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Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility

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ABSTRACT

The growing world population and its environmental impact motivate searching for new protein sources for the human diet. Agro-industrial by-products are potential sources due to high protein content. This study characterized meals from five sources (pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed), about the proximate composition, antinutritional factors (ANFs), amino acid profile (AA), and in vitro protein digestibility (IVPD). These by-products present protein content up to 40% and IVPD between 70-85%. ANFs results presented a low phytic acid content for all seed meals and high tannins content in grapeseed meal. In terms of essential AA, the chia seed meal did not show any deficiency. In contrast, the first limiting AA in sesame meal and brown flaxseed meal was lysine, and in pumpkin seed meal, grapeseed meal, and flaxseed meal were sulfur amino acids. These agro-industrial by-products are alternatives for replacing animal protein sources due to recovering high-quality proteins, minimizing adverse environmental impacts, and conserving scarce natural resources.

1. Introduction

Nowadays, food security is a primary challenge for humankind. The rising world population (about 10 billion by 2050) and limited environmental resources demand proteins from sustainable and renewable sources, searching for alternative foods (Kumar et al., 2021b; Yuliarti et al., 2021). Plant proteins, insects, algae, and by-products from food processing are novel protein sources that can meet this macronutrient's required intake (Sá et al., 2020, 2019). Besides, in terms of sustainability and carbon footprint, the plant proteins from food by-products are ideal for substituting animal protein sources.

Oilseed meals (also called oilseed press cakes), which are the by-products from edible oil industries after oil extraction, are underestimated as protein sources for human consumption (Kotecka-Majchrzak et al., 2020). Often discarded or conventionally used as feedstock for animal feed or as fertilizer, these by-products can present a high protein content (15 to 50 %) (Vinayashree and Vasu, 2021). Many studies evaluated oilseed by-products proteins as promising sources of protein for human nutrition and animal protein replacement, such as sunflower seed, rapeseed (or canola) (Jia et al., 2021), flaxseed, sesame seed (Terrien, 2017), pumpkin seed (Vinayashree and Vasu, 2021), mustard seed (Chakraborty et al., 2021), grapeseed (Fantozzi, 1981; Kamel et al., 1985), cottonseed (Kumar et al., 2021a), and peanut (Terrien, 2017). Furthermore, these oilseed by-products have great potential as an economic source of fatty acids and bioactive metabolites. Studies demonstrated the presence of significant amounts of carotenoids, phenolic compounds, tocopherols, and phytosterols in pumpkin seeds (Rabrenović et al., 2014; Veronezi and Jorge, 2012); linolenic and linoleic acid, and lignans in flaxseeds (Shim et al., 2014); polyunsaturated fatty acids and tocopherols in chia seeds (Gahfoor et al., 2018); phytosterols, polyunsaturated fatty acids, tocopherols, and lignans (e.g., phenylpropanoid compounds) in sesame seeds (Pathak et al., 2014); linolenic and linoleic acid, tocopherols, and catechins in grapeseeds (Al Juhaimi and Özcan, 2018). Also, these by-products may be called health foods which provide health benefits to consumers and can be used as food supplements because of their nutrients (Sunil et al., 2016). Therefore, the use of agricultural food by-products is a feasible alternative that can reduce waste disposal and increase limited sources of bioactive compounds and non-animal proteins (Pojić et al., 2018).

In this way, the hypothesis in this study is that these residues as high nutritional value proteins for human nutrition. To the best of the authors' knowledge, information about the nutrition quality of pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals is scarce. Also, no data is presented in the literature concerning the protein digestibility of the by-products, which brings the novelty of this study.

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These oilseed meals were selected based on an extensive literature review on the nutritional quality of plant proteins (Sá et al., 2020). Therefore, the present work aimed to determine the chemical composition, the presence of antinutritional factors (ANFs), the in vitro protein digestibility (IVPD), the amino acid (AA) profile, and the Amino Acid Score of the oilseed by-products.

2. Materials and methods

2.1. Chemicals

The chemicals used in this study are n-hexane (99% P.A., Neon®), ethyl ether (99.8%, Anidrol®), sulfuric acid (P.A., Anidrol®), acetic acid (P.A., Neon®), boric acid (P.A., Neon®), hydrochloric acid (P.A., Neon®), thioglycolic acid (P.S., Neon®), ethanol (99.5%, Anidrol®), methanol (P.A., Neon®), 2,2′-bipyridyl (purity 99%, Sigma, St. Louis, MO, USA), BAPNA (purity ≥ 98%, Sigma, St. Louis, MO, USA), selenium dioxide (purity > 99%, Sigma, St. Louis, MO, USA), dimethyl sulfoxide (P.A., Neon®), vanillin (Neon®), catechin hydrate (purity ≥ 96%, Sigma, St. Louis, MO, USA), copper sulfate II pentahydrate (P.A., Neon®), sodium hydroxide (P.A., Neon®), ammonium iron(III) sulfate dodecahydrate (purity 99%, Sigma, St. Louis, MO, USA), and sodium salt hydrate of phytic acid (≥ 90% phosphorus, Sigma, St. Louis, MO, USA).

