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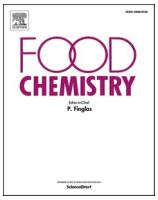

Transforming oilseed blends: the impact of low-moisture extrusion on antinutritional factors, protein structure, and nutritional value

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ABSTRACT

Oilseed cakes from hemp, rapeseed, and flaxseed are protein-rich, sustainable sources but are limited in food applications by antinutritional factors. This study blended meals from these oilseeds with pea or hemp protein ingredients (50:50 w/w) and applied low moisture extrusion (10 % and 20 %) at 122 °C to investigate their impact on physicochemical characteristics of oilseeds blends. Extrusion preserved protein content, reduced protein solubility by up to 44.5 %, and improved *in vitro* digestibility by up to 13.5 %. Antinutritional factors, including polyphenols (−10.18 % to −52.80 %), saponins (−4.48 % to −21.31 %), condensed tannins (−20.37 % to −41.05 %), and trypsin inhibitors (−2.26 TIU/mg to −13.31 TIU/mg), were significantly reduced, though phytic acid content was less affected. Extrusion decreased surface hydrophobicity, disrupted protein-protein interactions, altered secondary structures, and retained protein profiles under reducing conditions. These findings provided valuable scientific insights into the application of extrusion in enhancing nutritional value and modifying structure of plant-based meat alternatives.

1. Introduction

Meat production reached 350.75 million metric tons in 2024 and is expected to continue rising due to ongoing population growth (Statista, 2024). High levels of animal-based meat consumption have been associated with unhealthy dietary patterns (*e.g.*, high calorie and high fat) and adverse environmental impacts, including increased carbon footprint, land and water usage, and greenhouse gas emissions (González et al., 2020; Tuso et al., 2013). Therefore, development of plant-based meat alternatives is regarded as one of the most promising strategies to address the health and environmental concerns associated with traditional meat consumption. Currently, soy, pea and wheat proteins are the primary ingredients used in commercial plant-based meat products. These alternatives not only serve as nutritional substitutes for animal based meat but are also engineered to replicate its texture, flavour, and appearance, thereby providing a comparable sensory experience (Rubio et al., 2020).

Extrusion technology is a short-time, low-cost, continuous, energy-saving, and high-throughput processing, widely applied to produce plant-based meat analogues. The process of extrusion includes feeding, mixing, melting, die, and cooling. During this process, food materials are subjected to a combination of hydration, thermal, mechanical and pressure treatments (Bouvier & Campanella, 2014). High moisture extrusion (over 40 % moisture) is widely employed to create meat-like fibrous structure, which must be stored at chilled or frozen conditions (Guyony et al., 2022). Low moisture extrusion produces dried texturized plant proteins with spongy texture, which need to be rehydrated afterwards (Dekkers et al., 2018). Compared with the fibrous product produced by high-moisture extrusion, texturized plant proteins have a longer shelf-life due to their lower water activities, which limits microbial growth and reduces the rate of spoilage (Zhao et al., 2022).

Defatted oilseed meals are co-products of oil industry, often used as feedstock or fertilizer. They are underestimated protein sources with abundant bioactive and nutritional compounds and have not been widely used for human consumption (Usman et al., 2023). Oilseeds proteins are regarded as promising alternatives to animal proteins due to their good protein digestibility and abundance in sulphur amino acids (Kotecka-Majchrzak et al., 2020), However, lysine is present in

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relatively small amount in most oilseeds, such as sesame and flaxseed (Sá et al., 2021).

Proteins from a single plant-based protein source is usually not sufficient to provide a well-balanced amino acid composition. Therefore, combining multiple protein sources in food products is an important strategy to optimize amino acid profiles (Jiménez-Munoz, Tavares, et al., 2021). The combination of legumes (high in lysine and low in sulphur amino acids) and cereals (high in sulphur amino acids and low in lysine) is well-known to improve protein quality (Monnet et al., 2019). In addition, the amount of lysine, valine, isoleucine and leucine is limited in rapeseeds, but it can be compensated by pea protein to meet the standards (Zahari et al., 2021). This plant-based protein blend, with improved nutritional value, has been frequently used as ingredient for plant-based meat analogues production.

Nevertheless, plant-based protein ingredients contain various anti-nutritional factors (ANFs), such as phenolic compounds, phytic acid, tannins, saponins, oxalates, enzyme inhibitors, and lectins. Most ANFs present negative impacts on absorption of micronutrients (vitamins and minerals) and digestion of proteins (Manzanilla-Valdez et al., 2024). Therefore, food processing technologies are required to reduce or remove these compounds prior to human consumption. Traditional processing methods, including thermal treatment, soaking, germination, and fermentation, have been widely applied to improve nutritional quality (Samtiya et al., 2020). However, long processing time, undesirable change in functional properties and structure of protein and introduction of unwanted substances (*e.g.*, yeast and enzymes) are the drawbacks of these methods. Moreover, a single process cannot efficiently remove all the ANFs. Extrusion has been reported as a novel method for managing this issue. It heats the plant proteins for a short time at a high temperature and reduces the ANFs level without largely affecting the nutritional quality (Nikmaram et al., 2017). Using extrusion to reduce ANFs in oilseeds has been reported in the literature. For example, Vidal et al. (2022) reported that extrusion reduced polyphenol content in soybean and in sunflower by 17.5 % and by 32.9 %, respectively. In addition, tannins in linseed meal were reduced by up to 61.27 % after extrusion (Mukhopadhyay et al., 2007). However, the impact of extrusion on multiple ANFs in oilseeds or oilseed blends need to be studied systematically, taking the modification of physical and structural properties of proteins into consideration.

