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Nutritional properties of common bean protein concentrate compared to commercial legume ingredients for the plant-based market

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ABSTRACT

There is an enormous demand to develop new sources of proteins, mainly to supply the growing plant-based food market worldwide, with the push for more sustainable and healthier products. The objective of this study was to evaluate the composition and the nutritional properties of commercial soybean, pea, and fava bean protein ingredients and compare them with an in-house ingredient (flour and protein concentrate), obtained from the main Brazilian cultivar of common bean (*Phaseolus vulgaris*, Pinto bean). The protein content of the common bean concentrate (79.75%) was as high as other commercial proteins isolated from the pea and higher than the others concentrates. All the ingredients presented the minimum amounts of indispensable amino acids as required by FAO and all ingredients were rich in lysine and leucine, with the highest amounts found for pea (78.06 mg/g) and common bean (86.70 mg/g) concentrates. A diverse mineral composition was reported for all the ingredients and the common bean concentrate presented the highest iron content (342.6 mg/kg). In terms of antinutritional factors, the common bean flour and concentrate showed the highest values for trypsin inhibitor (18 and 27 TIU/mg, respectively) but the lowest ones for phytic acid (9 and 2 mg/g, respectively) compared to the other ingredients. Low amounts of oligosaccharides were found in most of the samples. All proteins from the ingredients were highly digested when evaluated *in vitro*, but phaseolins fraction protein from common bean samples remained partially undigested. Despite compositional differences between ingredients, all samples should be suitable as protein sources for plant-based food innovation.

1. Introduction

Many proteins used as ingredients in the food industry are derived from animals such as cow's milk, eggs, and meat from different animal species. However, there is a growing push to partially substitute these products with plant-based alternatives (Bessada et al., 2019; Boye et al., 2010). Soybean and soybean ingredients have been used thought many years as the main protein source for plant-based products, but consumers are restricting their use on food products due to health concerns such as soy allergy, isoflavones and GMO (de Paiva Gouvêa et al., 2023). In this regard, one of the approaches involves the use of innovative pulse-derived proteins, which can be suitable in the development of new food preparations resembling those of animal-origin, known to be "plant-based food products" (Kaushal et al., 2012; Ma et al., 2011; Pedrosa et al., 2020). Proteins extracted from pulses have been gaining prominence over animals' proteins due to their better sustainability rates, alignment with animal-friendly groups in terms of animal suffering, and nutritional concerns (Kumar et al., 2022).

As per the definition provided by FAO, the term "pulses" specifically refers to dry edible seeds from legumes that have a low-fat content (FAO, 2007) which includes various crops such as peas, diverse types of beans, fava beans, chickpeas, lentils, lupins, and others (Boye et al., 2010). From a nutritional standpoint, pulses are abundant sources of various essential components necessary for human health. These include protein, carbohydrates, and dietary fiber, as well as a range of vitamins and minerals (Bessada et al., 2019). Common beans (*Phaseolus vulgaris* L.) are cultivated and consumed on a worldwide basis and the most popular type in Brazil, the cultivar carioca, also known as pinto bean, represents

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almost 70% of the domestic market (Boye et al., 2010; Los et al., 2020).

Protein nutritional quality is a function of protein digestibility and indispensable amino acid composition that match the established standards (Sá et al., 2019). In terms of protein composition, legume proteins are considered good sources of leucine and lysine as indispensable amino acids, but are incomplete due to their lower levels of essential sulfur-containing amino acids such as methionine and tryptophan (Kumar et al., 2022). The digestibility is related with the legumes' tighter proteins ultrastructure associated to the linkages of antinutritional factors and dietary fibers that may negatively affect the protein digestible rate when compared to animal sources of proteins (Bessada et al., 2019; De Angelis et al., 2021; Khattab et al., 2009; Ohanenye et al., 2020).

One challenge associated with legumes is the presence of antinutrients, which impact their consumption. These compounds include enzyme inhibitors, phytic acid, oligosaccharides that promote flatulence, among others. Some of them may be inactivated or eliminated from the grains, by using simple culinary strategies such as applying heat or food processing, such as soaking the grains before cooking or fractioning the protein from the whole grain (Avilés-Gaxiola et al., 2018). The application of these techniques reduces the concentration of the antinutritional factors, making the bioactive compounds more bio accessible (Hall and Moraru, 2021).

Pulses proteins for the plant-based market are frequently accessible in the form of flours, concentrates, and isolates and the choice of the ingredient is not an easy task by the industry, as the ingredients have to present good technological performance and sensory profile, combined with the availability of the ingredient at an affordable cost and a favorable nutritional aspect.

In this sense, we have previously published a study on the technological properties of an in-house common bean flour and protein concentrate in comparison to other commercially available legume ingredients and could state that this source of pulse ingredient was technologically suitable for the market, and they could be an alternative source of protein to be locally produced and consumed in Brazil (de Paiva Gouvêa et al., 2023). The next question to be answered is to know if this in-house bean ingredient is nutritionally comparable to the same commercially available ingredients.

Evaluating these ingredients from a nutritional perspective is critical for the development of products that meet consumer's nutritional demands. To that end, this study aimed to assess the composition and nutritional properties of legume protein ingredients available on the market, as well as to compare them to the in-house common bean flour and protein concentrate, as a national alternative protein to the plant-based market.

2. Materials and methods

2.1. Materials

Six commercial protein ingredients available in the Brazilian market were used: soy protein isolate (SPI), soy protein concentrate (SPC), pea protein isolate (PPI), pea protein concentrate (PPC), clean taste fava bean protein concentrate (FBPC), and fava bean flour (FBF).