2.2. Sample Collection

Pumpkin seed (*Cucurbita moschata*) protein meal (PSM) and brown flaxseed (*Linum usitatissimum*) meal (FM1) was kindly provided by Vital Âtman Ltda., São Paulo, Brazil. Flaxseed meal (FM2) was donated by Cisbra Ltda., Rio Grande do Sul, Brazil. Chia seed (*Salvia hispanica*) meal (CSM) was kindly provided by Agropecuaria Produza S.A., Paraguay. Sesame seed (*Sesamum indicum* L.) meal (SSM) was donated by Sésamo Real Ind. Com. Prods. Alims. Ltda., São Paulo, Brazil. Grapeseed (*Vitis labrusca*) meal (GSM) and grapeseed meal flour (GSF) was kindly provided by Econatura Produtos Ecológicos e Naturais Ltda., Rio Grande do Sul, Brazil. The oilseed industries cited above employ cold-pressing extraction to obtain oil from the seeds, without organic solvents. The samples were ground and stored at -18 °C for further analysis.

2.3. Analytical Methods

2.3.1. Proximate composition

The proximate analysis of the raw oilseed by-products was carried out using official AOAC procedures (2012): moisture gravimetrically at 105 °C for 24 h (method 925.09); ash by calcination using a muffle furnace at 550 °C (923.03); lipid gravimetrically after n-hexane extraction (920.39); nitrogen by standard Kjeldahl method (954.01); and crude fiber (962.09). All determinations were performed in triplicates. Protein composition was calculated as nitrogen value multiplied by 6.25 as the conversion factor (AOAC International, 2012), and total carbohydrate content on a dry basis was estimated by calculating the percentile difference to crude proteins, lipids, ashes, and fibers. The Atwater conversion factors of 9 kcal/g (for lipids) and 4 kcal/g (for proteins and carbohydrates) (FAO, 2003) were used to estimate the energy value of the samples.

2.3.2. Antinutritional factors

2.3.2.1. Trypsin inhibitors. The determination of trypsin inhibition activity was performed according to Kakade et al. (1974). The trypsin assay contained trypsin from the bovine pancreas (salt-free lyophilized powder, ≥ 10.000 BAEE units/mg of protein, product no. T1426, Sigma, Chemical, St. Louis, MO, USA) and BAPNA reagent (Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride, purity ≥ 98%, product no. B4875, Sigma, Chemical, St. Louis, MO, USA) as substrates. One gram of finely ground sample (80 mesh) was extracted with 50 mL of NaOH 0.01 M for 3 h at room temperature. Supernatant aliquots of 1 mL were pipet into tubes, and 1 mL of distilled water was added. Distilled water (2 mL) was used as a reagent blank. Extracts were incubated with 2 mL of trypsin solution (0.02 mg/mL in 0.001 M HCl) and 5 mL of BAPNA reagent (0.4 mg/mL in Tris-buffer pH 8.2, containing $CaCl_2$) in a water bath at 37 °C. After 10 min, 1 mL of 30% (v/v) acetic acid was added to terminate the reaction. Trypsin inhibitor activity (TIA) was spectrophotometrically determined at 410 nm (UV/VIS spectrophotometer, Hitachi U-1900) against a reagent blank. The trypsin inhibition activity (TIA) was expressed as the trypsin inhibition unit (TIU) per milligram of the sample.

2.3.2.2. Tannins. The tannin content was estimated by the colorimetric method of vanillin-HCl, as described by Burns (1971). The tannins were extracted for 24 h at room temperature, which 1 g of finely ground sample (80 mesh) was mixed with 50 mL of methanol. Supernatant aliquots of 1 mL were pipet into tubes, and 5 mL of vanillin-HCl reagent was added. Then, the colored solution was measured spectrophotometrically at 500 nm (UV/VIS spectrophotometer, Hitachi U-1900). Catechin ((+)-Catechin hydrate, purity ≥ 96%, product no. 22110, Sigma, Chemical, St. Louis, MO, USA) was used as the reference standard, and the tannin concentration was expressed in mg catechin per gram of sample.