The aim of this study was to investigate the effects of low-moisture extrusion (10 % and 20 %) on six formulations: hemp, rapeseed, and flaxseed meals blended with either hemp protein concentrate or pea protein isolate. Specifically, this research aimed to evaluate: (1) the impact of low-moisture extrusion on antinutritional factors (ANFs) such as phytic acid, tannins, saponins, and trypsin inhibitors; (2) changes in protein and polyphenol content and molecular protein properties, including molecular weight profile by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and fast protein liquid chromatography (FPLC), solubility, secondary structure, protein-protein interactions, and protein quantity; and (3) the protein quality of the resulting ingredients, with a comprehensive assessment of amino acid profile, *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility-corrected amino acid score (IVPDCAAS), protein efficiency ratio (PER), amino acid score (AAS), biological value (BV), and essential amino acid index (EAAI). This study provides valuable insights into the nutritional and functional attributes of extruded oilseed blends for potential food applications.

2. Materials and methods

2.1. Materials

DL-dithiothreitol (DTT, BP172-5), methanol (M/4056/17), hydrochloric acid (H/1200/PB17), sulfuric acid (S/9240/PB17) and acetic acid glacial (A/0360/PB17) were purchased from Fisher Chemical (Loughborough, United Kingdom). Acetone (24201-2.5 L-M), formic acid (W248703-1KG-K), ethylenediaminetetraacetic acid (EDTA, E9884-100G), sodium phosphate monobasic monohydrate (S3522-500G), sodium phosphate dibasic (71640-1KG), Folin & Ciocalteu's phenol reagent (F9252-1 L), fast blue BB salt hemi (zinc chloride) salt (F3378-25G), sodium carbonate (31432-500G-R), gallic acid (G7384-100G), iron (III) chloride hexahydrate (236489-100G), 5-sulfosalicylic acid hydrate (390275-500G), citric acid (27109-100G0R), Nα-benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA, B4875-1G), 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS, 10417-5G-F), dimethyl sulfoxide (DMSO, 472301-1 L), trypsin from porcine pancreas (13,000–20,000 BAEE units/mg protein, T0303-1G), chymotrypsin from bovine pancreas (≥40 units/mg protein C4129), urea (U5128-5KG), protease from *Streptomyces griseus* (≥3.5 units/mg solid, P5147-1G) were purchased from Sigma-Aldrich (Gillingham, United Kingdom). Sodium hydroxide (28,244.262) and calcium chloride dihydrate (22,322.295) were purchased from VWR chemicals (Lutterworth, United Kingdom). Sodium chloride (0277.1000) was purchased from Avantor Sciences (Lutterworth, United Kingdom). Sodium phytate (sc-203,329) and ammonium sulfate (sc-29085 A) were purchased from ChemCruz® biochemicals (TE Huissen, the Netherlands). Diosgenin (012718) and dithiothreitol (DTT, M02712) were purchased from fluorochem (Hadfield, United Kingdom). Vanillin (BS-6341P) was purchased from BioServ™ (Rotherham, United Kingdom). Catechin (PHR1963) was purchased from Merck (Gillingham, United Kingdom). Ammonium hydroxide (255210010) was purchased from Acros Organics B.V.B.A (Kirtlington, United Kingdom). Potassium permanganate solution (4.80160.2500) was purchased from Supelco® (Gilliingham, United Kingdom).

2.2. Extrusion process

Raw and extruded protein blends used in this project were final products from an industrial trial performed by SPG innovation (Nottingham, United Kingdom). Briefly, cold-pressed defatted hemp (oil content: 5.27 ± 0.48 %), rapeseed (oil content: 7.12 ± 0.69 %), or flaxseed (oil content: 4.68 ± 0.39 %) meals were mixed with pea protein isolate or hemp protein concentrate at a ratio of 50:50 (w/w). These protein blends were extruded using a MPF24 Twin screw extruder (Baker Perkin Ltd., Peterborough, United Kingdom). The oilseed blends were pre-heated at 80 °C and subsequently extruded at 122 °C. The screw speed was maintained within a range of 500–800 rpm, the rate of powder feed was 8.0 kg/h, and 10 % or 20 % (w/w) moisture were added. Increasing the feed moisture to 20 % in flaxseed blends resulted in a notable rise in viscosity, leading to adhesion of the blends to the screw. This caused a sudden increase in resistance force and prolonged extrusion time. As more flaxseed blends accumulated on the screw, it either obstructed the barrel or was abruptly propelled forward due to the continuous addition of ingredients, ultimately resulting in non-uniform production. Due to the above, only 10 % moisture extrusion was conducted for flaxseed blends.

2.3. Protein content and moisture content

The total nitrogen content of raw and extruded protein blends was determined by the Dumas' method using an Elementar Vario Max Cube (AOAC, 1990). A conversion factor of 6.25 was selected as the value for converting nitrogen content to protein content. EDTA was used as a high nitrogen standard, while rice flour was chosen as a low nitrogen standard.

Regarding moisture content, 2 g of raw and extruded protein blends were placed in oven at 80 °C overnight. Weight of the sample before and after drying process was measured and used to estimate the moisture content (AACC, 2000).