Whole grains without defects of common beans (*Phaseolus vulgaris*, Pinto bean) were donated from a local producer and ground in a Perten LM3100 hammer mill with a 0.8 mm sieve opening (Perten Instruments AB, Huddinge, Sweden) to obtain the common bean flour (CBF). The common bean protein concentrate (CBPC) was obtained by alkaline extraction followed by acid precipitation and was spray-dried according to Lima et al. (2023). All ingredients were stored in a cold chamber at 8 °C (±2 °C) until use.

Based on the information provided by the suppliers combined with physical and chemical analyses from our previous study with these ingredients, it was possible to trace the probable processing steps to obtain the tested ingredients, which were by (i) wet extraction followed by spray-drying for SPI, SPC, PPI, and CBPC; (ii) grinding followed by air classification and some further processing for ingredients PPC and FBPC, and (iii) grinding for CBF and FBF.

2.2. Proximate composition

The proximate composition of the ingredients was determined according to official AOAC (2010), as follows: moisture (925.09), ash (923.03), total fiber (985.29), protein (001.11) with correction factor of 6.25 x N, and fat with automatic extraction (Am 5.04) according to AOCS (2009). Total carbohydrate was calculated by difference.

2.3. Mineral profile

The cavity microwave-assisted method 999.10 (AOAC, 2012) was used for mineralization and quantification by ICP-OES method 990.08 (AOAC, 2012) with some modifications. Briefly, 0.5–0.6 g of each sample was weighed directly into XPress type PFA® digestion tubes (CEM, United States) and 6 mL of 69% nitric acid EMSURE® (ACS, Ph Reag. Eur, MERCK KGaA, Germany). The samples were digested in a cavity microwave, model MARS5 (CEM, United States) with maximum power of 1600 W, heating ramp of 20 min–180 °C, and plateau of 180 °C for 20 min. The digest was quantitatively transferred to a 50 mL volumetric flask, completing the volume with ultrapure water. Quantifications of the elements Na, K, Ca, Mg, Mn, P, Fe, Zn, and Cu were conducted on an inductively coupled argon plasma optical emission spectrometer (ICP-OES), model Optima 2100DV (PerkinElmer, United States), cyclonic nebulization chamber and concentric nebulizer, with sequential optics and dual-view torch visualization. The equipment conditions were: RF Power (W) 1300; nebulizer flow (L/min) 0.60; plasma flow (L/min) 15; sample flow (L/min) 1.50; Concentric MEINHARD® Type C nebulizer; MEINHARD® Cyclonic (glass) nebulization chamber.

2.4. Anti-nutritional factors

The oligosaccharides were extracted with water under heating and agitation (250 rpm/55 °C) for 30 min, and then the proteins were precipitated with the addition of acetonitrile and centrifugation for 15 min, 6000 rpm. Then quantification was performed by HPLC (Mobile phase 60% acetonitrile in water for raffinose, stachyose, and verbascose; column at 40 °C, flow 1.4 mL/min) (Macrae, 1998). Trypsin was extracted and quantified by method Ba 12–75 (AOCS, 2009). Phytic acid was extracted and quantified by method 986.11 (AOAC, 2010) with some modifications. The collection of phytates is done with a 2.0 M HCl solution in a 25 mL volumetric flask and direct reading of phosphorus (P) by IPC (inductively coupled plasma). The result is expressed in Phytates (mg/g).

2.5. Total amino-acids

The analysis was performed according to Liu et al. (1995), and method 994.12 (AOAC, 2010). The protein hydrolysis was done as acid hydrolysis (HCl, 6M), basic hydrolysis (NaOH, 4.2M) for the quantification of tryptophan, and prior oxidation (performic acid) and subsequent acid hydrolysis to the quantification of sulfur amino acids. The hydrolyses were done in glass ampoules sealed under vacuum and kept at 110 °C for 20 h. The separation of tryptophan was performed on a C18 column with fluorometric detection. Sulfur amino acids and those resistant to acid hydrolysis were derivatized with 6 aminoquinolylsuccimidyl-carbamate (AQC), separated by reversed-phase liquid chromatography and detected by fluorescence.

2.6. In vitro digestion

The simulation of *in vitro* gastrointestinal digestion was performed in

3 stages (oral, gastric, and enteric stages), as proposed by the international consensus Infogest (Minekus et al., 2014; Brodkorb et al., 2019) with some modifications. The powder ingredients were pre-hydrated until a thick porridge with similar visual viscosity was obtained. To achieve similar visual consistency, different concentrations were used, as follows (g of ingredient/g of slurry): SPI 0.2 g/g, SPC 0.25 g/g; PPI 0.25 g/g, PPC 0.4 g/g; FBPC 0.4 g/g, FBF 0.5 g/g; CBPC 0.33 g/g, CBF 0.5 g/g. Corrections to the results were made based on these concentrations.

Then, 5 g from each hydrated ingredient was submitted to digestion, at 37 °C. The oral phase was performed by using salivary amylase, in a 2:1 ratio, for 2 min. Afterwards, the material was submitted to the gastric phase, performed with pepsin PA, pH 2.0, for 2h, followed by the intestinal phase, at pH 7.5, in the presence of pancreatin and bile acids, for 2h. After digestion, the samples were centrifuged at 9500 rpm, for 15 min and the supernatant (bio accessible extract) was frozen until analysis. The quantification of soluble protein before and after the digestion was performed according to Bradford (1976) and aromatic amino acids, by Chang-Lee et al. (1989).