2.3.2.3. Phytic acid. The phytic acid content was estimated by the methodology of Haug and Lantzsch (1983). The samples were extracted for 24 h at room temperature, which 1 g of finely ground sample (80 mesh) was mixed with 50 mL of 0.2 N HCl. Supernatant aliquots of 0.5 mL were pipet into tubes, and 1 mL of ferric solution (ammonium iron(III) sulfate dodecahydrate, purity 99%, product no. 221260, Sigma, Chemical, St. Louis, MO, USA) was added, and tubes were put in a boiling water bath (100 °C) for 30 min. At room temperature, 1.5 mL of the 2,2′-bipyridine (purity ≥ 99%, product no. D216305, Sigma, Chemical, St. Louis, MO, USA) solution was added. Then, the colored solution was measured spectrophotometrically at 519 nm (UV/VIS spectrophotometer, Hitachi U-1900). Sodium salt hydrate of phytic acid (≥ 90% phosphorus, product no. 68388, Sigma, Chemical, St. Louis, MO, USA) was used as a phytate reference solution for the standard calibration curve. The phytic acid was estimated as μg per gram of sample.

2.3.3. In vitro protein digestibility (IVPD)

The Hsu et al. (1977) method with minor modifications (Tinus et al., 2012) was used to determine the IVPD of oilseed by-products. The protein suspension (6.25 mg/mL of distilled water) was adjusted to pH 8.0 with 0.1 N NaOH or 0.1 M HCl while stirring at 37°C. An enzyme mix containing 1.6 mg of trypsin (porcine pancreatic trypsin type IX-S, 13.000-20.000 BAEE units/mg protein, product no. T0303, Sigma, Chemical, St. Louis, MO, USA), 1.3 mg of peptidase (porcine gastric mucosa pepsin, 3.200–4.500 units/mg protein, product no. P6887, Sigma, Chemical, St. Louis, MO, USA), and 3.1 mg of α-chymotrypsin (bovine pancreatic chymotrypsin type II, ≥ 40 units/mg protein, product no. C4129, Sigma, Chemical, St. Louis, MO, USA) per mL was maintained in an ice-bath and adjusted to pH 8.0. The enzymatic solution was added to the protein solution at a 1:10 v/v ratio and stirred at 37 °C. A rapid decrease in pH value occurred due to the amino acid carboxyl groups releasing from the protein chain by the proteolytic enzymes. The pH mixture was measured after 10 min using a portable pH meter (model testo 205, Testo Instrument Co.). IVPD as a percentage of digestible protein was estimated according to pH variation after 10 min (ΔpH_{10min}), as shown in Equation 1.

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10\,min} \quad (1)$$

2.3.4. Amino acid composition

The determination of total amino acids of the raw material was performed by reverse phase column (C18 from Phenomenex) chromatography in a high-performance liquid chromatograph (HPLC, SHIMADZU®),

Table 1
Proximate composition and energy values of oilseed by-products (raw samples).

Samples	Moisture (%)	% Dry weight basis					Energy (kcal/100g)
		Protein[1]	Lipids	Ash	Crude fiber	Carbohydrate[2]	
PSM	8.84 ± 0.02^{f}	40.9 ± 0.1^{d}	14.1 ± 0.1^{c}	4.92 ± 0.01^{c}	27.5 ± 0.5^{c}	12.57	340.87
FM1	10.14 ± 0.06^{g}	28.6 ± 0.7^{a}	11.6 ± 0.1^{b}	5.57 ± 0.03^{d}	11.9 ± 0.6^{b}	42.33	388.12
FM2	8.42 ± 0.01^{e}	28.3 ± 0.8^{a}	13.6 ± 0.6^{bc}	6.08 ± 0.01^{e}	10.3 ± 0.5^{ab}	41.72	402.47
CSM	8.06 ± 0.05^{d}	27.5 ± 0.8^{a}	5.4 ± 0.4^{a}	7.15 ± 0.05^{f}	26.7 ± 0.9^{c}	33.25	291.60
SSM	4.27 ± 0.03^{a}	35.3 ± 0.7^{c}	32.8 ± 0.1^{d}	8.26 ± 0.06^{g}	8.2 ± 0.1^{a}	15.44	498.16
GSM	4.58 ± 0.01^{b}	9.4 ± 0.3^{b}	7.1 ± 0.5^{a}	2.31 ± 0.01^{a}	58.6 ± 0.7^{e}	22.59	191.86
GSF	5.34 ± 0.02^{c}	9.9 ± 0.1^{b}	5.8 ± 0.1^{a}	2.48 ± 0.01^{b}	51.4 ± 0.4^{d}	30.42	213.48

All values are means ± standard deviation.
$^{a–g}$ Different letters in the same column indicate a significant difference ($p < 0.05$ by Tukey's test).
[1] N x 6.25.
[2] The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight - (g crude protein + g lipids + g ash + g crude fiber)].
PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

according to the method described in Hagen et al. (1989). The release of individual amino acids occurs in acid hydrolysis at 110 °C for 22 h, using 6 M of hydrochloric acid and phenol solutions. After the hydrolysis, α-aminobutyric acid (Sigma-Aldrich®, St. Louis, MO, USA) is added as an internal standard. The identification of the amino acids was performed by comparison with an external standard (Pierce, PN 20088). The internal standard α-aminobutyric acid was used for the quantification of total amino acids, according to White et al. (1986) method.

