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Extraction methods and nutritional characterization of protein concentrates obtained from bean, chickpea, and corn discard grains

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ABSTRACT

Protein concentrates obtained from discarded grain flours of white chickpea Sinaloa (*Cicer arietinum)* (CC), "Azufrazin" bean (*Phaseolus vulgaris)* (BC), and white corn (*Zea mays)* (MC), were characterized biochemically through bromatological analyses (protein, lipid, fiber, moisture, ashes, and nitrogen free extract), HPLC techniques (amino acids content), and spectrophotometry (anti-nutrients: phytic acid, trypsin inhibitors, and saponins). The percentage of protein obtained from CC, BC, and MC was 71.23, 81.10, and 55.69%, respectively. Most peptides in the BC and CC flours had a molecular weight of <1.35 kDa, meanwhile, MC peptides were heavier (1.35 to 17 kDa). The amino acids (AA) profile of flours and protein concentrates were similar; however, all the protein concentrates showed an increased AA accumulation (300 to -400%) compared with their flours. The protein concentrates from BC registered the highest AA accumulation (77.4 g of AA/100 g of protein concentrates). Except for the phytic acid in CC and trypsin inhibitor in CC and MC, respectively, the rest of the protein concentrates exhibited higher amounts of the anti-nutrients compared with their flours; however, these levels do not exceed the reported toxicity for some animals, mainly when used in combination with other ingredients for feed formulations. It is concluded that CC and BC protein concentrates showed better nutritional characteristics than MC (level of protein, size of peptides, and AA profile). After biochemical characterization, protein concentrates derived from by-products have nutritional potential for the animal feed industry.

1. Introduction

According to Arévalos et al. (2019), agricultural grains and seeds are products destined for the food industry and for sowing and propagation of plant species, respectively. In the food industry, plant-origin proteins are cheaper than those of animal origin. However, their use is limited because they are deficient in some essential amino acids (EAA), particularly sulfur-containing amino acids; besides, plant proteins contain anti-nutritional factors that can be noxious or affect food utilization, making the diet less digestible. Therefore, they are classified as nutritionally lower or low-quality proteins (Hughes et al., 2011; Hua et al., 2019). The denaturation of proteins derived from vegetables is a process commonly used to increase their dietary digestibility and reduce anti-nutritional factors, obtaining a product of higher bioavailability, better protein accessibility, and lower cost (VioqueSánchez-Vioque et al., 2001).

Plant-based proteins are a cheaper alternative to be included in animal diets, primarily when obtained from low-quality agricultural grains, so these are considered agricultural by-products (Ducrocq et al., 2022). Due to diverse factors such as smaller size and different colors, among others (Dobrzański and Rybczyński, 2011), grains like chickpeas, beans, and corn could present quality standards below those required for export marketing. Other criteria to consider these agricultural grains as by-products can include broken grains, severely deformed grains, and attacked or infected by plagues, which represent economic losses for agricultural producers (Manrique Klinge, 2017).

Chickpea (*Cicer arietinum*) is a good source of protein and

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Abbreviations

CC	Chickpea Concentrate
BC	Bean Concentrate
MC	Corn Concentrate
EAA	Essential Amino acids
NEAA	Non-essential amino acids
ANFs	Anti-nutritional factors
RCF	Relative Centrifugal Force
MPS	Minimal protein solubility

carbohydrates; this gran has multifunctional activities related to its content of phytochemicals (Xing et al., 2020; Wang et al., 2021). However, it is limited in some EAA like valine, threonine, and tryptophan (Kaur and Prasad, 2021; Iqbal et al., 2006). Nevertheless, it is considered an option for animal nutrition. The bean (*Phaseolus vulgaris*) is a legume characterized by its high protein content and hydrosoluble vitamins and is a good source of polyunsaturated fatty acids (Zhang et al., 2021; Garcia-Mora et al., 2015). However, its nutritional characteristics depend on the variety and the geographical location where it is cultivated. Notwithstanding, beans are also characterized by their deficiency in some amino acids (AA), like methionine, cysteine, and tryptophan (Camacho-Espinoza et al., 2010). Despite this, beans have been considered a nutritional source of interest for the aquaculture industry (Novriadi et al., 2019; Bu et al., 2018; Khalifa et al., 2018). In turn, corn (*Zea mays*) contains low percentages of crude protein (Eleazu et al., 2021; Oboh et al., 2010). It is a cereal commonly used in animal diets due to its levels of some AA, like methionine and cysteine; however, it is deficient in lysine (Loy and Lundy, 2019).

Due to the EAA deficiency presented in vegetable sources, diverse methodologies have been studied to make vegetal-origin proteins more efficiently assimilated by organisms (Donadelli et al., 2019). One of these alternatives is the use of protein concentrates (Gharibzahedi and Smith, 2021; Diaz et al., 2022; Sá et al., 2020), which represents both economic and environmental advantage over the dependence on animal-origin proteins (Loveday, 2020). Proteins in the form of concentrates contain 50 to 70% protein, and the protein isolates are above 90%; thus, when the diet source is of vegetal origin, the obtained ingredients can pose different benefits like conserving functional properties, elimination of anti-nutritional factors (ANFs), and maximal possible extraction of proteins (Beski et al., 2015; Sánchez-Chino et al., 2019; VioqueSánchez-Vioque et al., 2001) Therefore, the objective of this research is to obtain and characterize nutritionally protein concentrates from discarded grains of chickpeas, beans, and corn, to assess their nutritional potential for the formulation animal diets.