2.7. Characterization of protein fractions

The PROTEAN II xi cell electrophoresis system from the BIO-RAD brand was used and the gel preparation is described by Laemmli (1970). For the electrophoresis analysis, 2 mg of each of the ingredients were used, added of 1 mL of the sample buffer solution (TRIS-HCl; sodium dodecyl sulfate (SDS); glycerol; mercaptoethanol; bromophenol blue). For the digested samples, 200 μL of the supernatant after enteric digestion was taken and 100 μL of sample buffer solution was added. Aliquots of 30 μL of the samples were applied to a polyacrylamide gel at a concentration of 12%, for 8 h under a voltage of 100V. The low range and high range standards are from BIO-RAD, as follows: Phosphorylase b (104.856 kDa), BSA (82.345 kDa), ovalbumin (47.489 kDa), carbonic anhydrase (33.620 kDa), soybean trypsin inhibitor (27.118 kDa) and lysozyme (17.543 kDa) for low range; Myosin (201653 kDa), B-galactosidase (114505 kDa), BSA (82174 kDa) and ovalbumin (46906 kDa) for high range.

2.8. Statistical analysis

Results were subject to the Analysis of Variance (ANOVA) and Tukey's test, with a significance level of 5% to identify differences between means in composition, physicochemical and functional properties using the STATISTICA software, version 7.0 (StatSoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Proximate composition of ingredients

The proximate composition of the ingredients is shown in Table 1. As expected, the protein isolates SPI and PPI showed the highest protein content (85.44% and 79.90%, respectively) when comparing all the ingredients. Bean concentrate (CBPC) also presented a high protein value (79.75%), despite being produced only by alkaline extraction followed by acid precipitation, with no further fractionation processes. All the other concentrates presented lower amounts of proteins. The concentrate from soybeans (SPC) presented 69.37% protein, while the ones from pea (PPC) and fava beans (FBPC) had 50.30% and 58.10%, respectively. The flours from fava beans (FBF, 31.26%) and common beans (CBF, 21.83%) were much lower in protein content, as expected.

The ingredients showed a low percentage of lipids varying between 0.34 % (SPI) and 3.53 % (PPC). Pulses are already low lipid sources of grains and soybeans as an oleaginous source of legume; usually undergo a prior defatting process, so the low-fat values were expected.

Both flours exhibit high percentages of carbohydrate (CBF – 51.21%; FBF – 56.37%) and, among the protein ingredients; it ranged from 9.07 % (CBPC) and 22.84 % (PPC). Dietary fiber content also differed among the samples with CBF having the highest amount of fiber (21.23%) and SPI with the lowest fiber content (0.69%). CBPC exhibited a lower amount of fiber, carbohydrate, and lipid compared to CBF. Wet fractionation processes are considered efficient routes to concentrate protein, significantly reducing the amount of fiber and carbohydrates and the success of it will depend on the parameters and conditions used (Eze et al., 2022).

Table 1
Proximate composition of the ingredients.

Samples	Proximate composition (%, dry basis)				
	Fiber	Ashes	Protein	Lipid	Carbohydrate
SPI	0.69 ± 0.08^{h}	4.35 ± 0.11^{b}	85.44 ± 2.75^{a}	0.34 ± 0.13^{d}	9.18 ± 2.83^{e}
SPC	11.83 ± 0.41^{d}	6.15 ± 0.07^{a}	69.37 ± 0.80^{c}	0.83 ± 0.12^{d}	11.82 ± 1.24^{e}
PPI	2.98 ± 0.24^{g}	4.62 ± 0.03^{b}	79.90 ± 0.90^{b}	0.49 ± 0.06^{d}	12.01 ± 0.60^{e}
PPC	17.45 ± 0.08^{b}	5.88 ± 0.09^{a}	50.30 ± 0.65^{e}	3.53 ± 0.30^{a}	22.84 ± 0.53^{c}
CBPC	6.75 ± 0.30^{f}	1.76 ± 0.03^{d}	79.75 ± 0.69^{b}	2.67 ± 0.33^{b}	9.07 ± 0.80^{e}
CBF	21.23 ± 0.34^{a}	3.96 ± 0.25^{c}	21.83 ± 0.32^{g}	1.78 ± 0.13^{c}	51.21 ± 0.36^{b}
FBPC	14.81 ± 0.57^{c}	5.92 ± 0.05^{a}	58.10 ± 0.26^{d}	2.62 ± 0.28^{b}	18.56 ± 0.75^{d}
FBF	7.97 ± 0.59^{e}	3.68 ± 0.02^{c}	31.26 ± 0.17^{f}	0.72 ± 0.09^{d}	56.37 ± 0.73^{a}

Different letters, in the same column, mean significant difference (Tukey test, $p \leq 0.05$). SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; PPC: pea protein concentrate; CBPC: common bean protein concentrate; CBF: common bean flour; FBPC: fava bean protein concentrate; FBF: fava bean flour.

3.2. Mineral profile

The macro and microminerals from the ingredients are presented in Table 2. The mineral composition varied greatly among the eight samples. The range of each mineral evaluated was: sodium (9823.3–31.7 mg/kg), potassium (19356.2–988.4 mg/kg), magnesium (2625.5–290.2 mg/kg), calcium (6242.4–614.8 mg/kg), manganese (30.6–9.5 mg/kg), iron (342.2–55.4 mg/kg), zinc (103.7–14.0 mg/kg), copper (21.3–7.7 mg/kg) and phosphorus (9760.9–3698.3 mg/kg).

Obtaining CBPC significantly reduced the amount of minerals by comparing it to the beans flour (CBF), but an increase in sodium (CBF – 31.7 mg/kg; CBPC – 1899.5 mg/kg) and iron (CBF – 55.4 mg/kg; CBPC – 342.2 mg/kg) were observed. The wet extraction process used to obtain protein concentrates and isolates ends up removing most of the minerals originally present in the raw materials. However, it increases sodium content due to salt formation (Kornet et al., 2021a), as also observed in protein isolates from pea and soybean (9823.3 mg/kg and 8948.4 mg/kg respectively) and soybean and common bean concentrates (6168.3 mg/kg and 1899.5 mg/kg). The flours and other dried-fractionated ingredients were lower in sodium.