The amino acid composition of the raw samples was used to estimate the Amino Acid Score as [mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern] × 100 (WHO/FAO/UNU Expert Consultation, 2007). The lowest Amino Acid Score calculated reflects the first limiting amino acid in the protein source (Nosworthy et al., 2017).

2.4. Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc.) was used to perform the experimental data statistical analysis, adopting a confidence level of 95% in all cases. The Tukey's test was used to compare the chemical composition, ANFs, and IVPD of oilseed by-products. Results are expressed as mean ± standard deviation of replicated samples.

3. Results and discussions

3.1. Proximate composition

The chemical composition and energy values of the oilseed by-products are presented in Table 1. In terms of protein content, the dry weight basis results for PSM are higher in comparison for edible whole pumpkin seeds reported in the literature (30.2–36.5%) (El-Adawy and Taha, 2001; Rogerson, 2017) and fibers (4.4–12.1%) (El-Adawy and Taha, 2001; García-Aguilar et al., 2015). For FM1 and FM2, fiber and protein content are higher than edible whole flaxseed reported in the literature (4.8% and 20.3%, respectively) (Kajla et al., 2015). Wu et al. (2012) also studied the composition of flaxseed meal, and the protein content was higher (32.7%) than the results presented here. The CSM and SSM results for protein content are similar to those found in the literature for edible whole chia seeds (Olivos-Lugo et al., 2010) and sesame seeds. The protein results for GSM and GSF are also similar to other studies (8.2–11.8%) (Fantozzi, 1981; Kamel et al., 1985). The PSM, FM1, FM2, CSM, and SSM contain high protein content (35–41%). Similar results are reported in the literature for oilseed meals (up to 50%) (Sarker et al., 2015; Terrien, 2017). These results are also comparable to other meals, such as watermelon seed (27.6%) (Lakshmi and Kaul, 2011), rapeseed (32.8%) (Jia et al., 2021), black mustard seed (38.2%), and yellow mustard seed (28.8%) (Sarker et al., 2015). Furthermore, comparing these sources to the traditional plant protein sources in the human diet, soybean, common beans, and peas present protein content of 35.3%, 19.9%, and 21.7%, respectively (Terrien, 2017).

Table 2
Protein, ash, and fiber composition of oilseed by-products in a dry weight and lipid-free basis.

Samples	% Dry weight and lipid-free basis		
	Protein	Ash	Crude fiber
PSM	47.6	5.7	32.0
FM1	32.4	6.3	13.5
FM2	32.8	7.0	11.9
CSM	29.1	7.6	28.2
SSM	52.5	12.3	12.2
GSM	10.1	2.5	63.0
GSF	10.5	2.6	54.4

PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

Nevertheless, the protein intake in the human diet is predominantly animal-based proteins, such as UHT milk (3.5% of protein in whole product (w.p.) / 27.8% in a dry weight basis (d.b.)) (Pestana et al., 2015), eggs (6.5% (w.p.) / 10.9% (d.b.)) (Murcia et al., 1999), meat from chicken breasts (20.9% (w.p.) / 58.6% (d.b.)) (Fakolade, 2015), and meat from beef steaks (23.1% (w.p.)/ 84.6% (d.b.)) (Wahrmund-Wyle et al., 2000). Therefore, the results presented here for the PSM, FM1, FM2, CSM, and SSM show the potential of these residues as high protein sources in food formulations and human nutrition.

Additionally, all the oilseed meals present high amounts of dietary fibers (8–59 g/100 g). In terms of dietary fibers, a high source contains > 6 g/100 g (WHO, 2004); therefore, all by-products evaluated in this work are considered a high dietary fiber source.