2. Materials and methods

2.1. Samples

The discard grains of white chickpea Sinaloa (*Cicer arietinum*), "Azufrasin" bean (*Phaseolus vulgaris*), and white corn (*Zea mays*) were provided by Productores Unidos del Río Petatlán, municipality of Guasave, Sinaloa, Mexico. Grains were pulverized in an industrial mill (Perc Grindmaster® Gr-500) (Louisville, KY, US) and sieved through a cylindrical sifter to obtain a fine flour of 460 μm particle size.

2.2. Standardization of variables for the production of protein concentrates

The chickpea and bean flours were dissolved in distilled water at a 1:10 (w/v) ratio, adding a 2 N NaOH solution and stirring for 30 min at 25° (pH 11 for chickpeas and pH 9.5 for beans), following the methodology with some modifications, described by Sánchez-Chino et al. (2019) and Félix et al. (2019) to obtain the chickpea protein concentrate, and that of Valdez-Ortiz et al. (2012) and Piñuel et al. (2019) with some modifications, to obtain the bean protein concentrate. Solutions were centrifuged at 3350 rcf for 20 min at 25 °C; afterward, the supernatant of each flour was recovered and distributed in different containers. The isoelectric precipitation was adjusted to three different pH values (3.5, 4, and 4.5). This was achieved by adding 2 N HCl and leaving it to rest for 16 h at 4 °C. The mixtures were centrifuged at 3350 rcf for 30 min at 25 °C to obtain the frozen sediments at -60 °C and, finally, lyophilized for 48 h.

To obtain the corn protein concentrate, the methodology described by Medina et al. (1990) with some modifications was used. The flour was dissolved with distilled water at a 1:5 (w/v) relation, adding 2 g of cupric sulfate ($CuSO_4$), then the mixture was agitated for 30 min at 46 °C, adding 2N NaOH to adjust the pH to 9.5. Then, the solution was centrifuged at 3350 rcf for 20 min at 25 °C. The supernatant was recovered, and the pH was adjusted (3.5, 4, and 4.5) by adding a solution of 2 N HCl. Then, the sample was left to rest for 16 h at 4 °C. After this time, new centrifugation was performed at 3350 rcf for 30 min at 25 °C to obtain the sediment that was frozen and lyophilized for 48 h.

The obtained protein yield was determined using the following formula described by González et al. (2021).

$$Yield\ (\%) = \frac{(EP)}{(TP)} \times 100$$

Where: *EP = extracted protein (g), and TP = total protein in the flour of the grains (g).*

2.3. Chemical-proximal analyses

The chemical-proximal analyses of the three grains' flours and protein concentrates were determined according to the methods described in AOAC, 2019. Protein was determined using the micro-Kjeldahl method, and lipid was estimated by Soxhlet extraction with anhydrous ether. Ash was analyzed using a muffle furnace to (600 °C) for 2 h, and crude fibre by the phenol–sulphuric acid method. The difference determined carbohydrate (NFE = Nitrogen free extract).

2.4. Characterization of protein concentrates

The peptide and amino acid profile and anti-nutrient levels were determined only in the chickpea, beans, and corn concentrates that showed the highest protein concentration after treatment with different pH levels.

2.4.1. Peptides profile

This analysis was performed following the methodology described by Martínez Montaño et al. (2020), using HPLC (Varían™ ProStar) equipped with a Bio SEC-5™ (4.6 × 300 mm; Agilent) molecular exclusion column and monitoring the absorbance at 254 nm with a diodes array detector. Samples were eluted with a buffer of 150 mM sodium phosphate at a pH of 7, using an isocratic flow of 0.4 mL/min. The molecular weight was determined concerning the elution time of a molecular weight marker constituted by thyroglobulin (MW = 670 kDa), gamma-globulin (MW = 158 kDa), ovalbumin (MW = 44 kDa), myoglobin (MW = 17 kDa), and vitamin B12 (MW = 1.35 kDa).

2.4.2. Amino acids profile

The amino acid content of the meal samples was determined according to the chapter #2.2.56 (Council of Europe, 2005). Each 25 mg sample was supplemented with 200 μL of 6 N HCl with 0.06% of phenol to be digested for 24 h at 110 °C under a nitrogen atmosphere. Afterward, the samples were rehydrated with 0.1 N HCL and filtered through a PTFE-B of 0.45 μm mesh. Before the chromatographic analysis, the

Table 1
Chemical-proximal analyses of chickpea (*Cicer arietinum),* Azufrazin bean (*Phaseolus vulgaris*), and corn (*Zea mays*) flours.