Considering the macrominerals, those needed by the body in concentrations higher than 100 mg/day (Farag et al., 2023), potassium was the most abundant element found in PPC (19312.6 mg/kg), FBPC (19180.3 mg/kg), FBF (19356.2 mg/kg), CBF (12476.5 mg/kg), and SPC (10889.3 mg/kg). PPC and FBPC presented the highest amount of magnesium (2625.5 mg/kg and 2568.5 mg/kg respectively), while SPC had the highest concentration of calcium (6242.4 mg/kg), and PPI for phosphorus (9760.9 mg/kg).

For microminerals, those needed by the body on a concentration lower than 100 mg/day, PPC had the highest concentration for manganese (30.6 mg/kg), CBPC had the highest concentration for iron

Table 2
Mineral content of the ingredients.

	Macrominerals (mg/Kg)					Microminerals (mg/Kg)			
Samples	Sodium	Potassium	Magnesium	Phosphorus	Calcium	Manganese	Iron	Zinc	Copper
SPI	8948.4 ± 112.0^{b}	988.4 ± 22.2^{f}	441.6 ± 8.3^{f}	6826.4 ± 58.2^{e}	3651.1 ± 51.4^{b}	8.6 ± 0.1^{e}	118.2 ± 7.3^{c}	24.3 ± 0.2^{e}	10.0 ± 0.1^{e}
SPC	6168.3 ± 109.9^{c}	10899.3 ± 93.8^{c}	1871.6 ± 36.0^{c}	7459.1 ± 75.2^{d}	6242.4 ± 12.1^{a}	24.6 ± 0.6^{b}	114.2 ± 2.4^{c}	62.2 ± 0.9^{c}	11.4 ± 0.5^{d}
PPI	9823.3 ± 98.6^{a}	4622.9 ± 56.3^{d}	831.4 ± 19.5^{e}	9760.9 ± 45.2^{a}	614.8 ± 5.1^{g}	11.7 ± 0.1^{d}	177.4 ± 0.3^{b}	86.0 ± 0.9^{b}	7.7 ± 0.0^{f}
PPC	97.7 ± 40.7^{e}	19312.6 ± 171.3^{a}	2625.5 ± 14.1^{a}	8210.2 ± 26.3^{c}	979.3 ± 6.0^{e}	30.6 ± 0.2^{a}	70.0 ± 0.7^{d}	63.2 ± 0.1^{c}	14.0 ± 0.2^{c}
CBPC	1899.5 ± 48.7^{d}	2795.3 ± 11.4^{e}	290.2 ± 9.3^{g}	3698.3 ± 14.3^{g}	686.2 ± 6.8^{f}	20.0 ± 2.0^{c}	342.2 ± 3.2^{a}	38.3 ± 8.3^{d}	16.2 ± 0.6^{b}
CBF	31.7 ± 12.3^{e}	12476.5 ± 92.6^{b}	1669.4 ± 13.5^{d}	3982.5 ± 34.8^{f}	1389.9 ± 36.6^{c}	9.5 ± 0.1^{e}	55.4 ± 0.7^{e}	14.0 ± 0.3^{f}	8.0 ± 0.1^{f}
FBPC	168.6 ± 2.6^{e}	19180.3 ± 141.0^{a}	2568.5 ± 45.6^{ab}	9048.1 ± 40.1^{b}	1083.1 ± 5.3^{d}	24.1 ± 0.3^{b}	68.8 ± 0.6^{d}	103.7 ± 0.3^{a}	21.3 ± 0.2^{a}
FBF	171.0 ± 8.0^{e}	19356.2 ± 58.2^{a}	2533.9 ± 27.3^{b}	9111.9 ± 68.7^{b}	1095.7 ± 13.4^{d}	24.3 ± 0.2^{b}	70.5 ± 2.8^{d}	103.1 ± 2.2^{a}	21.3 ± 0.3^{a}

Different letters in the same column mean significant differences between samples (Tukey test, $p \leq 0.05$). SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; PPC: pea protein concentrate; FBPC: fava bean protein concentrate; FBF: fava bean flour; CBPC: common bean protein concentrate; CBF: common bean flour.

(342.2 mg/kg) and FBPC and FBF presented the highest concentration of zinc (103.7 mg/kg and 103.1 mg/kg) and copper (21.3 mg/kg and 21.3 mg/kg). Additionally, as beans are considered a rich source of iron, it is worth mentioning the high amount of iron (342.2 mg/kg) found on the in-house bean concentrate.

The overall mineral content of the FBPC was similar to the FBF and had no significant difference in any mineral studied, as the concentrate was obtained from its flour, by air classification. However, some studies show that the use of air classification can cause a reasonable change in mineral concentrations (De Angelis et al., 2021; Saldanha do Carmo et al., 2022) and this result was probably due to a poor protein concentration on this ingredient. The mineral content in the soy and pea ingredients is similar to others found in the literature (Chamba et al., 2015; Kornet et al., 2021b).

3.3. Amino acid profile

The amino acid composition plays a significant role on the physical, technological, and nutritional properties of protein ingredients. The proportion and sequence of hydrophilic (Thr, Ser, Arg, Lys, His, Gly, Asp, Cys, Glu) and hydrophobic amino acids (Ala, Tyr, Val, Leu, Ile, Pro, Met, Phe, Trp) define properties such as solubility, protein surface tension, water and oil holding capacities (Brishti et al., 2020), but also the protein folding, an important aspect in terms of digestibility, as digestive enzymes must reach their substrates catalytic regions to promote the protein hydrolysis (Sá et al., 2019).