2.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Molecular weight distribution of protein profiles in oilseeds blends was determined by SDS-PAGE (Laemmli, 1970), using protein blends mixed with Laemmli buffer (BIO-RAD, 1610737) with/without 50 mM DTT to make a solution with 1 mg/mL protein. After heating at 95 °C for 5 min, the sample was centrifuged at 15,000 rpm (5424R, Eppendorf) for 10 min. Fifteen μL of supernatant (per lane) was loaded to AnykD™ Criterion™ TGX™ Precast Midi Protein Gel (BIORAD, 5671124) together with Protein™ standard. Electrophoresis was performed at 200 V for 30 min using 1× running buffer (BIO-RAD Tris/Glycine/SDS buffer 1,610,772). Afterwards, the precast gels were stained by Bio-safe Coomassie G-250 Stain (1610787) and destained with Milli-Q water. Finally, the gels were scanned using a Gel Doc™ XR+ system.

2.5. Antinutritional factors of raw and extruded protein blends

2.5.1. Polyphenol contents (fast blue assay)

Polyphenol contents were determined according to Pico et al. (2020). One g of protein blends was dissolved in 8 mL of 80 % methanol with 0.1 % formic acid, then stirred for 15 min. After centrifugation at 3,500 rpm (ROTINA 380, Hettich) at room temperature, supernatant was collected and 40 μL of 2 % EDTA solution was added to stabilize flavon-3-ols. Pellets were re-suspended in 70 % acetone with 0.1 % formic acid for a second extraction. Supernatants of the two extractions were mixed to be used for solid phase extraction.

For solid-phase extraction, the column (Oasis HLB 1 cc Vac Cartridge) was conditioned with 3 mL of 1 % formic acid in methanol and 3 mL of 1 % formic acid in Milli-Q water. Then 3 mL of extracted solution were loaded to the column and the extract was collected. After the column was washed using 1 mL of 50 mM NaH_2PO_4 (pH 3), 3 mL of 0.1 % formic acid were loaded for elution and then mixed with the previously collected solution.

Polyphenol content was quantified by Fast blue assay. Two hundred μL of sample was mixed with 20 μL of 0.1 % Fast blue BB reagent, and then 20 μl of 5 % NaOH was added. The absorbance was measured at 420 nm after 2 h incubation at room temperature in the dark. Gallic acid (0–500 μg/mL) was used as a standard in both assays.

2.5.2. Phytic acid

Phytic acid content was determined according to Hande et al. (2013), with some modifications. An amount of 0.5 g of protein blend was fully dissolved in 10 mL of 2.4 % HCL. After shaking for 16 h, samples were centrifuged at 5,000 rpm (ROTINA 380, Hettich) for 10 min. Supernatant was mixed with 1 g of NaCl, and then stored at 4 °C for 1 h. Afterwards, 150 μL of diluted samples (dilution factor: 25, diluted by Milli-Q water) was mixed with 50 μL of wade reagent (0.03 % $FeCl_3$ + 0.3 % sulfosalicylic acid). The absorbance was measured at 500 nm after 15 min incubation at room temperature. Sodium phytate (0–0.6 mg/mL) was used as a standard. The conversion factor of sodium phytate to phytic acid is 18.38 %.

2.5.3. Condensed tannins

Condensed tannins were determined according to Makkar and Becker (1993), with some modifications. One g of protein blend was fully dissolved in 10 mL of 4 % HCL in methanol. After 18 h of extraction, samples were centrifuged at 5,000 rpm (ROTINA 380, Hettich) for 10 min. Fifty μL of supernatant was mixed with 100 μL of 10 % H_2SO_4 in methanol, followed by the addition of 100 μL of 1 % vanillin in methanol. The absorbance was measured at 500 nm after a 15 min incubation. Catechin (0–1 mg/mL) was used as a standard.

2.5.4. Saponins

Saponins were quantified according to Hiai et al. (1976), with some modifications. An amount of 0.5 g of protein blend was fully dissolved in 10 mL of 80 % methanol. After shaking for 16 h, samples were centrifuged at 5,000 rpm (ROTINA 380, Hettich) for 10 min. The extraction was repeated twice using 5 mL of 80 % methanol. Subsequently, 100 μl of collected supernatant was mixed with 50 μl of 80 % methanol. Then, 0.25 mL of vanillin reagent and 2.5 mL of 72 % sulfuric acid was added to the extracted solution. The absorbance was measured at 520 nm after samples were heated at 60 °C for 10 min. Diosgenin (0–0.5 mg/mL) was used as a standard.

2.5.5. Trypsin inhibitors

Trypsin inhibitors were determined according to Liu et al. (2021), with some modifications. An amount of 0.5 g of sample (< 80 mesh) was dissolved in 25 mL of 10 mM NaOH. After shaking for 3 h at 400 rpm, 1 mL of extracted solution (or Milli-Q water, used as reference) was mixed with 2.5 mL of BANPA solution, and then 1 mL of trypsin solution was added for hydrolysis. After incubation for 10 min at 37 °C, the reaction was terminated by adding 0.5 mL of acetic acid. For the blanks of sample and reference, acetic acid was added before the addition of trypsin solution. Sample concentration was diluted using 10 mM NaOH to ensure the percentage of inhibition was within the range 30 % - 70 %. The absorbance of supernatant was measured at 410 nm. The trypsin activity was expressed as the trypsin inhibition unit (TIU) per mg of the sample and calculated using the following equation:

$$\mathrm{TUI}\Big/\mathrm{mg} = \frac{(Absorbance\ of\ (Reference - Reference\ blank) - Absorbance\ of\ (Sample - Sample\ blank))\ x\ 50}{mg\ sample.}$$