	Chickpea	Bean	Corn
Protein (%)	22.34 ± 0.20[a]	21.58 ± 0.61[a]	9.13 ± 0.04[b]
Lipids (%)	6.28 ± 0.07[a]	3.50 ± 0.36[c]	5.33 ± 0.36[b]
Fiber (%)	2.78 ± 0.06[c]	3.18 ± 0.34[b]	5.39 ± 0.01[a]
Moisture (%)	8.22 ± 0.21[c]	10.04 ± 0.11[b]	10.67 ± 0.06[a]
Ash (%)	3.71 ± 0.02[b]	4.26 ± 0.07[a]	1.68 ± 0.02[c]
NFE (%)	66.52 ± 1.69[b]	69.27 ± 1.03[a]	66.93 ± 0.89[b]

NFE: Nitrogen free extract. Average ± standard deviation values of triplicate analyses. Different lowercase letters indicate statistical differences among grains; Tukey test, $P < 0.05$.

samples were derivatized using the OPA reagent (o-phthalaldehyde) and an HPCLC equipment (Varían™ ProStar) with a C-18 reverse phase column (AdvanceBio AAA™, Agilent, 4.4 × 100 mm). A gradient was used, employing as eluent A the buffer of 10 mM dibasic sodium phosphate (Na_2HPO_4), 10 mM of decahydrate sodium borate ($Na_2B_4O_7$ 10 H_2O), and 5 mM of sodium azide (NaN_3), at a pH of 8.2 and, as eluent B, a mixture of methanol:acetonitrile:water, 45:45:10 (v:v:v). The flow rate was 1.5 mL/min. Absorbance was monitored at a wavelength of 338 nm using a diodes array detector. A standard amino acids mixture (Agilent 5061-3332) was used for determining the amino acid concentrations.

2.4.3. Anti-nutritional factors

Phytic acid was determined according to the methodology described by Vaintraub and Lapteva (1988), and results were expressed as phytic acid equivalents per gram of a sample, dry weight (PAE/g).

Trypsin inhibitor levels were expressed in trypsin inhibitor units per milligram of sample (ITI/mg).

The calculation was made using the following formula:

$$UTI = \frac{Abs\ trypsin - Abs\ sample}{0.01}$$

$$\frac{UTI}{mg\ sample} = \frac{(Abs.\ Max.\ Tryp. - Abs\ sample) * Dilution\ factor}{0.01 * mg\ of\ sample\ in\ 2\ mL}$$

One unit of trypsin inhibitor (UTI) was defined as the amount of inhibitor that inhibits 1 μg of pure trypsin and determined through spectrophotometry using the technique of Liu (2019). The analysis of condensed tannins was performed following the modified technique of Deshpande and Cheryan (1985). Results are reported as milligram equivalents of catechin per 100 g of dry sample (mg EC/100 g). Saponins were quantified with the colorimetric technique proposed by Hiai et al. (1976) using vanillin and H_2SO_4 to generate chromophore groups in steroidal and triterpenoid saponins. Results were expressed as milligram diosgenin equivalents per 100 g dry sample (mg ED/100 g).

2.5. Statistical analysis

The statistical analysis was performed using the Statistica 7® software to determine whether significant differences existed among the treatments. Data were initially subjected to an analysis of normality and homogeneity of variance using the Shapiro-Wilk and the Bartlett tests, respectively. Because data were distributed normally, an ANOVA variance analysis was performed; the Tukey's test was used to compare the means, with a significance level set at $P < 0.05$. Results for anti-nutritional and comparisons between flours and concentrates were analyzed with a Student-t-test ($P < 0.05$). All analyses were made using the IBM® SPSS Statistics software. All values were presented as means ± standard deviation in tests carried out in triplicate.

Table 2
Proximal composition and yields of chickpea, bean, and corn protein concentrates.

Chickpea concentrate						
Proximal composition					Yields	
pH	Protein[a] (%)	Lipids[a] (%)	Fiber[a] (%)	Ash[a] (%)	Concentrate yield[a] (g/kg)	Protein Yield[b] (%)
3.5	69.42 ± 0.76[b]	18.93 ± 0.11[b]	3.45 ± 0.67[a]	3.74 ± 0.16[a]	220.48	31.07
4	70.05 ± 0.32[b]	18.84 ± 0.43[b]	2.06 ± 0.19[b]	3.76 ± 0.35[a]	230.54	31.35
4.5	71.23 ± 0.48[a]	19.50 ± 0.40[a]	2.43 ± 0.46[b]	3.45 ± 0.82[a]	210.98	31.88
Bean concentrate						
Proximal composition					Yields	
pH	Protein[a] (%)	Lipids[a] (%)	Fiber[a] (%)	Ash[a] (%)	Concentrate yield[a] (g/kg)	Protein Yield[a] (%)
3.5	78.84 ± 0.14[b]	10.20 ± 0.81[a]	1.69 ± 0.29[c]	4.13 ± 0.13[a]	200	36.53
4	79.21 ± 0.04[b]	6.27 ± 0.72[b]	2.67 ± 0.41[b]	3.78 ± 0.12[b]	200.51	36.7
4.5	81.10 ± 0.66[a]	2.39 ± 0.25[c]	7.21 ± 0.57[a]	3.07 ± 0.06[c]	200.39	37.58
Corn concentrate						
Proximal composition					Yields	
pH	Protein[a] (%)	Lipids[a] (%)	Fiber[a] (%)	Ash[a] (%)	Concentrate yield[a] (g/kg)	Protein Yield[a] (%)
3.5	54.37 ± 0.55[b]	27.07 ± 0.68[a]	9.63 ± 0.34[a]	4.24 ± 0.10[c]	150.1	59.55
4	55.44 ± 0.26[ab]	16.37 ± 0.32[b]	2.56 ± 0.24[b]	3.78 ± 0.42[b]	130.06	60.72
4.5	55.69 ± 0.38[a]	12.33 ± 0.41[c]	2.23 ± 0.61[b]	4.54 ± 0.12[a]	120.98	60.99

Average ± standard deviation values of triplicate analyses. Different lowercase letters indicate statistical differences among grains; Tukey test, $P < 0.05$.