In terms of vegetable materials, it is well known that any genotype composition can vary depending on the climate, production site, soil type, cultural practices, and even the process of oil extraction (e.g., the use of high or low temperatures, presence of organic solvents, equipment features, pressing capacity, among others conditions), which could bring significant differences on the composition of these oilseeds. This uncertainty in genotype expression may justify some differences presented in this work compared to the literature. The oilseed meal samples were obtained employing cold-pressing extraction without organic solvents. This extraction technique presents great advantages (e.g., higher quality of the oil extracted); however, it can present lower oil yields than oil extraction using high temperature and organic solvents. The results of

Table 3
Antinutritional factors concentration and *in vitro* protein digestibility of oilseed by-products (raw samples).

Samples	TIA (TIU/mg sample)	Tannins (mg catechin/g sample)	Phytic acid (μg/g sample)	IVPD (%)
PSM	12.7 ± 1.0^{b}	n.d.^{a}	37.0 ± 0.1^{e}	85.4 ± 0.5^{b}
FM1	30.8 ± 3.0^{a}	n.d.^{a}	28.1 ± 0.1^{b}	83.3 ± 0.1^{ab}
FM2	33.6 ± 4.0^{a}	n.d.^{a}	27.0 ± 0.8^{b}	83.9 ± 0.1^{ab}
CSM	11.0 ± 1.0^{b}	n.d.^{a}	18.7 ± 0.8^{c}	81.1 ± 0.8^{a}
SSM	39.4 ± 4.0^{a}	n.d.^{a}	23.0 ± 0.4^{d}	81.4 ± 0.2^{a}
GSM	29.2 ± 1.0^{a}	282 ± 6^{c}	n.d.^{a}	70 ± 1.0^{c}
GSF	36.9 ± 2.0^{a}	163 ± 8^{b}	n.d.^{a}	71 ± 2.0^{c}

n.d. = not detected.
All values are means ± standard deviation.
$^{a–e}$ Different letters in the same column indicate a significant difference ($p < 0.05$ by Tukey's test).
PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

Table 4
Amino acid composition (g/100 g of protein) of the raw oilseed by-products.

AA composition (g/100 g protein)	Samples						
	PSM	FM1	FM2	CSM	SSM	GSM	GSF
Essential (EAA)							
Histidine (His)	1.48 ± 0.06^{AB}	2.47 ± 0.01^{C}	1.32 ± 0.05^{A}	2.10 ± 0.40^{BC}	2.56 ± 0.01^{C}	1.69 ± 0.04^{AB}	1.71 ± 0.05^{AB}
Isoleucine (Ile)	4.05 ± 0.03^{A}	4.50 ± 0.01^{C}	4.62 ± 0.03^{D}	4.01 ± 0.01^{A}	3.99 ± 0.01^{A}	4.38 ± 0.01^{B}	4.38 ± 0.01^{B}
Leucine (Leu)	6.60 ± 0.03^{D}	5.97 ± 0.01^{B}	6.21 ± 0.01^{C}	6.77 ± 0.05^{A}	6.72 ± 0.04^{A}	7.28 ± 0.01^{E}	7.43 ± 0.02^{F}
Lysine (Lys)	4.66 ± 0.02^{E}	4.11 ± 0.01^{C}	4.27 ± 0.01^{D}	4.87 ± 0.05^{F}	3.00 ± 0.01^{B}	3.67 ± 0.01^{A}	3.61 ± 0.01^{A}
Threonine (Thr)	1.39 ± 0.08^{B}	4.04 ± 0.01^{A}	4.19 ± 0.01^{A}	3.95 ± 0.02^{A}	3.85 ± 0.01^{A}	1.80 ± 0.20^{BC}	2.00 ± 0.20^{C}
Valine (Val)	4.69 ± 0.03^{A}	5.32 ± 0.01^{C}	5.40 ± 0.03^{C}	4.91 ± 0.03^{D}	4.75 ± 0.01^{A}	5.15 ± 0.01^{B}	5.19 ± 0.01^{B}
Total sulfur amino acids (Met + Cys)	1.30 ± 0.05^{A}	3.39 ± 0.02^{B}	1.70 ± 0.10^{A}	3.65 ± 0.08^{BC}	4.50 ± 0.20^{C}	1.20 ± 0.50^{A}	0.84 ± 0.01^{A}
Total aromatic amino acids (Phe + Tyr)	10.09 ± 0.04^{D}	7.20 ± 0.01^{A}	7.56 ± 0.01^{AB}	8.75 ± 0.05^{C}	8.48 ± 0.02^{BC}	7.50 ± 0.02^{AB}	7.00 ± 0.70^{A}
Non-essential (NEAA)							
Alanine (Ala)	3.63 ± 0.05^{C}	4.69 ± 0.01^{A}	4.82 ± 0.01^{A}	5.14 ± 0.02^{D}	4.65 ± 0.02^{A}	4.30 ± 0.10^{B}	4.34 ± 0.09^{B}
Arginine (Arg)	14.00 ± 1.00^{C}	10.00 ± 0.01^{A}	10.46 ± 0.04^{A}	10.99 ± 0.06^{A}	13.46 ± 0.02^{C}	7.85 ± 0.01^{B}	7.82 ± 0.02^{B}
Aspartic acid (Asp)	11.94 ± 0.03^{E}	11.26 ± 0.02^{C}	11.39 ± 0.04^{D}	10.21 ± 0.03^{A}	9.61 ± 0.02^{B}	10.10 ± 0.03^{A}	10.17 ± 0.02^{A}
Glutamic acid (Glu)	20.40 ± 0.10^{A}	21.31 ± 0.01^{C}	21.88 ± 0.03^{D}	19.30 ± 0.10^{B}	20.56 ± 0.04^{A}	25.40 ± 0.05^{E}	25.69 ± 0.04^{F}
Glycine (Gly)	7.30 ± 0.70^{A}	6.52 ± 0.01^{A}	6.57 ± 0.01^{A}	5.23 ± 0.08^{B}	5.25 ± 0.02^{B}	9.39 ± 0.04^{C}	9.60 ± 0.10^{C}
Proline (Pro)	3.65 ± 0.01^{A}	4.06 ± 0.02^{B}	4.36 ± 0.02^{C}	4.23 ± 0.01^{BC}	3.82 ± 0.03^{A}	5.40 ± 0.08^{D}	5.68 ± 0.09^{E}
Serine (Ser)	4.90 ± 0.20^{A}	5.15 ± 0.03^{AB}	5.16 ± 0.01^{AB}	5.92 ± 0.03^{B}	4.82 ± 0.02^{A}	4.89 ± 0.02^{A}	4.60 ± 0.50^{A}
Total EAA (g/100 g protein)	34.26 ± 0.07^{C}	37.01 ± 0.01^{B}	35.30 ± 0.10^{D}	39.00 ± 0.10^{E}	37.80 ± 0.20^{B}	32.70 ± 0.30^{A}	32.20 ± 0.60^{A}
Total NEAA (g/100g protein)	65.74 ± 0.07^{E}	62.99 ± 0.01^{A}	64.70 ± 0.10^{D}	61.00 ± 0.10^{C}	62.20 ± 0.20^{A}	67.30 ± 0.30^{B}	67.80 ± 0.60^{B}
Total AA (g/100g sample)	36.10 ± 0.10^{F}	28.93 ± 0.02^{A}	29.09 ± 0.08^{A}	25.90 ± 0.20^{D}	34.40 ± 0.06^{E}	9.12 ± 0.01^{B}	9.67 ± 0.02^{C}