2.6. In vitro protein digestibility

In vitro protein digestibility was measured using the protocol reported by Tinus et al. (2012), with slight modifications. Briefly, 10 mL of sample solution containing 62.5 ± 0.5 mg protein was pre-heated to 37 °C, and its pH was adjusted to 8.0 using 0.1 M NaOH. Then, the pH of the samples was monitored and maintained at 8.0 for 10 min. Meanwhile, an enzyme cocktail (10 mL) with 16 mg of trypsin (13,000–20,000 BAEE units/mg protein), 31 mg of chymotrypsin (40 units/mg protein) and 13 mg of protease from *Streptomyces griseus* Type XIV (P3.5 units/mg) were prepared, with pH adjusted to 8.0 and kept in a water bath at 37 °C. After that, 1 mL of enzyme cocktail was added to 10 mL of sample solution. pH of the sample solution was recorded every 30 s for 10 min. The change in pH ($pH_{0min} - pH_{10min}$) during 10 min digestion was used to calculate the *in vitro* protein digestibility (IVPD), using the equation shown as follow (Manzanilla-Valdez et al., 2024).

$$IVPD\ (\%) = 65.66 + 18.10 \times (pH_{0min} - pH_{10min})$$

2.7. Protein-protein interactions

Protein-protein interactions of plant blends were determined according to Chiang (2007) with slight modifications. Twenty mg of protein blends was dissolved in 10 mL of 1) 0.035 M PBS; 2) 0.035 M PBS + 8 M Urea; 3) 0.035 M PBS + 50 mM DTT; 4) 0.035 M PBS + 1.5 % SDS;

5) 0.035 M PBS + 8 M Urea +5 mM DTT; 6) 0.035 M PBS + 8 M Urea +1.5 % SDS; 7) 0.035 M PBS + 8 M Urea +5 mM DTT + 1.5 % SDS; 8) 0.035 M PBS + 50 mM DTT + 1.5 % SDS. These samples were shaken for 2 h and then centrifuged at 4000 rpm (ROTINA 380, Hettich) for 30 min. The soluble protein content in samples was measured using BCA kit (Pierce™ BCA Protein Assay kit) and Bio-Rad Protein Assay Kit. Bovine serum albumin (0–2 mg/mL) was used as a standard.

2.8. Surface hydrophobicity

Surface hydrophobicity was determined according to the method described by Yan et al. (2021). One g of protein blends was dissolved in 10 mL of 0.01 M PBS (pH 7.0), then diluted to 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL protein concentration. One mL of protein solution was mixed with 5 μL of ANS reagents (2.53 mg/mL), and then the fluorescence at 360 nm (excitation) and 460 nm (emission) was measured using TECAN. Gain value was set to 69, optimized by the fluorescence intensity of bovine serum albumin (BSA). The slope of the linear curve (protein concentration *versus* fluorescence intensity) indicated the protein surface hydrophobicity of samples tested.

2.9. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy

Grounded protein blend flours were directly placed on the top of diamond crystal plate of FTIR spectroscopy (ALPHA II, Bruker, Germany). The spectra between 4,000 and 400 cm^{-1} were recorded. FTIR spectra were acquire using OPUS 8.7 software (Bruker Optic GmbH, Bruker, Bremen, Germany). The secondary structure of the oilseed blends was determined by deconvolution of the Amide I band (1700–1600 cm^{-1}) using OrginPro 2021 software (OriginLab Corp., MA, USA).

2.10. Fast protein liquid chromatography (FPLC)–gel filtration chromatography

The FPLC procedure was followed as Carrasco-Castilla et al. (2012). Five-hundred μL of protein blends (0.2 mg/mL protein concentration) was injected for gel filtration chromatography, which was carried out using a AKTA-purifer FPLC system equipped with a Superdex peptide 10/300 GL column (Cat: 17-5176-01, GE Healthcare). A 0.75 M ammonium bicarbonate solution was used for eluent and elution was monitored at 215 nm. Blue dextran (2,000 kDa), cytochrome C (12.5 kDa), aprotinin (6,512 Da), bacitracin (1,450 Da), cytidine (246 Da) and glycine (75 Da) were used as molecular weight standards.

2.11. Amino acid composition

The amino acid composition was measured according to Carrasco-Castilla et al. (2012). Two mg of protein blends were hydrolysed by 4 mL of 6 M HCl at 110 °C for 24 h under nitrogen. After derivatization with diethyl ethoxymethylenemalonate, amino acids were determined by HPLC with a 300 mm × 3.9 mm i.d. reversed phase C18 column (Novapack C_{18} 4 μm; Waters, Milford, MA. USA). Tryptophan was quantified by HPLC after basic hydrolysis (Yust et al., 2004). D,L-α-aminobutyric acid was used as an internal standard.

2.12. Protein quality parameters

Amino acid score (AAS), essential amino acid index (EAAI), predicted biological value (BV), and protein efficiency ratio (PER) were calculated according to the following equations

$$AAS = \frac{mg\ of\ limited\ amino\ acid\ in\ 1\ g\ of\ total\ protein}{mg\ of\ this\ amino\ acids\ in\ 1\ g\ of\ requirement\ pattern}$$

$$EAAI = \sqrt[9]{\frac{(Lys\ x\ Thr\ x\ Val\ x\ (Met + Cys)\ x\ Ile\ x\ Leu\ x\ (Phe + Tyr)\ x\ His\ x\ Trp\ )\ (sample)}{(Lys\ x\ Thr\ x\ Val\ x\ (Met + Cys)\ x\ Ile\ x\ Leu\ x\ (Phe + Tyr)\ x\ His\ x\ Trp\ )\ (standard)}}$$

$$BV = 1.09(EAAI) - 11.7$$

$$PER_1 = -0.684 + 0.456(Leu) - 0.047(Pro)$$

$$PER_2 = -0.468 + 0.454(Leu) - 0.105(Tyr)$$

$$PER_3 = -1.816 + 0.435(Met) + 0.780(Leu) + 0.211(His) - 0.944(Tyr)$$

$$PER_4 = 0.08084(Thr + Val + Met + Ile + Leu + Phe + Lys) - 0.1094$$

$$PER_5 = 0.0632(Thr + Val + Met + Ile + Leu + Phe + Lys + His + Arg + Tyr) - 0.1539$$

The *in vitro* protein-digestibility corrected amino acid score (IVPD-CAAS) was calculated by AAS × IVPD (Ma et al., 2024).