[a] Average ± standard deviation of triplicate analyses.

[a] Grams of dry weight obtained of the protein concentrate per kilogram of processed flour (g/kg).

[b] : Relation extracted protein/initial protein of the flour (%).

3. Results and discussion

3.1. Proximal analyses and yields of flours and protein concentrates

Table 1 shows the results of the proximal content in the flours of the three grains. The protein content in the chickpea, bean, and corn flours was 22.34 ± 0.22, 21.58 ± 0.22, and 9.13 ± 0.04%, respectively, which increased in the concentrates (Sánchez-Chino et al., 2019) (Table 2). The percentage of crude proteins and protein yield of the three concentrates was higher when precipitated isoelectrically at a pH of 4.5. The BC yielded the highest amount of proteins (81.10 ± 0.66%), whereas the highest yield (60.99%) was observed in the MC. Comparatively, the MC showed the lowest protein concentration (55.69 ± 0.38%). However, its protein yields were higher than those of BC and CC because the initial protein level is statistically ($P < 0.05$) lower than in the chickpea and bean flours. Considering that the protein yield is a relation of the level of protein obtained from the concentrates divided by the initial protein levels of the flours, the yields of the corn concentrate with the three

Table 3
Chromatographic profile of peptides of chickpea, bean, and corn flours and their respective concentrates at a pH value of 4.5.

kDa	Area (%)					
	Chickpea		Bean		Corn	
	Flour	Concentrate	Flour	Concentrate	Flour	Concentrate
>670	9.94	0.07	4.03	1.36	0.34	3.34
670 – 158	15.58	5.20	14.13	17.32	7.02	1.69
158 – 44	14.40	5.72	21.92	24.98	12.87	12.23
44- 17	6.85	1.55	8.21	2.30	8.30	15.91
17–1.35	40.56	30.86	37.45	18.10	45.44	46.34
<1.35	12.64	56.57	14.24	35.91	26.00	20.40