$^{A–F}$ Different letters in the same line indicate a significant difference between the raw samples for each amino acid ($p < 0.05$ by Tukey's test).
PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

lipids (on a dry weight basis) demonstrated that oil residual is still presented in the oilseed meals, where SSM has the higher content (32.8%), and CSM has the lower content (5.4%), which dilutes the concentration of other nutrients in the proximate composition. The elimination of the oil residual factor will increase the concentration of the other constituents. Therefore, the concentrations of protein, ash, and crude fiber on a lipid-free basis were calculated, and the results are presented in Table 2.

3.2. Antinutritional factors

The presence of trypsin inhibitors, phytates, and tannins in food by-products from plant origin are unfavorable for protein digestion; therefore, they must be removed to increase protein digestibility (Sá et al., 2019). The significance of phytates and tannins lies in the extent of their influence on the bioaccessibility of minerals, and the trypsin inhibitors, as the term itself indicate, inhibit protein absorption on binding with proteases (Lakshmi and Kaul, 2011). These compounds are naturally synthesized due to plant physiology, at the beginning of seed formation (trypsin inhibitors) or the plant healing process (tannins) and during maturation (phytic acid) (Sá et al., 2019). The characterization of the oilseed by-products in terms of these so-called antinutritional factors, such as trypsin inhibition activity, the tannin, and phytic acid concentration, is shown in Table 3.

The raw residues from the oil extraction industries showed elevated trypsin inhibitor activity (11–39.4 TIU/mg). These results are higher in comparison for rapeseed meal (1.74 TIU/mg) (Mansour et al., 1993) and other oilseeds, such as paprika seed flour (1.96 TIU/mg), watermelon seed flour (1.46 TIU/mg), and pumpkin seed flour (1.39 TIU/mg) (El-Adawy and Taha, 2001). The trypsin inhibitor activities for oilseed by-products are also higher when compared to some traditional sources of plant proteins, such as pea (1.84–2.2 TIU/mg) (Frias et al., 2011) and lentil (5.12 TIU/mg) (Samaranayaka, 2017). The results are similar to those found for common beans (18.1 TIU/mg) (Nikmaram et al., 2017). However, the TIA presented here for oilseed residues are lower than soybean (41.5–96.9 TIU/mg) (Lusas and Rhee, 1995; Samaranayaka, 2017), *Mucuna pruriens* seeds (78.7 TIU/mg) (Siddhuraju et al., 1996), and karkade seed flour (41 TIU/mg) (Abu-tarboush and Ahmed, 1996).