2.13. Statistical analysis

Results were expressed as mean ± standard deviation and analysed by Prism GraphPad 9.0. FTIR, FPLC and amino acid profiles were carried out in duplicates. All the other experiments were conducted in triplicates. Data was analysed using analysis of variance (ANOVA). When statistical difference was observed, Tukey's multiple comparison test was subsequently applied to detect the difference among the mean of each sample at a significance level of 95 % ($p < 0.05$). Principal component analysis and correlation analysis were also performed using Prism GraphPad 9.0.

3. Results and discussion

3.1. Protein, moisture, and molecular weight distribution

The protein and moisture content of protein blends before and after extrusion are shown in Table 1. A slight change (ranging between −2.0 % to +3.7 %) was observed in protein content of extruded protein blends. Low impact of extrusion on protein content was also stated by Gui et al. (2012), and a decrease (ranging between 2 % and 10 %) in protein content was found after extrusion. Only moisture in HP and FHP increased after 10 % moisture extrusion, due to the high temperature applied in extrusion led to water evaporation (Gu et al., 2020). Although moisture content in extrudates was significantly increased after 20 % moisture extrusion, loss of moisture during the extrusion was also found in this thermal-mechanical process.

The M_w distribution of raw and extruded oilseeds blends under reduced and non-reducing conditions are shown in Fig. 1. Two large protein bands (~71 kDa and ~ 89 kDa) associated to convicilin were

Table 1
Protein and moisture content of protein blends before and after 10 % or 20 % moisture extrusion.

Samples	Raw		10 % moisture extrusion		20 % moisture extrusion	
	Protein (g/100 g dw)	Moisture %	Protein (g/100 g dw)	Moisture %	Protein (g/100 g dw)	Moisture %
HP	62.4 ± 2.0^{b}	7.10 ± 0.05^{c}	61.4 ± 0.2^{b}	10.09 ± 0.15^{b}	66.1 ± 1.5^{a}	14.88 ± 0.19^{a}
HHP	41.4 ± 0.3^{a}	8.43 ± 0.06^{b}	39.5 ± 0.3^{b}	8.58 ± 0.16^{b}	42.2 ± 0.3^{a}	13.34 ± 0.17^{a}
RP	59.8 ± 0.3^{b}	8.48 ± 0.13^{b}	61.6 ± 0.6^{a}	7.64 ± 0.13^{c}	60.0 ± 1.0^{ab}	12.36 ± 0.15^{a}
RHP	40.0 ± 0.5^{a}	8.77 ± 0.17^{b}	41.2 ± 0.5^{a}	8.94 ± 0.17^{b}	41.5 ± 0.3^{a}	14.15 ± 0.20^{a}
FP	63.2 ± 1.2^{a}	6.63 ± 0.10^{a}	61.2 ± 0.4^{a}	5.66 ± 0.07^{b}	NP	NP
FHP	45.3 ± 1.2^{a}	8.12 ± 0.08^{b}	46.4 ± 0.3^{a}	9.32 ± 0.12^{a}	NP	NP

Different small letters within the columns (protein g/100 g dw and moisture %) indicate significant differences (p-value <0.05).
HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); NP – not produced.

observed in all pea blends. They had a higher M_w than all the protein profiles in hemp blends (Schmidt et al., 2022). Apart from RP and FP, Edestin (~43 kDa) and hemp albumin (~11 kDa) were clearly seen in other oilseed blends. Meanwhile, Edestin acidic subunit (~29 kDa) and basic subunit (~18 kDa) were observed under reducing conditions, together with a M_w protein bands of ~4 kDa (Shen et al., 2020). Under reducing conditions, rapeseed blends have the most bands, which were associated to Cruciferin (15 kDa–37 kDa) and Napin (~6 kDa) (Rahman et al., 2021). Meanwhile, flaxseed protein contributed to broader bands with M_w ~32 kDa and ~20 kDa (El-Beltagi et al., 2011).

Under non-reducing conditions, only a few bands totally disappeared (highlighted using red colour) after extrusion, while most bands become narrower with a blurry colour and low intensity. Fang et al. (2014) also claimed that most of the bands are still found in extruded soy protein. Protein profiles of oilseed blends after 10 % and 20 % moisture extrusion were similar. The only difference was found in Fig. 1b **line 11**, a thinner band (~47 kDa) was shown after 20 % moisture extrusion, which did not appear after 10 % moisture extrusion. The changes in bands under non-reducing conditions might be because of protein depolymerization and denaturation caused by extrusion, which led to the unfolding of the protein structures (Jiang et al., 2023). Partial aggregation may have resulted from the depolymerized proteins forming a network through disulphide bonds (Morel et al., 2002; Zhang et al., 2022). Due to DTT breaking these disulphide bonds, it was inferred that the bands of oilseed blends under reducing conditions are similar to unextruded samples.