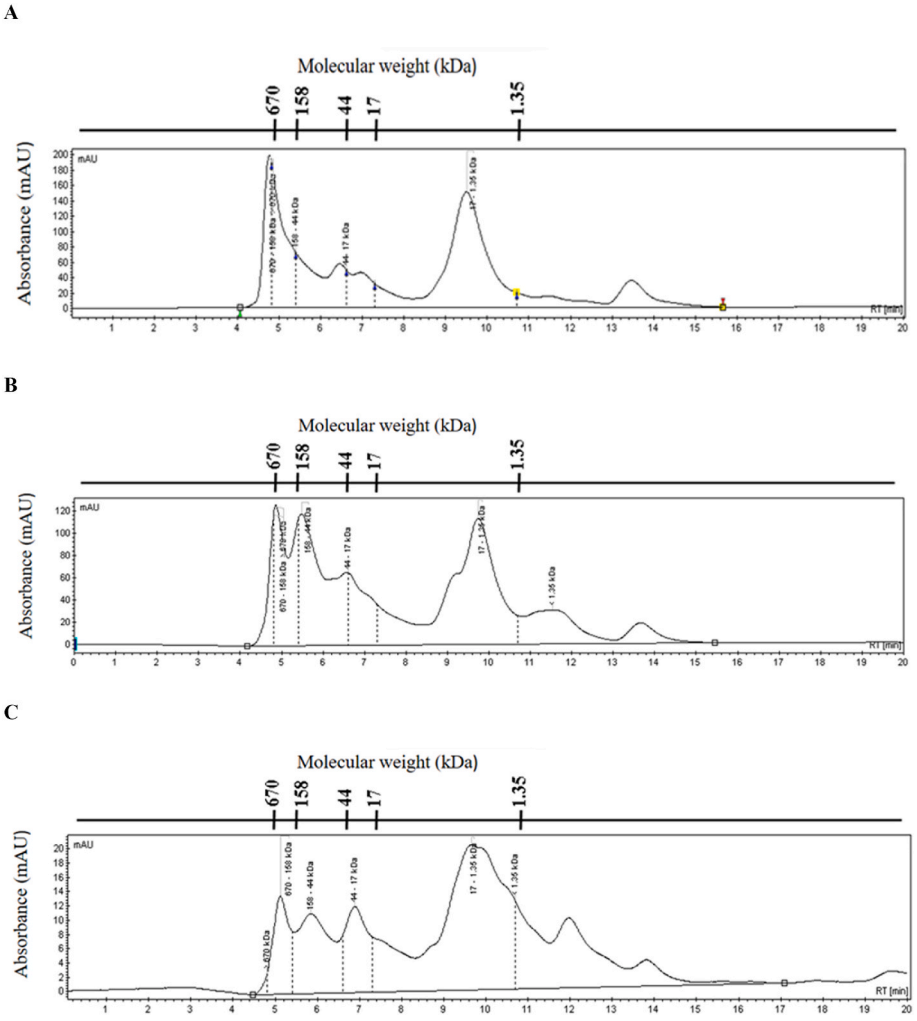

Fig. 1. Chromatographic profiles of peptides of flours from (A) chickpea (*Cicer arietinum*), (B) bean (*Phaseolus vulgaris*), and (C) corn (*Zea mays*). The graph shows the signal from the UV monitor at 254 nm.

tested pH levels were higher. It is worth mentioning that all the yields were higher than those reported by González et al. (2021) and Jarpa--Parra et al. (2014). The protein level in the MC (89.58%) was lower than those reported by Medina et al. (1990). Although these authors followed the same protein extraction conditions, they used shelled and germinated corn.

The pH directly influenced the protein content and, thus, the yield of each grain; in this case, the highest levels of proteins were obtained at an isoelectric point of 4.5 because when dealing with proteins like albumins and globulins, these have a minimal solubility at pH between 4 and 5. Sánchez-Vioque et al. (1999) obtained the minimal protein solubility (MPS) for chickpea protein at pH 4.3 (except albumins which are soluble at 4.3), meanwhile, Kusumah et al. (2020) reported that MPS for *Phaseolus vulgaris* and *Ph. radiatus* beans was found at pH 4.56 and 4.81, respectively. Besides, Gu and Glatz (2007) worked at pH of 4.0 to obtain the MPS for corn proteins. Moreover, different studies on protein concentrates have determined that the 4.5 pH value positively influences variables like the properties of foaming, emulsion, and solubility (Jarpa-Parra et al., 2014; Boye et al., 2010). On the other side, the amount of crude protein in CC and BC was higher than that reported by Sánchez-Chino et al. (2019) and Torres-Fuentes et al. (2011) with the same grains, applying an alkaline extraction at pH 11 coinciding with our findings; but, in our case, without the use of enzymes like pancreatin and pepsin.

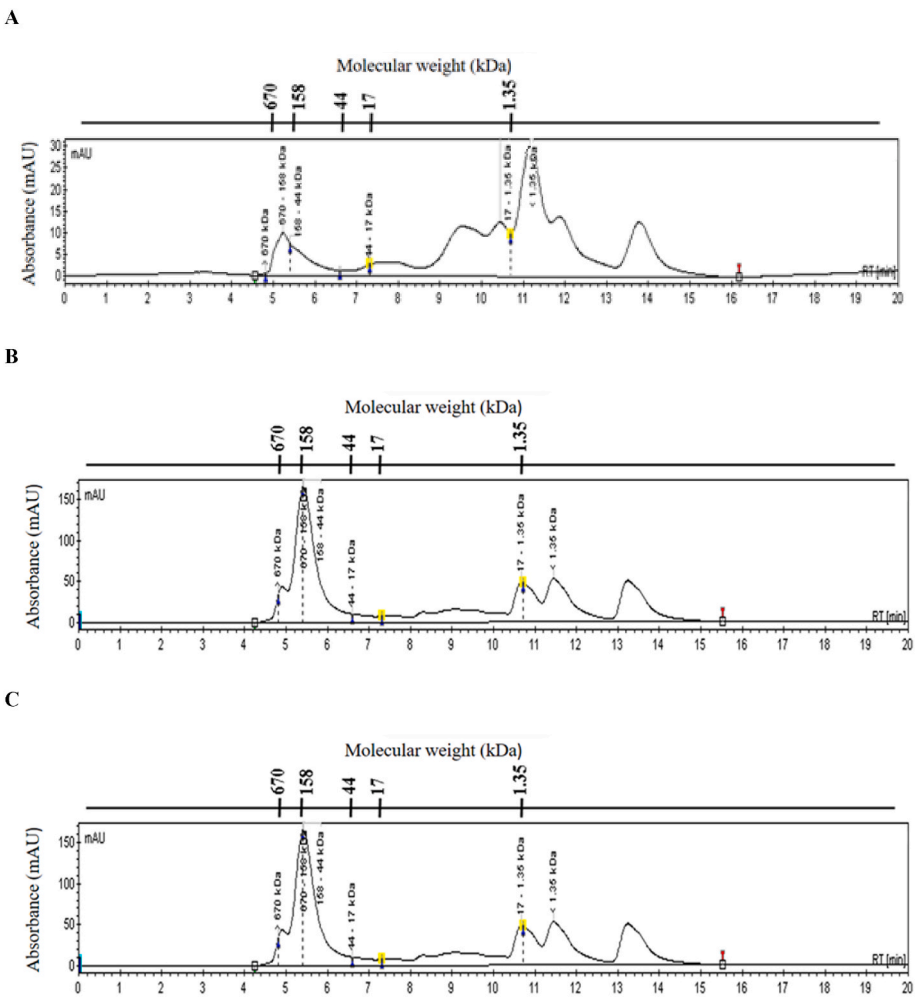

Fig. 2. Chromatographic profiles of peptides of protein concentrates from (A) chickpea (*Cicer arietinum*), (B) bean (*Phaseolus vulgaris*), and (C) corn (*Zea mays*). The graph shows the signal from the UV monitor at 254 nm.

