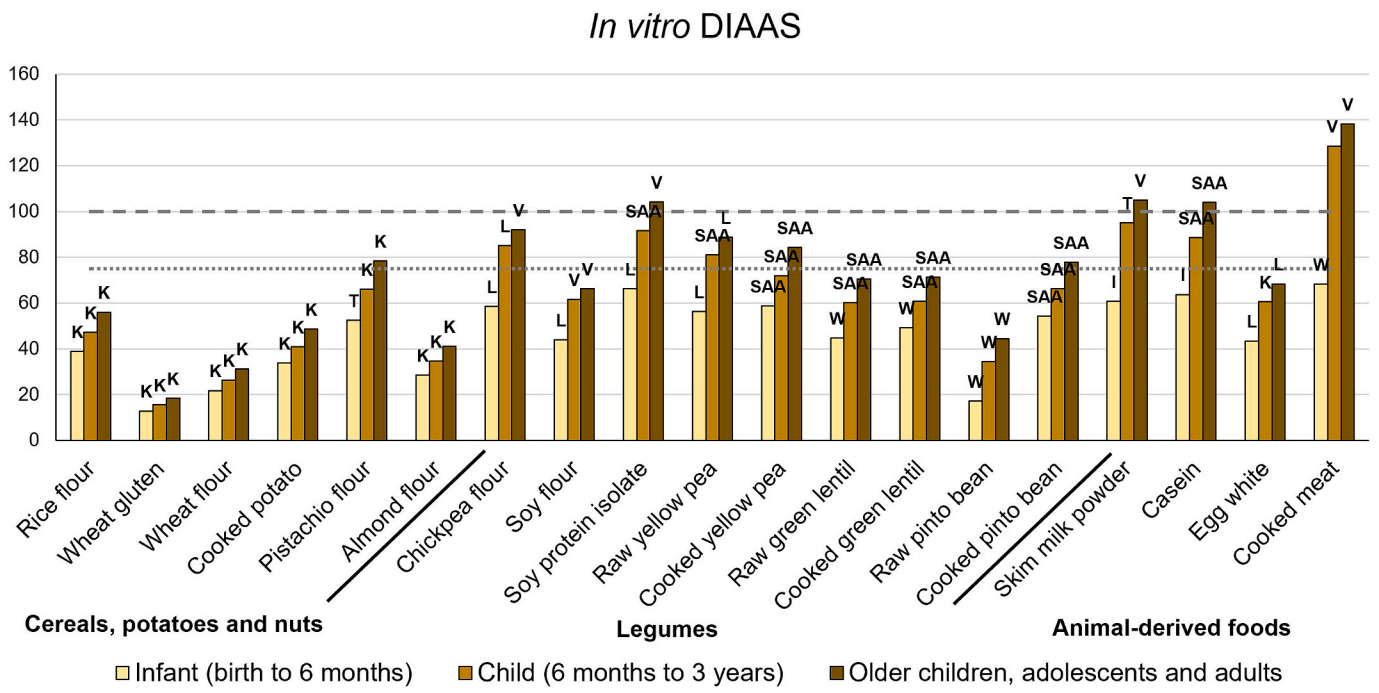


Fig. 7. Digestible indispensable amino acid scores (DIAAS) of the studied substrates for infants (birth to 6 months), children (6 months to 3 years), and older children, adolescents and adults, and the corresponding limiting amino acid (one-letter-coded). Values are expressed as percentage (%) and represent the lowest average digestible indispensable amino acid ratio (DIAAR) calculated from triplicate digestions. Dotted horizontal lines represent the theoretical threshold for classifying dietary proteins as "good" (75%) or "excellent" (100%) according to FAO (2013). I: isoleucine; K: lysine; L: leucine; SAA: sulfur amino acids (methionine and cysteine); T: threonine; V: valine; W: tryptophan.

source with DIAAS values exceeding 100% in vivo (Mathai et al., 2017), contrasting with our in vitro results. Likewise, Fanelli et al. (2024) reported higher standardized ileal digestibility values and DIAAS over 100% in fried, boiled and scrambled whole eggs measured in pigs compared to the present study. These differences could be related to physical matrix effects or to sample processing differences that might have affected amino acid digestibility, underscoring the importance of using the identical food matrix for in vitro-in vivo comparisons. Therefore, the low digestibility of egg white powder cannot be extrapolated directly to cooked whole. Sousa, Portmann, et al. (2023) also reported DIAAR values above 100% in beef burgers measured in vitro, whereas in vivo pig studies found slightly lower DIAAS values for children (92%, leucine) and adults (99%, leucine) (Bailey et al., 2020).