3.2. Polyphenols and anti-nutritional factors

3.2.1. Polyphenols

Polyphenols are naturally occurring compounds found in a wide range of plants. They are well-known dietary antioxidant and anti-inflammatory compounds with potential health benefits. As shown in Table 2**,** higher total phenolic content (TPC) was found in hemp blends than in pea blends. Nasrollahzadeh et al. (2022) also observed that the TPC in five commercial hemp protein concentrates (1,983.3–2,727.0 mg/100 g) were higher than in pea protein ingredient (1,478.5 mg/100 g), while their values were much higher than those in this study. Teh et al. (2014) reported similar TPC values in hemp (733 mg GAE/100 g), flaxseed (774.33 mg GAE/100 g) and canola (modified version of rapeseed, 2,104 mg GAE/100 g). In this study, compared with flaxseed blends, hemp blends were found to have a 9.5 % and 13.7 % higher TPC content. The highest TPC values (1,637.0 and 2,000.7 mg GAE/100 g) were found in rapeseed blends, which may be resulting from the abundant phenolic compounds in rapeseeds. Borges-Martínez et al. (2021) reported TPC in pea being 584.3 mg GAE/100 g, which is slightly higher than the value observed in this study, but TPC could be reduced after protein extraction at industrial scale enrichment technologies (Pedrosa et al., 2020).

After 10 % moisture extrusion, a 16.7 %–52.80 % reduction in TPC value was found in oilseed bends. It is noticeable that around half of polyphenols in flaxseed blends disappeared after the treatment. Šárka et al. (2021) summarised the impact of extrusion on TPC and found that this process could decrease the TPC in polished rice flour (−54 %), broken rice flour (−32 %) and wheat flour (−35 %), while increasing the TPC in soybean (20–22 %) and has no effect on the TPC of soaked rice. Extrusion breaks the cell wall, disrupts the covalent bonds and make the polyphenols more extractable (Apea-Bah & Beta, 2018). In oilseed blends, the heat-labile polyphenols degrade due to the barrel temperature which is over 80 °C (Zadernowskl et al., 1999). Meanwhile, polyphenols interact with other food nutrients to form insoluble complexes. In Hu et al. (2018)'s study, soluble and soluble-conjugated polyphenols were reduced by 30.8 % and 31.0 %, respectively. However, insoluble polyphenols showed a 60.7 % increase after extrusion at 120 °C. After increasing the feeding moisture from 10 % to 20 %, a change of TPC was found in RP (−19.4 %) and RHP (+26.07 %). Abd El-Hady and Habiba (2003) reported an increase in TPC of faba bean, while moisture content increased from 18 % (713 mg/100 g) to 22 % (750 mg/100 g). However, the TPC in pea (430 mg/100 g -402 mg/100 g), chickpea (520 mg/100 g–490 mg/100 g) and kidney beans (621 mg/100 g–610 mg/100 g) all decreased. In addition, Kaur et al. (2015) also reported that higher moisture can effectively decrease the polyphenols content, but increasing the moisture from 14 % to 20 % had no effect on TPC in wheat at 140 °C. Therefore, the formulation, barrel temperature and feed moisture are all important parameters affecting the change of polyphenols in extruded products.

3.2.2. Phytic acid

Phytic acid is the major storage form of phosphorus in plants. It interacts with iron, zinc, calcium, and magnesium to form insoluble complex and then reduce the absorption of these minerals. Oilseeds were reported to contain abundant amount of phytic acid. For example, 2–5 % phytic acid has been reported in defatted rapeseeds (Thompson, 1990); 3.5 % phytic acid was detected in hemp (Mattila et al., 2018), and 2.3–3.3 % phytic acid was reported in flaxseed (Oomah et al., 1996). Similarly, 3.57 %–5.35 % phytic acid were detected among six oilseeds blends. Pea protein (1.5 g/100 g) was reported to have a lower phytic acid content, compared with hemp protein concentrates (3.4 g and 3.6 g/100 g) (Nasrollahzadeh et al., 2022). That can explain that the oilseed blend with pea presented a lower phytic acid content than the ones formulated with hemp.

Although extrusion was reported to reduce the amount of phytic acid by 54.5 % in cereal brans on average (Kaur et al., 2015), a limited effect on phytic acid content was found before and after extrusion. The highest reduction was found in HP (−11.7 %, 20 % moisture extrusion), followed by HHP (−10.47 %, 20 % moisture extrusion). However, an increase in the phytic acid content was found in HP (compared with HP10) and RP (compared with RP10 and RP20). This might be because the phytic acid was more bio-accessible after extrusion due to the disrupted food matrix (Ti et al., 2015). A typical strategy to thermal decomposition of phytic acid is heating at 150 °C for one hour (Daneluti & Matos,