The amino acid composition of the ingredients is presented in Fig. 1 and all the ingredients had a similar amino acid profile with glutamate (163.55–190.84 mg/g; 16–19%), aspartate (110.66–121.07 mg/g; 11–12%), and arginine (63.44–100.84 mg/g; 7–10%) being the major

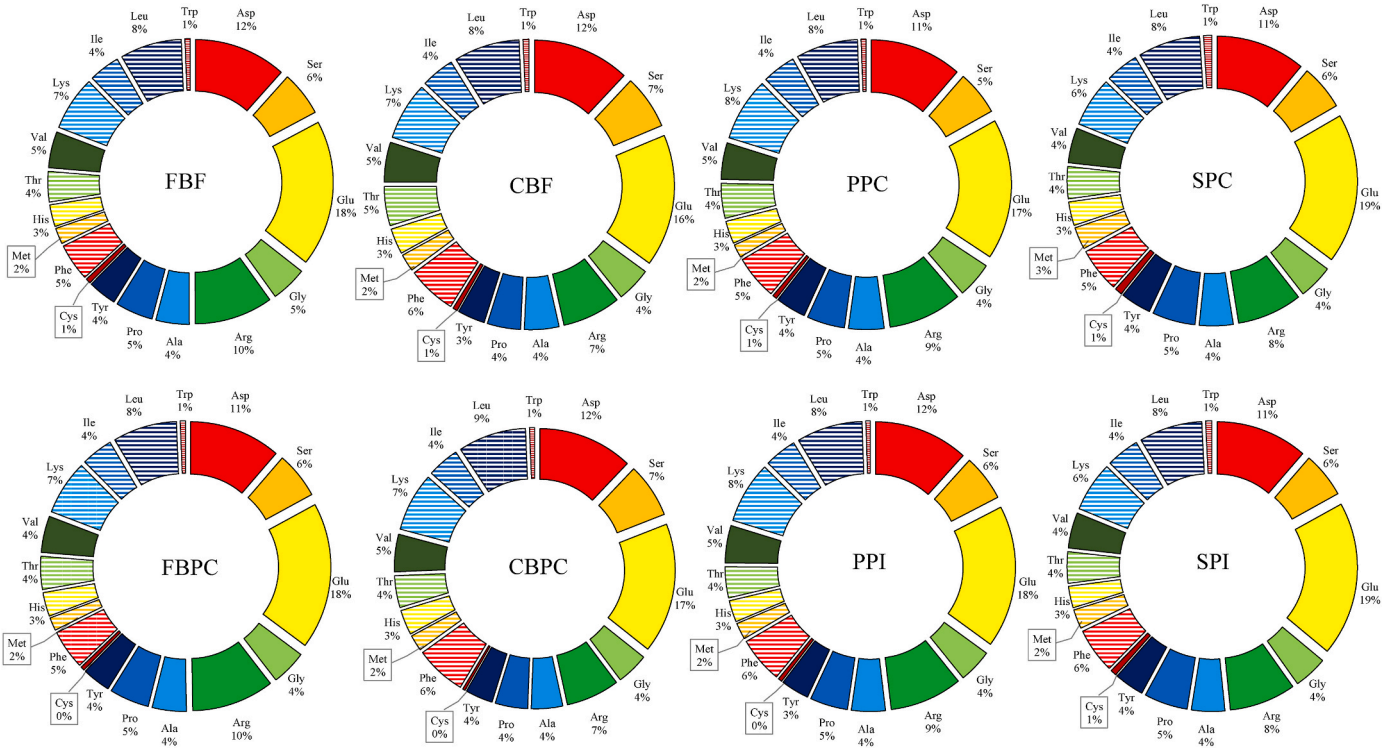

Fig. 1. Amino acid composition of the ingredients.
Units were converted in %, calculated from mg of amino acid/g of protein. SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; PPC: pea protein concentrate; FBPC: fava bean protein concentrate; FBF: fava bean flour; CBPC: common bean protein concentrate; CBF: common bean flour. Amino acids: Aspartate (Asp); Serine (Ser); Glutamate (Glu); Glycine (Gly); Arginine (Arg); Alanine (Ala); Proline (Pro); Tyrosine (Tyr); Cysteine (Cys); Phenylalanine (Phe); Methionine (Met); Histidine (His); Threonine (Thr); Valine (Val); Lysine (Lys); Isoleucine (Ile); Leucine (Leu); Tryptophan (Trp). FAO indispensable amino acids recommendation: His 2%; Thr 2%; Val 4%; Lys 5%; Ile 3%; Leu 6%; Trp 1%; Phe + Tyr 4%; Met + Cys 2%. (FAO, 2019).

found ones from the hydrophilic group and leucine (76.34–86.70 mg/g; 8–9%) and phenylalanine (47.13–64.97 mg/g; 5–6%) being the major ones from the hydrophobic group.

Fig. 1 also shows the minimum requirements for the indispensable amino acids as defined by FAO (2019) when considering the protein requirement of 0.66 g of protein/Kg/day (milligram of AA/gram of protein: His 15; Thr 23; Val 39; Lys 45; Ile 30; Leu 59; Trp 6; Phe + Tyr 38; Met + Cys 22). Basically, all pulses ingredients presented the minimum amounts of the indispensable amino acids for functional claims if considering the ingredients by themselves into a supplement formulation, for example. As they will probably be used in different food preparations, the total amounts will depend the amount of the ingredient added on each formulation and that will have to be considered when adding a claim into a final product.