3.2. Peptides profile

More than 50% of the molecular weight of the peptides contained in the flours of the three grains ranged from <1.35 to 17 kDa, and the same tendency was observed in their concentrates (Table 3; Figs. 1–2). However, CC and MC peptides with this molecular size represented more than 66%. For the chickpea and bean flours -legumes that contain globulins of 7S and 11S subunits-, their molecular weight range from 150 to 190 kDa (Barac et al., 2015; Dhawan et al., 1991) and became fractioned at a pH value of 4.5. This procedure reduces their size, as occurred with CC and BC. The molecular weight of the corn flour peptides is given by the presence of zeins, mainly δ-zeins (approximately 10 kDa) and β-zeins (15 kDa) (Espinosa et al., 2015; Wu et al., 2009). As for CC and BC, the dilution process of the corn flour protein in acidic conditions (pH 4.5) increase the number of smaller peptides due to a possible protein denaturing and hydrolysis by the very acidic or very alkaline pH conditions. This process interrupts the quaternary and tertiary structures, mainly in proteins like globulin; these conditions lead to the production of amino acids or low molecular weight peptides (Quelal et al., 2019; Rodsamran and Sothornvit, 2018). The cleavage of α and β subunits of 11S protein from globulins into polypeptides with MW of 43.6 kDa to 5.9 kDa has been reported by Sánchez-Vioque et al. (1999) when chickpea flour was subjected to an alkaline extraction and subsequent precipitation of the proteins at the isoelectric point.

In the protein concentrates, the reduction in the number of peptides in the range of 158 to >670 kDa confirms the effect of the acid treatment of flours. Our results indicate that the acid treatment at pH 4.5 fragmented the peptides with the highest molecular weight contained in the proteins (González et al., 2021; Hadidi et al., 2020; Aryee and Boye, 2016; Benítez et al., 2008; Ordóñez et al., 2008), reducing them to <1.35 kDa peptide size. Such peptides are characterized by their high bioavailability (easy digestion and absorption) (Day et al., 2022; González et al., 2021; Zaretabar et al., 2021), which is a fundamental criterion for the formulation and elaboration of commercial diets (Espinoza and Castillo, 2022).

3.3. Amino acids profile

As observed in Table 4, the amino acid profiles of the three grains and their respective concentrates were similar, as reported by Chew et al. (2003). The chickpea, bean, and corn proteins are characterized by their deficiency in sulfur-containing amino acids (methionine and cysteine) (Reyes-Moreno et al., 1993). Within the EAA, the highest amino acid percentages in the bean concentrate were registered for arginine and leucine (7.7 ± 0.2%, 7.6 ± 0.3%, respectively), coinciding with Lee et al. (2022). Also, this tendency was observed by Espinosa-Ramírez and Serna-Saldívar (2019), Mune et al. (2011), and Awadalkareem et al. (2008) in rice isolates, protein isolates of chickpeas, and Bambara bean concentrate, respectively. BC presented the highest EAA levels among all flours and concentrates.

Concerning NEAA, glutamic acid was the amino acid with the highest presence in all flours and concentrates. Just like that, some amino acids

Table 4
Composition of total amino acids (AA; g/100 g of protein) of chickpea, bean, and corn flours and their respective protein concentrates obtained at pH 4.5.