Although the present experiments were not designed to identify the

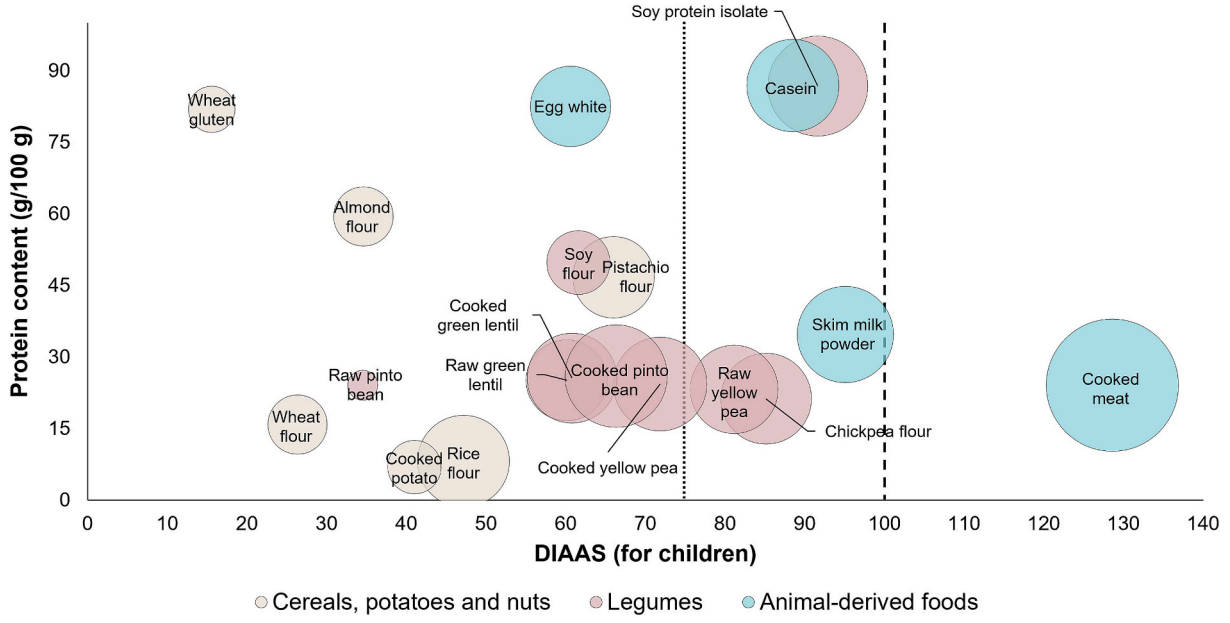


Fig. 8. Bubble chart summarizing the overall quality of the studied dietary protein sources. In vitro DIAAS values for children (6 months to 3 years) are represented in the x-axis, total protein content of the food, expressed as g protein/100 g food, is represented in the y-axis, and bubble size represents the total digestible indispensable amino acid content of the food normalized according to the formula: [$x_{normalized}$ = (x - $x_{minimum}$)/range of x], where x = [mg amino acid/g protein]. Food groups are color-coded, and DIAAS 75 and 100 are indicated with dotted vertical lines.

processing variables responsible for these differences, the results demonstrate that apparently similar protein sources can yield distinct amino acid digestibility profiles. Matrix-matched comparisons and a more detailed characterization of processing history would therefore strengthen future in vitro to in vivo comparisons.