3.4. Antinutritional factors

Many plant food sources, including pulses, have some antinutritional factors that exert a negative impact on their nutritional quality. Conventional food processing and even culinary strategies which include soaking, dehulling, boiling, pressure cooking as well as germination and fermentation may be used to reduce the levels of phytate, protease inhibitors, phenolics, condensed tannins, lectins, and saponins (Avilés-Gaxiola et al., 2018).

Trypsin inhibitors (TIs) are one of the most relevant protease inhibitors (Mohan et al., 2016; Vagadia et al., 2017) and they are undesirable as they reduce the activity of important digestive proteases and interfere on the digestion and absorption of food proteins (Avilés-Gaxiola et al., 2018; Hall and Moraru, 2021; Kubo et al., 2021).

Common beans ingredients showed the highest values of trypsin inhibitors: CBPC and CBF with 26.98 TIU/mg and 18.18 TIU/mg, respectively (Table 3). The other ingredients ranged from 3.55 TIU/mg (PPI) to 6.87 TIU/mg (SPI). Even presenting high values for TI, CBPC and CBF exhibited similar values to the ones found for beans in the literature, which are around 18.1–24.17 TIU/mg (Shi et al., 2017; Nikmaram et al., 2017; Pedrosa et al., 2020). The other ingredients also showed similar values for TI found in the literature (Áviles-Gaxiola et al., 2018; Shi et al., 2017; Liu and Ruiz, 2021; Vogelsang-O'Dwyer et al., 2020; Gonzales De Mejia et al., 2005).

Table 3
Anti-nutritional content in the ingredients.

Samples	Anti-nutritional factors		Oligosaccharides		
	Trypsin Inhibitor (TIU/mg)	Phytic acid (mg/g)	Verbascose (g/100g)	Raffinose (g/100g)	Stachyose (g/100g)
SPI	6.87 ± 0.40^{c}	13.47 ± 0.46^{d}	**	**	**
SPC	6.72 ± 0.21^{c}	16.77 ± 0.58^{c}	**	0.39 ± 0.01^{a}	1.58 ± 0.06^{b}
PPI	3.55 ± 0.60^{c}	19.43 ± 0.63^{b}	0.54 ± 0.01^{d}	0.06 ± 0.01^{c}	0.60 ± 0.01^{d}
PPC	4.46 ± 0.18^{c}	17.07 ± 0.30^{c}	2.18 ± 0.03^{c}	0.05 ± 0.00^{c}	3.55 ± 0.08^{a}
CBPC	26.98 ± 2.49^{a}	1.77 ± 0.09^{f}	**	**	0.70 ± 0.01^{d}
CBF	18.18 ± 3.40^{b}	8.63 ± 0.38^{e}	0.20 ± 0.01^{e}	0.09 ± 0.00^{b}	3.41 ± 0.04^{a}
FBPC	5.68 ± 0.16^{c}	22.55 ± 0.73^{a}	3.61 ± 0.06^{a}	0.10 ± 0.00^{b}	1.06 ± 0.05^{c}
FBF	6.22 ± 0.40^{c}	13.05 ± 1.19^{d}	2.76 ± 0.14^{b}	0.03 ± 0.00^{d}	1.07 ± 0.06^{c}

* Not quantified (signal close to the noise level); ** Not Detected. Different letters in the same column mean significant difference between samples (Tukey test, $p \leq 0.05$). SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; PPC: pea protein concentrate; FBPC: fava bean protein concentrate; FBF: fava bean flour; CBPC: common bean protein concentrate; CBF: common bean flour.

Phytic acid (PA) is a characteristic and abundant constituent of legume seeds (Gonçalves et al., 2016). It is the main storage form of phosphorus, although it is not bioavailable for humans. In the human gut, phytic acid reduces the bioavailability of minerals and limits the digestibility of proteins and starch by inhibiting proteases and amylases and by forming complexes with minerals (calcium, zinc, iron, and magnesium), making them also biologically unavailable for absorption (Gonçalves et al., 2016; Sarkhel and Roy, 2022).

The PA concentration was significantly different among all ingredients with the bean ingredients presenting a much lower concentration (8.63 mg/g for CBF and 1.77 mg/g for CBPC) in comparison to the other ingredients. The others ingredients ranged from 13.05 (FBF) to 22.55 (FBPC) mg/g of PA. Obtaining CBPC by isoelectric precipitation reduced the PA concentration considerably. Ruckmangathan et al. (2022) studied the reduction of PA when obtaining a pulse protein concentrate by isoelectric precipitation technique and obtained a concentrate with approximately 60% reduction in PA concentration. Saldanha do Carmo et al. (2022) observed that fava bean protein concentrates obtained by air classification showed an increased in the PA concentration. The other ingredients showed a similar PA composition to that found in the literature (Alonso et al., 2000; Coda et al., 2015; Kumar et al., 2010; Millar et al., 2019; Wang and Guo, 2021).

Raffinose, stachyose, and verbascose are oligosaccharides naturally found in legume seeds and, despite not being an antinutritional factor, their ingestion may cause flatulence, a very undesirable feature that brings certain restrictions regarding the variety and amounts of legume consumption (Wang et al., 2003). Most of the ingredients presented very low or even not quantified amounts of the oligosaccharides (Table 3). SPC had the highest raffinose content (0.39 g/100g) while PPC and CBF had the highest stachyose content (3.55 g/100g and 3.41 g/100g respectively) and FBPC has the higher concentration for verbascose (3.61 g/100g).