Amino acid	HG	CG	HF	CF	HM	CM
EAA						
ARG	3.7 ± 0.1^{d}	6.7 ± 0.0^{b}	2.4 ± 0.1^{e}	7.7 ± 0.2^{a}	0.7 ± 0.0^{f}	5.0 ± 0.2^{c}
HIS	0.7 ± 0.0^{d}	1.8 ± 0.0^{b}	0.7 ± 0.0^{d}	3.0 ± 0.1^{a}	0.3 ± 0.0^{e}	1.4 ± 0.21^{c}
LYS	1.5 ± 0.0^{c}	5.1 ± 0.0^{b}	1.6 ± 0.0^{c}	6.0 ± 0.2^{a}	0.3 ± 0.0^{e}	1.2 ± 0.0.1^{d}
MET	0.3 ± 0.0^{d}	1.0 ± 0.0^{b}	0.4 ± 0.0^{de}	1.2 ± 0.1^{a}	0.2 ± 0.0^{e}	0.7 ± 0.0^{c}
PHE	1.4 ± 0.0^{d}	4.5 ± 0.0^{b}	1.4 ± 0.0^{d}	5.3 ± 0.1^{a}	0.5 ± 0.0^{e}	2.1 ± 0.1^{c}
ILE	0.8 ± 0.0^{d}	3.2 ± 0.0^{b}	0.9 ± 0.0^{d}	3.3 ± 0.0^{a}	0.3 ± 0.0^{d}	1.2 ± 0.0^{c}
LEU	1.8 ± 0.0^{d}	5.7 ± 0.0^{b}	2.0 ± 0.0^{d}	7.6 ± 0.3^{a}	1.4 ± 0.0^{e}	3.7 ± 0.1^{c}
VAL	0.9 ± 0.0^{d}	1.4 ± 0.8^{b}	1.0 ± 0.0^{d}	3.5 ± 0.1^{a}	0.4 ± 0.0^{e}	1.7 ± 0.1^{c}
THR	0.8 ± 0.0^{d}	2.6 ± 0.0^{b}	0.9 ± 0.9^{d}	2.8 ± 0.2^{a}	0.3 ± 0.0^{e}	1.4 ± 0.0^{c}
ΣEAA	11.9 ± 0.1^{e}	32.0 ± 0.8^{b}	11.2 ± 0.1^{d}	40.4 ± 0.5^{a}	4.6 ± 0.1^{f}	18.5 ± 0.2^{c}
NEAA						
ALA	1.1 ± 0.0^{d}	3.2 ± 0.0^{b}	1.1 ± 0.0^{d}	3.4 ± 0.0^{a}	0.8 ± 0.0^{e}	2.9 ± 0.1^{c}
ASP	1.6 ± 0.1^{d}	8.4 ± 0.0^{b}	1.7 ± 0.0^{d}	5.9 ± 0.3^{a}	0.4 ± 0.0^{e}	2.1 ± 0.1^{c}
GLU	4.0 ± 0.1^{c}	13.8 ± 0.1^{a}	3.6 ± 0.2^{c}	13.3 ± 0.4^{a}	1.8 ± 0.1^{d}	6.9 ± 0.2^{b}
GLY	1.5 ± 0.1^{d}	2.9 ± 0.0^{c}	1.5 ± 0.1^{d}	5.2 ± 0.2^{a}	0.5 ± 0.0^{e}	3.1 ± 0.0^{b}
SER	1.4 ± 0.0^{e}	3.6 ± 0.9^{b}	1.6 ± 0.0^{d}	6.0 ± 0.0^{a}	0.5 ± 0.0^{f}	2.4 ± 0.0^{c}
TYR	0.6 ± 0.0^{e}	2.2 ± 0.0^{b}	0.7 ± 0.0^{d}	3.2 ± 0.0^{a}	0.3 ± 0.0^{f}	1.4 ± 0.1^{c}
ΣNEAA	10.2 ± 0.1^{d}	34.0 ± 0.9^{b}	10.2 ± 0.1^{d}	36.9 ± 0.5^{a}	4.4 ± 0.1^{e}	18.7 ± 0.1^{c}
Total	22.3	66.2	21.6	77.4	9.0	37.3

Average ± standard deviation values of triplicate analyses. Different lowercase letters indicate statistical differences among grains; Tukey test, $P < 0.05$.
EAA. Essential amino acids; NEAA. Non-essential amino acids; ARG: arginine; HIS: histidine; LYS: lysine; MET: methionine; PHE: phenylalanine; ILE: isoleucine; LEU: leucine; VAL: valine; THR: threonine; ALA: alanine; ASP: aspartic acid; GLU: glutamic acid; GLY: glycine; SER: serine; TYR: tyrosine.

Table 5
Levels of anti-nutrients in chickpea, bean, and corn flours and protein concentrates at a pH value of 4.5.

Ingredient	Phytic acid[a]	Trypsin inhibitors[b]	Saponins[c]
Chickpea flour	15.67 ± 0.37^{a}	7.93 ± 0.34^{a}	20.75 ± 1.92^{b}
Chickpea concentrate	13.67 ± 0.01^{b}	7.28 ± 0.2^{a}	25.6 ± 1.95^{a}
Bean flour	17.4 ± 0.24^{b}	9.23 ± 0.25^{b}	13.56 ± 1.68^{b}
Bean concentrate	18.18 ± 0.07^{a}	20.36 ± 1.4^{a}	17.98 ± 0.81^{a}
Corn flour	22.13 ± 0.47^{b}	1.56 ± 0.08^{a}	15.24 ± 0.95^{b}
Corn concentrate	40.17 ± 0.71^{a}	1.1 ± 0.08^{b}	34.73 ± 2.85^{a}

Mean ± standard deviation value, different letters among columns indicate statistically significant differences based on the Student-t test ($P < 0.05$).
[a] Values expressed in milligrams of phytic acid/grams of sample.
[b] Values expressed in trypsin inhibitors units (TIU)/milligrams of a sample.
[c] Average ± standard deviation values of triplicate analyses. Values expressed in milligrams of diosgenin/grams of sample.

increase after the protein extraction process, coinciding with other reports on protein concentrates (Zambrano et al., 2022; Gorissen et al., 2018; Brishti et al., 2017); however, the amino acid profile varies according to the level of purity and extraction of the vegetable protein (Baune et al., 2022).

3.4. Anti-nutritional factors

Diverse studies have shown the presence of anti-nutritional factors in protein isolates and concentrate obtained from seeds of legumes and non-legumes (Bora, 2014; Martínez Augustin and Martínez de Victoria, 2006), which can be toxic and affect the bioavailability of amino acids (Hua et al., 2019; Ngugi et al., 2017; Gilani et al., 2005). Therefore, it is necessary to confirm their presence and content in the ingredients that make up commercial diets.