3.2.3. General protein quality evaluation

Fig. 8 provides a visual overview of the protein quality of all the substrates studied, based on their in vitro DIAAS for children (x-axis), total crude protein content (y-axis), total content of digestible indispensable amino acids (bubble size), and product category (color). The same figure based on amino acid requirements for adults is given in Supplementary material Fig. S1. Confirming the previous results, the figure clearly illustrates that animal-derived foods exhibit the highest protein quality, legumes occupy an intermediate position, and cereals, potatoes and nuts show the lowest quality. Cooked meat stands out as the best protein source due to its high bioaccessibility of indispensable amino acids, despite its lower total protein content compared with some other substrates. Casein and soy protein isolate are represented as good protein sources, mainly due to their high protein content and digestible indispensable amino acid availability relative to requirements, although both contain certain limiting amino acids. Skim milk powder is also a great source of digestible indispensable amino acids but provides a limited protein amount per 100 g of food. In contrast, egg white, while rich in total protein, provides a comparatively limited amount of digestible indispensable amino acids. All legumes (excluding soy protein isolate) present a similar overall quality, with relatively low protein concentrations and intermediate DIAAS values. The exception was raw pinto bean, which was limited in most indispensable amino acids in terms of both total content and digestibility. Soy flour contained slightly more protein than other legumes but exhibited a lower proportion of digestible indispensable amino acids. Among the remaining plant-based foods, pistachio flour emerged as the best option, combining a relatively high DIAAS with a higher total content of digestible indispensable amino acids. Other plant sources were generally low either in total protein concentration or in digestible indispensable amino acids, resulting in an overall lower protein quality.

Fig. 8 also highlights that DIAAS and protein concentration describe different dimensions of nutritional value. DIAAS expresses the capacity of one gram of protein to meet indispensable amino acid requirements, whereas protein content and the amount of digestible indispensable amino acids per 100 g describe the quantity provided by the food. Accordingly, a protein-rich ingredient may remain limited by one indispensable amino acid, as illustrated by wheat gluten, while a product with a lower protein concentration may provide a more favorable amino acid balance. These measures should therefore be interpreted together, particularly when comparing foods or formulating products, rather than using a single value to rank protein sources.

Until now, protein quality has been analyzed for individual substrates, identifying a limiting amino acid for entire product groups, such as lysine in cereals or sulfur-containing amino acids in most legumes. However, entire meals containing multiple protein sources are consumed on a daily basis. Analyzing these combined sources using the same methodological approach will reveal how protein quality changes for these combinations (Herreman et al., 2020; Moughan & Lim, 2024).

Finally, the present results characterize the specific products and preparation conditions investigated and should not be interpreted as category-wide reference values. Differences among varieties, commercial batches, processing conditions, and particle sizes may influence both amino acid composition and digestibility. The replicate digestions characterize within-study methodological variability but do not capture the broader variability among products available on the market. Future studies including multiple products or batches and standardized, matrix-matched processing conditions would help determine the generalizability of the observed protein quality patterns.

4. Conclusion

The present study shows that animal foods have the highest in vitro DIAAS, except for freeze-dried egg white, which showed low digestibility, likely due to matrix effects or chemical modifications during processing. Values for legumes were moderate, while cereals, nuts and potatoes exhibited low protein concentrations and/or low indispensable amino acid contents, with potatoes also showing limited protein digestibility. Cooking pinto beans significantly improved protein digestibility. These results mirror similar experimental data using in vivo models. Overall, these findings highlight the importance of assessing not only protein concentration but also protein quality, including amino acid composition and digestibility, when evaluating dietary protein sources and formulating nutritional recommendations. Results support the adoption of in vitro methods for protein quality assessments, which could be particularly useful in regions that require protein quality analysis on a routine basis to validate “source of protein” claims. Further studies should investigate in vitro digestibility of intact food and entire meals to better mimic real consumption conditions.

CRediT authorship contribution statement

Ana Blanco-Doval: Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Raquel Sousa:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Sébastien Dubois:** Formal analysis. **Erin M. Goldberg:** Writing – review & editing, Resources, Project administration. **Mingyan Jing:** Writing – review & editing, Project administration, Methodology. **Adam J. Franczyk:** Writing – review & editing. **Christopher P.F. Marinangeli:** Writing – review & editing, Funding acquisition, Conceptualization. **Elaine S. Krul:** Writing – review & editing. **James D. House:** Resources, Funding acquisition, Conceptualization. **Reto Portmann:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Lotti Egger:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christopher P.F. Marinangeli is an employee of Protein Industries Canada and former employee of Pulse Canada and Kellogg Canada.

Christopher P.F. Marinangeli serves on the protein committee for the Institute of Food and Nutrition Science.

He was not involved in the generation of the data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.121110.

Data availability

Data will be made available on request.

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