The sum of the three oligosaccharides in CBF is 3.70 g/100g. This value is lower than that reported by Khattab et al. (2009) for Egyptian (6.0 g/100g) and Canadian (6.19 g/100g) kidney bean and by Hall et al. (2017) for black bean (6.1 g/100g) and pinto bean (7–11 g/100g). As CBPC, SPI, SPC, and PPI were obtained through wet process, the water may have removed the oligosaccharides along the processing routes. Vogelsang-O'Dwyer et al. (2020) compared a protein isolate from fava beans obtained by the wet route with a protein-enriched flour obtained by air classification and noted that the IEP (isoeletric precipitation) method reduced the presence of oligosaccharides on the ingredients while the protein enriched flour maintains the proximate composition.

3.5. In vitro digestion

All the ingredients were submitted to *in vitro* simulation of gastrointestinal digestion by using the Infogest international consensus (2016). The ingredients behavior after digestion was evaluated by measuring the soluble protein decrease together with the increase of the aromatic amino acid found after digestion (Fig. 2).

The ingredients with higher digestibility rates considering the percentage of soluble protein reduction were FBPC (87.2 %), FBF (84.3 %) and CBPC (69.1 %), followed by PPC (60.3 %), SPI (52.3 %), PPI (51.3 %), CBF (50.7 %) and SPC (26.4 %) showed the lowest reduction. PPC, PPI, and CBPC were the ingredients that showed the highest aromatic amino acid amounts (>350% increase), indicating a high cleavage on the proteins after digestion.

Protein digestibility for beans flours shows different rates when comparing the results with the literature. Chávez-Murillo et al. (2018) observed that the digestibility of protein in black bean flours produced in Mexico was 64%, Choe et al. (2022) found that the digestibility of protein of black beans, kidney bean, navy bean and pinto bean from North Dakota State were approximately 35%, 45%, 40% and 39%, respectively. The digestibility of CBPC was lower than of pinto bean protein isolate (71%) (Tan et al., 2014), which may be related to several

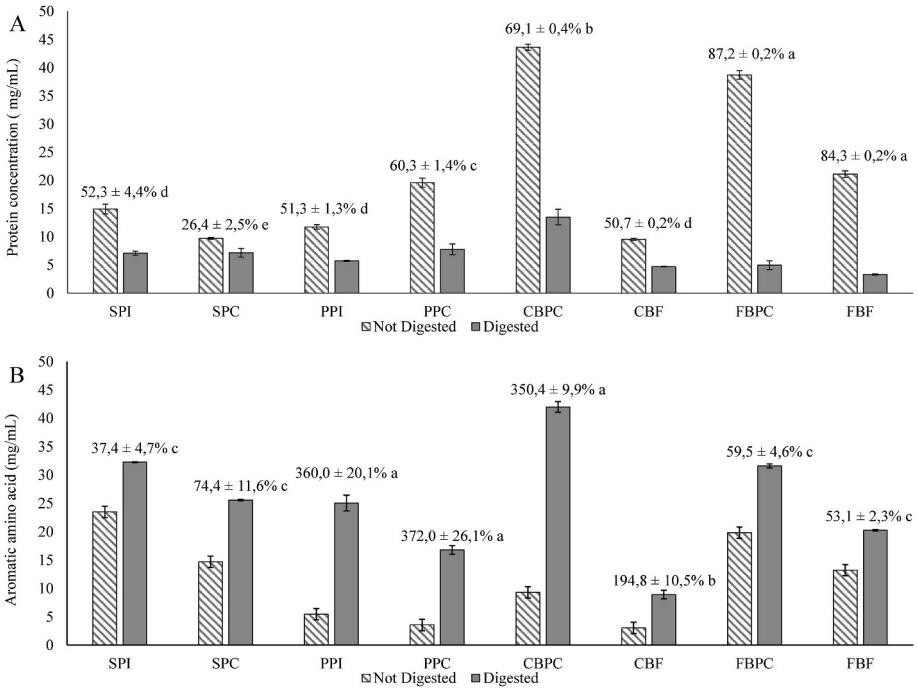

Fig. 2. Quantification of total soluble protein (A) and total aromatic amino acids (B) before and after *in vitro* digestion of the ingredients.
The number above samples represents the percentage of decrease (A) and increase (B) of protein content. Different letters mean significant differences between samples ($p \leq 0.05$). SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; PPC: pea protein concentrate; CBPC: common bean protein concentrate; CBF: common bean flour; FBPC: fava bean protein concentrate; FBF: fava bean flour.

aspects, such as the way the digestion was carried out, the way the responses were measures, the concentration of antinutritional factors from the samples or even, the amounts of phaseolins.

Phaseolin is a bean vicilin-like globulin that shows resistance toward enzymatic hydrolysis due to its unique compact structure and the presence of glycosylation that leads to increased stability of a three-dimensional structural by impeding the proteolytic enzyme to access some peptide bonds on the protein surface (Tang et al., 2009).

SPI, SPC, and PPI presented significantly lower digestibility compared to the literature (Rivera del Rio et al., 2022; Zhao et al., 2022). This result may be directly related to the low solubility of the protein present in these ingredients. In our previous research about the technological properties of these same ingredients (de Paiva Gouvêa et al., 2023) we observed that they did not present solubility greater than 30% between pH 2 and pH 9. This directly affects the digestion because the insoluble protein is potentially not digested while the soluble part is more likely to be digested (Ayala-Rodríguez et al., 2022). The fava bean ingredients present great protein digestibility similar to that found in the literature (Ayala-Rodríguez et al., 2022; Vogelsang-O'Dwyer et al., 2020).

The ingredients were also evaluated by SDS-PAGE and the results are found in Fig. 3. Prior digestion (Fig. 3A), each ingredient from the same pulse variety showed similar protein profiles. The common bean flour and concentrate showed a predominant band between 53 and 43 kDa, related to the 7S protein or phaseolin. The smaller bands between 38 - 40 kDa and 19–22 kDa can be related to acidic and basic legumin, respectively. Rui et al. (2011) found the same profile for nine *Phaseolus* species.