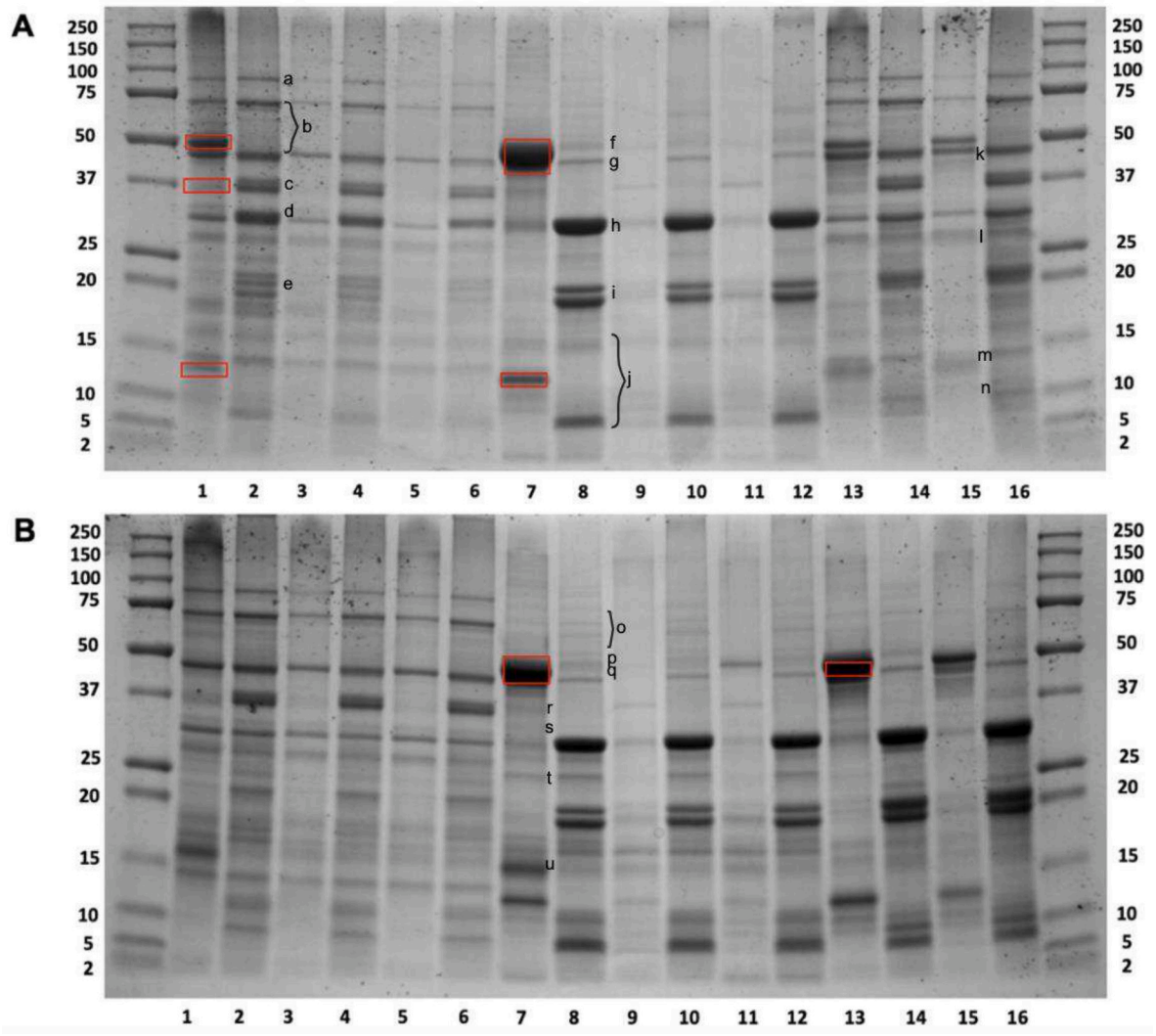

Fig. 1. SDS-PAGE patterns of soluble protein fractions from A: 1–2, HP (non-reducing and reducing; 3–4, HP10 (10 % moisture extrusion, non-reducing and reducing); 5–6, HP20 (20 % moisture extrusion, non-reducing and reducing); 7–8, HHP (non-reducing and reducing; 9–10, HHP10 (10 % moisture extrusion, non-reducing and reducing); 11–12, HHP20 (20 % moisture extrusion, non-reducing and reducing); 13–14, FP (non-reducing and reducing) and 15–16, FP10 (10 % moisture extrusion, non-reducing and reducing). B: 1–2, RP (non-reducing and reducing; 3–4, RP20 (10 % moisture extrusion, non-reducing and reducing); 5–6, RP20 (20 % moisture extrusion, non-reducing and reducing); 7–8, RHP (non-reducing and reducing; 9–10, RHP10 (10 % moisture extrusion, non-reducing and reducing); 11–12, RHP20 (20 % moisture extrusion, non-reducing and reducing); 13–14, FHP (non-reducing and reducing) and 15–16, FHP10 (10 % moisture extrusion, non-reducing and reducing). Protein bands (a) 89.3 kDa Pea-Convicilin; (b) 57.8–70.5 kDa Pea-Vicilin (α, β and γ); (c) 35.6 kDa Pea-Legumin (acid subunit); (d) 31.8 kDa Pea-Lectin; (e) 17.9 kDa Pea-Legumin (basic subunit); (f) 52.3 kDa Hemp-Edestin (11S globulin); (g) 42.7 kDa Hemp-7S globulin; (h)28.6 kDa Hemp-Acidic subunit (AS); (i)18.6 kDa Hemp-Basic subunit (BS); (j) 8.5–14.4 kDa Hemp-Albumin; (k) 48.3 kDa Flaxseed-48 kDa protein; (l) 26.6 kDa Flaxseed-acidic subunits of 11S protein; (m) 13.3 kDa Flaxseed-basic subunits of 11S protein; (n) 11.9 kDa Flaxseed-conlinin and oleosins; (o) 59.4–73.2 kDa Rapeseed-Cruciferin; (p) 45.1 kDa Rapeseed-Steroleosin; (q) 41.8 kDa Rapeseed-Caleosin; (r) 34.5 kDa Rapeseed-Cruciferin α polypeptide; (s) 32.5 kDa Rapeseed-Cruciferin β polypeptide; (t) 23.4 kDa Rapeseed-Oleosin and (u) 15.6 kDa Rapeseed-Napin.

2013). In this study, a lower processing temperature and short processing times failed to efficiently reduce the phytic acid content in oilseeds plants.