The highest level of tannin was noticed in GSM (282 mg/g), which was already expected due to the grape be a rich source of tannins. The other oilseed by-products did not contain tannins. The results for GSM and GSF were higher in comparison to rapeseed meal (9–15 mg/g) (Wanasundara et al., 2017), and other oilseeds, such as pumpkin seed

Table 5
Amino Acid Score according to the WHO/FAO/UNU requirement pattern for adults (g/100g protein), of the raw oilseed by-products.

Amino acids	Requirement pattern[1] (g/100g protein)	Amino Acid Score (%)[2]						
		PSM	FM1	FM2	CSM	SSM	GSM	GSF
		Essential						
Histidine (His)	1.5	99 ± 4^{AB}	165 ± 1^{C}	88 ± 4^{A}	138 ± 26^{BC}	170 ± 1^{C}	113 ± 3^{AB}	114 ± 3^{AB}
Isoleucine (Ile)	3.0	135 ± 1^{A}	150 ± 1^{C}	154 ± 1^{D}	134 ± 1^{A}	133 ± 1^{A}	146 ± 1^{B}	146 ± 1^{B}
Leucine (Leu)	5.9	112 ± 1^{D}	101 ± 1^{B}	105 ± 1^{C}	115 ± 1^{A}	114 ± 1^{A}	123 ± 1^{E}	126 ± 1^{F}
Lysine (Lys)	4.5	104 ± 1^{E}	91 ± 1^{C}	95 ± 1^{D}	108 ± 1^{F}	67 ± 1^{B}	82 ± 1^{A}	80 ± 1^{A}
Threonine (Thr)	2.3	61 ± 4^{B}	176 ± 1^{A}	182 ± 1^{A}	172 ± 1^{A}	167 ± 1^{A}	80 ± 11^{BC}	89 ± 10^{C}
Tryptophan (Trp)	0.6	-	-	-	-	-	-	-
Valine (Val)	3.9	120 ± 1^{A}	137 ± 1^{C}	139 ± 1^{C}	126 ± 1^{D}	122 ± 1^{A}	132 ± 1^{B}	133 ± 1^{B}
Total sulfur amino acids (Met + Cys)	2.2	59 ± 3^{A}	154 ± 1^{B}	80 ± 5^{A}	166 ± 3^{BC}	204 ± 10^{C}	53 ± 24^{A}	38 ± 1^{A}
Total aromatic amino acids (Phe + Tyr)	3.8	266 ± 1^{D}	190 ± 1^{A}	199 ± 1^{AB}	230 ± 1^{C}	223 ± 1^{BC}	197 ± 1^{AB}	184 ± 19^{A}
First limiting amino acid	-	Met ± Cys	Lys	Met ± Cys	-	Lys	Met ± Cys	Met ± Cys

$^{A–F}$ Different letters in the same line indicate a significant difference between the raw samples for each essential amino acid ($p < 0.05$ by Tukey's test).
PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.
[1]WHO/FAO/UNU (2007) Expert Consultation Report for adults.
[2]Amino Acid Score: (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern) × 100.

flour (1.7 mg/g), watermelon seed flour (2.4 mg/g), and paprika seed flour (4.8 mg/g) (El-Adawy and Taha, 2001). The concentration of tannin in GSM is also higher when compared to traditional sources of plant proteins, such as pea (2.06 mg/g) (Frias et al., 2011) and common bean (0.65 mg/g) (Espinosa-Páez et al., 2017).

The highest level of phytic acid was noticed in PSM (0.0037 g/100 g). This result is lower than those found in the studies for pumpkin seed flour (2.37 g/100 g) (El-Adawy and Taha, 2001) and pumpkin seeds (0.299 g/100 g) (Giami, 2004). The results presented here, in terms of phytic acid composition, are also lower than other oilseed meals, such as watermelon seed (0.99 g/100 g) (Lakshmi and Kaul, 2011), and rapeseed (or canola) (3.3 g/100 g) (Wanasundara et al., 2017), and traditional sources of plant proteins, such as pea (0.35–1.19 g/100 g) (Frias et al., 2011), soybean (1–2 g/100 g) (Gilani and Lee, 2003), chickpea (0.12–1.5 g/100 g) (Dadon et al., 2017), common bean (1.59 g/100 g) (Alonso et al., 2000), and rice (0.74 g/100 g) (Albarracín et al., 2015). The low phytic acid content may be a consequence of the oil extraction processing that changes the original chemical composition due to chemical affinity. This result highlights the importance of the oil extraction step on improving protein digestion by reducing this ANF.