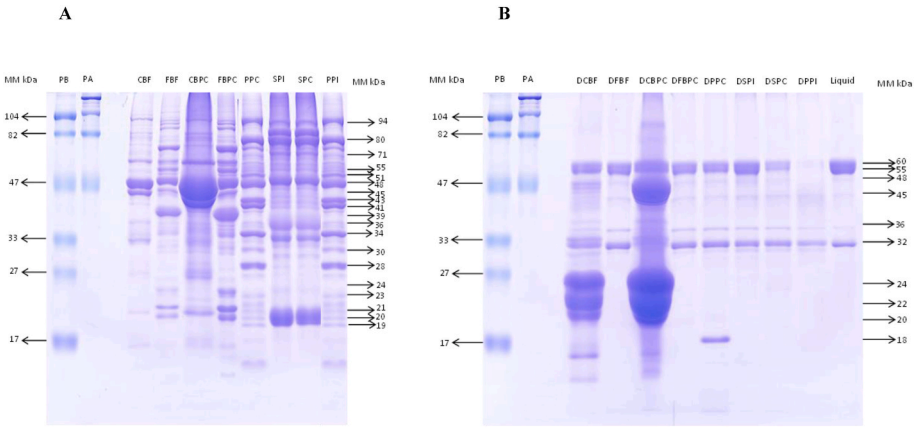

Fig. 3. SDS-PAGE of ingredients prior (A) and after (B) *in vitro* digestion.
PA: High Mw range standard; PB: Low Mw range standard, CBF: common bean flour; FBF: fava bean flour; CBPC: common bean protein concentrate; FBPC: fava bean protein concentrate; PPC: pea protein concentrate; SPI: soy protein isolate; SPC: soy protein concentrate; PPI: pea protein isolate; MM: molar mass; liquid: enzymatic solutions used for digestion.

Pea ingredients have bands at ~95 kDa, ~80 - 70 kDa, ~50 and ~30 kDa, ~40 and ~20 kDa related to lipoxygenase, convicilin, vicilin, and legumin, respectively (Bogahawaththa et al., 2019). Soybean ingredients showed strong bands between 88 and 80 kDa and 47 kDa related to conglycinin and traces of 34 - 20 kDa related to glycinin (Tan et al., 2014; X. Tang et al., 2021). The fava bean ingredients showed ~76 kDa, ~60 kDa, ~51 kDa, ~40 kDa, and ~20 kDa bands related to convicilin, legumin, vicilin, α-legumin, and β-legumin, respectively (Ayala-Rodríguez et al., 2022; Vogelsang-O'Dwyer et al., 2020).

After digestion, all protein chains from pea, fava bean, and soybean ingredients were successfully cleaved into smaller peptides as the protein bands are no longer seen in the electrophoresis gel (Fig. 3B). PPC still presented a small band in 18 kDa, probably related to legumin or some polypeptides from other protein chains.

The phaseolin presented in both CBF and CBPC showed to be partially resistant to the digestion process. As previously mentioned, phaseolin is a glycosylated trimeric cluster protein devoid of disulfide bonds that has been reported as a major protein found in the *Phaseolus* beans and each subunit has ~50 kDa (Tan et al., 2014) as is resistant to the digestive enzymes. Comparing the protein pattern prior and after digestion it is possible to see the trimeric protein band (Fig. 3A) being degraded into its monomeric units, in the region of ~20 kDa (Fig. 3B), showing a partial hydrolysis of the protein. After all, the protein fractions that were reached by the enzymes were digested, but a great part of the monomeric subunits remained undigested, in accordance with the literature (Bessada et al., 2019).

4. Conclusions

There were notable variations in proximate composition, antinutritional factors, and mineral content among the commercial ingredients and the new potential ones from Brazilian common beans. The bean concentrate was very high in protein when compared to the other commercial concentrates and the iron content was also relevant. In terms of antinutritional factors, the beans ingredients presented the highest trypsin inhibitors amounts, but the lowest phytic acid ones. Basically, all the ingredients presented low levels of flatulence-promoting oligosaccharides and they all achieved minimal amounts of FAO recommendation for the indispensable amino acids considering the ingredients composition.

In terms of *in vitro* digestion, all commercial ingredients were largely digested. SPC presented the lowest rates when analyzing protein degradation and aromatic amino acid quantitation. The phaseolins from common beans ingredients were not fully digested as they are known to present a very tight ultrastructure in its native form. In this sense, we strongly encourage further research to improve the digestibility of the common bean protein concentrate, including exploring the use of ultrasound, heat, high-pressure or enzymatic techniques to overcome the protein resistance.

In conclusion, each ingredient has its point for and against and the choice of one to compose a food formulation will depend on the desired technological properties and on the nutritional expectations of each ingredient from a food formulation. After all, all tested ingredients showed to be suitable for the plant-based market.

CRediT authorship contribution statement

Lucas de Paiva Gouvêa: Formal analysis, Investigation, Methodology, Writing – review & editing. **Rodrigo Fernandes Caldeira:** Formal analysis. **Tatiana de Lima Azevedo:** Formal analysis. **Rosemar Antoniassi:** Formal analysis, Investigation, Methodology. **Melicia Cintia Galdeano:** Investigation, Methodology, Writing – review & editing. **Ilana Felberg:** Investigation, Methodology, Writing – review & editing. **Janice Ribeiro Lima:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Caroline Grassi Mellinger:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

Declarations of interest: none.

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Data availability

Data will be made available on request.

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