The phytic acid content of CC diminished significantly (12.77%; $P < 0.05$; Table 5) compared with its flour, whereas the trypsin inhibitors diminished in 8.2%; however, this was not significant. As described by Garg et al. (2020) and Xu and Chang (2008), the series of treatments or steps during the isolation of chickpea proteins reduces the ANF, like the levels of glucosinolates and phytate, which can be attributed mainly to the binding of phytates to the sodium ions, because NaOH was used during the elimination of soluble compounds.

The levels of saponins increased significantly ($P < 0.05$; Table 5) in the CC from 20.75 ± 1.92 in the flour to 25.6 ± 1.95 mg of diosgenin per gram of sample in the concentrates. This effect is mainly because a large part of these ANFs remains bound to the rest of the proteins that sediment during the isoelectric precipitation, triggering an increase in antinutritionals as a function of the high levels of proteins in the concentrates (Ngugi et al., 2017; Francis et al., 2001).

The three ANFs analyzed in the BC increased significantly ($P < 0.05$). The phytic acid of the BC flour increased by 0.78 mg/g of the sample because ANFs -like the phytic acid-have a negative charge with a wide pH range (from 3 to 1.5 and 8 to pH 7.5), binding to proteins and cations. When the pH is below the isoelectric point, the phytic acid binds to net positively-charged proteins, forming an insoluble binary complex through electrostatic interactions (Mondor et al., 2009; Cheryan and Rackis, 1980). The trypsin inhibitors and saponins also increased (11.13 UTI/mg of sample and 4.42 mg of diosgenin/g of sample, respectively) compared to the bean flour (Table 5).

The concentrates procurement process gave rise to significant changes ($P < 0.05$) in the levels of phytic acid in MC, which increased from 22.13 ± 0.47 in the flour to 40.17 ± 0.71 mg/g sample. The heat treatment to obtain the concentrate could be responsible for the complete deactivation of the phytase enzyme, as mentioned by Sharma et al. (2022) and Omosebi et al. (2018). Likewise, the saponin levels increased in all concentrates because these compounds can be transported with the protein if extractions are made only with water without applying any other type of treatment like cooking; hence, a concentrate produced only with water extraction can contain high levels of saponins (Dersjant-Li, 2021). On the other hand, the trypsin inhibitors showed a significant diminution ($P < 0.05$), from 1.56 ± 0.08 UTI/mg in the flour to 1.1 ± 0.08 UTI/mg of corn concentrated, because these are thermolabile ANFs (Eleazu et al., 2021; Chukwuma et al., 2016, Table 5).

The results obtained were similar to those reported in chickpea, bean, and corn grains in studies already existing in the literature (Samtiya et al., 2020; Saurabh et al., 2021).

4. Conclusions

The extraction condition used in the study produced the highest protein concentrate levels (corn = 55.69%, chickpea = 71.23%, and bean = 81.10%. The chickpea and bean concentrates showed a higher percentage of peptides of low molecular weight (<1.35 kDa: 56.57% chickpeas; 35.91% beans), whereas, in the corn concentrates, the highest percentage of peptides ranged from 1.35 to 17 kDa (46.34%). The flours presented a higher concentration of peptides with a molecular weight of 1.35 to 17 kDa. The extraction process showed that the phytic acid and saponin levels were higher in chickpea, bean, and corn concentrates. In contrast, the trypsin inhibitors showed a diminution in the chickpea and corn concentrates. Due to the presence of nutritional properties in the chickpea and bean concentrates, such as high levels of

protein and low-weight peptides, these can become of great interest to the food industry because they are proposed to have better digestibility.

Nevertheless, the all-year-round availability of discarded grains could be a limitation for farm feed producers. It is also recommended to perform more studies on the production of corn concentrates because, despite obtaining protein levels above those of the initial flour, the presence of ANFs like phytic acid and saponins in higher amounts could lead to nutritional and palatability disadvantages. The yield obtained from the protein concentrates is superior to that previously reported; it is of great importance since a methodology is proposed for protein concentrate production from discharged raw material. As part of the agri-food industry, discarded grains like chickpeas, beans, and corn, present quality standards below those required for human consumption and marketing and are considered by-products that can be used for the farm-animals feed-producing industry. These ingredients represent both an economic and an environmental advantage because the increase in their utilization could be a guideline to reduce the dependence on animal-origin proteins.

CRediT authorship contribution statement

Griselda Karina González-Félix: Writing – original draft, writing - reviewing, Formal analysis. **Silvia Luna-Suárez:** Conceptualization, Methodology, reviewing. **Manuel García-Ulloa:** Writing – review & editing. **Emmanuel Martínez-Montaño:** Methodology, reviewing. **Fernando Barreto-Curiel:** Methodology, reviewing. **Hervey Rodríguez-González:** Investigation, Project administration, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hervey Rodriguez Gonzalez reports financial support, administrative support, equipment, drugs, or supplies, statistical analysis, travel, and writing assistance were provided by National Polytechnic Institute.

Data availability

No data was used for the research described in the article.

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