Results appointed in this study are different from those into the literature, which could be directly associated with the oil extraction process leading to remove some oilseeds ANFs. However, it does not exclude the intrinsic differences due to climate, soil type, production site, cultural practices, and others. At the present moment, to the best of the authors' knowledge, no data in the literature was reported about the composition of antinutritional factors in pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals. The antinutritional factors evaluated in terms of the content of phytic acid, tannins, and trypsin inhibitor activity indicated the PSM, FM1, FM2, CSM, SSM, GSM, and GSF as promising sources of proteins for humans.

3.3. In vitro protein digestibility (IVPD)

The IVPD is a useful tool for evaluating the nutritive quality of a food protein, combined with the amino acid composition and bioavailability (Sá et al., 2019). The results of IVPD for all the raw oilseed meals evaluated are shown in Table 3.

The protein digestibility presented significant differences ($p < 0.05$) among the by-product raw samples, where PSM presented the highest IVPD (85%), and the GSM and GSF presented the lowest (70%). The results of IVPD presented here for the PSM were higher compared to another study (71.3%) (Venuste et al., 2013). The same occurred to the results of FM1 and FM2, where Wu et al. (2012) found 66% of IVPD. The result for CSM was similar to those found in the literature for chia seeds (77.5%) (López et al., 2018); and results for GSM and GSF were similar to grapeseeds (58–77%) reported in the literature (Fantozzi, 1981). The IVPD result for the SSM was also higher than another study (74.1%) (El-Adawy, 1995).

All by-products IVPD results presented in this work are similar to the protein digestibility of other kinds of residues, such as black and yellow mustard cakes (80.3% and 77.4%, respectively) (Sarker et al., 2015). These results corroborate the potential of these oilseed by-products to be an alternative protein source for human consumption.

3.4. Amino acid composition

The amino acid (AA) composition of the raw samples of oilseed by-products is presented in Table 4. The total AA content shows similarity to those results presented for protein content (Table 1), which corroborates the analysis's veracity. For each amino acid evaluated, the raw samples of oilseed by-products showed a statistical difference between them ($p < 0.05$). However, the total essential amino acids (EAA) and non-essential amino acids (NEAA) showed excellent results for these alternative sources of protein.

According to the Amino Acid Score, shown in Table 5, the oilseed meals have a good profile of EAA, although they presented some deficiency in some amino acids. Nevertheless, following the pattern of essential amino acids (WHO/FAO/UNU Expert Consultation, 2007), the CSM met the nutritional requirements entirely. The limiting amino acids of the PSM were sulfur amino acids (first limiting amino acid), threonine, and histidine. The first limiting amino acid for FM1 and SSM was lysine, and for FM2, GSM and GSF were sulfur amino acids.

The FM1, FM2, and SSM results were very close to those found for the whole flaxseed seed and the defatted sesame seed, respectively. Also, the CSM results in this study were higher than the chia seed after isolation procedures (Sá et al., 2020). However, at the present moment and to the best of the authors' knowledge, very few data in the literature was reported regarding the amino acid composition in pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals.

4. Conclusions

Among all the oilseed by-products evaluated, CSM has the most excellent amino acid profile, since it is a full source of essential amino acids. Other oilseeds by-products assessed are also good sources, pre-

senting the first limiting amino acid as the lysine (SSM and FM1) or sulfur amino acids Met and Cys (PSM, GSM, and FM1). The trypsin inhibitor activity in all by-products was similar to the value found in the primary sources of plant proteins. Tannins presented high content only in GSM, as expected, and, worthy of highlighting, the phytic acid concentration was lower than most plant protein sources in the literature. Furthermore, the protein digestibility ranged from 70 to 85%, a relatively high value for a plant protein source. Even so, additional processing interventions can improve these IVPD values, which is an opportunity for the food industry.

Pumpkin seed, flaxseed, brown flaxseed, chia seed, sesame seed, and grapeseed as by-products from the oil extraction industries are high nutritional value protein sources. They may be claimed as sustainable protein sources for human consumption due to three key factors: relatively low content of antinutritional factors, valuable content of essential amino acids, and good digestibility. They could become an extra income, minimize waste disposal, and be used as a technological ingredient for food formulations.

Declaration of Competing Interest

The authors state that they have no conflict of interests.

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