3.2.3. Condensed tannins

Reducing the bioavailability of protein and diminishing weight gains are the major anti-nutritional effects of tannins (Butler, 1992). Mattila et al. (2018) observed that rapeseed press cake contained slightly higher condensed tannins (expressed as proanthocyanidins) (119 mg/100 g) compared with oil hemp seed (105 mg/100 g), while no condensed tannins were detected in whole flaxseed. In this study, rapeseed blends were found to have 47.2 % and 67.2 % more condensed tannins compared with hemp mixed with pea and hemp protein powder respectively. In addition, the lowest amount of tannins was found in FP (97.4 mg/100 g). Higher tannin contents (500–1,500 mg/100 g) in pea were reported by Jain et al. (2009). However, Wang et al. (1998) reported that condensed tannins in field pea were barely detected, which might be attributed to different cultivars and environmental conditions. In addition, Alonso et al. (1998) reported condensed tannin levels of three pea seeds varying between 13.5 mg/100 g and 23.8 mg/100 g. Moreover, the co-product (residual flour) remaining after protein isolate production tended to have lower condensed tannins that the original flour. For instance, the tannin content in pigeon pea flour was 2.70 mg/100 g, which decreased to 0.80–1.07 mg/100 g after undergoing protein isolation technologies (Adenekan et al., 2018).

Extrusion effectively reduced the condensed tannins content of oilseed blends by 20.4 %–36.8 % after 10 % moisture extrusion and 26.8 %–41.1 % after 20 % moisture extrusion. Much higher reduction in

Table 2
Polyphenols and antinutritional factors (phytic acid, condensed tannins, saponins, and trypsin inhibitors) of oilseed blends before and after 10 % or 20 % moisture extrusion.

Oilseed Blends	Treatment	Total Polyphenols (mg GAE/per 100 g dw)	Phytic Acid (g/100 g dw)	Condensed Tannins (mg/100 g dw)	Saponins (mg/100 g dw)	Trypsin inhibitors (TUI/mg dw)
HP	Raw	705.5 ± 17.9^{Da}	4.63 ± 0.25^{Bb}	139.9 ± 1.9^{Da}	587.5 ± 29.5^{Da}	8.22 ± 1.21^{Ca}
	10 % moisture extrusion	572.1 ± 26.2^{Db}	5.25 ± 0.26^{Aa}	111.4 ± 6.7^{Cb}	550.4 ± 23.9^{Cb}	1.60 ± 0.14^{Cb}
	20 % moisture extrusion	633.7 ± 20.9^{Db}	4.09 ± 0.15^{Cc}	101.2 ± 5.4^{Bb}	561.2 ± 18.8^{Cb}	0.56 ± 0.07^{Cc}
HHP	Raw	879.3 ± 16.4^{Ca}	5.35 ± 0.22^{Aa}	172.1 ± 7.9^{Ca}	805.2 ± 14.6^{Ba}	13.85 ± 1.51^{Ba}
	10 % moisture extrusion	732.7 ± 15.3^{Cb}	5.09 ± 0.32^{Aab}	108.1 ± 7.7^{Cb}	648.8 ± 44.5^{Bb}	1.06 ± 0.07^{Dc}
	20 % moisture extrusion	773.6 ± 15.9^{Cc}	4.79 ± 0.10^{Ab}	107.1 ± 8.1^{Bb}	646.0 ± 17.3^{Bb}	1.89 ± 0.15^{Bb}
RP	Raw	$1{,}637.0 \pm 83.0^{Ba}$	3.57 ± 0.25^{Cc}	205.9 ± 13.9^{Ba}	624.0 ± 25.7^{Da}	9.03 ± 1.17^{Ca}
	10 % moisture extrusion	$1{,}287.4 \pm 51.8^{Bb}$	4.03 ± 0.12^{Cb}	142.2 ± 5.5^{Bb}	500.9 ± 26.3^{Cb}	1.52 ± 0.17^{Cb}
	20 % moisture extrusion	$1{,}161.8 \pm 42.9^{Bc}$	4.43 ± 0.13^{Ba}	151.6 ± 6.7^{Ab}	491.0 ± 12.7^{Eb}	0.21 ± 0.01^{Dc}
RHP	Raw	$2{,}000.7 \pm 93.2^{Aa}$	4.36 ± 0.19^{Ba}	287.7 ± 13.2^{Aa}	973.3 ± 46.0^{Aa}	16.20 ± 1.35^{Aa}
	10 % moisture extrusion	$1{,}499.7 \pm 56.9^{Ac}$	4.43 ± 0.32^{Aa}	191.1 ± 6.0^{Ab}	823.3 ± 10.1^{Ab}	2.89 ± 0.32^{Ab}
	20 % moisture extrusion	$1{,}696.2 \pm 90.9^{Ab}$	4.00 ± 0.23^{Ca}	169.6 ± 8.5^{Ac}	878.7 ± 19.7^{Aa}	3.38 ± 0.13^{Ab}
FP	Raw	635.3 ± 18.4^{Fa}	4.25 ± 0.14^{Ba}	97.4 ± 5.4^{Ea}	533.7 ± 39.8^{Ea}	3.90 ± 0.27^{Da}
	10 % moisture extrusion	347.3 ± 15.7^{Eb}	4.05 ± 0.18^{Ca}	71.8 ± 5.5^{Eb}	436.6 ± 36.1^{Db}	1.64 ± 0.17^{Cb}
FHP	Raw	756.6 ± 32.7^{Ea}	5.10 ± 0.12^{Aa}	145.2 ± 6.0^{Da}	749.9 ± 39.0^{Ca}	10.68 ± 1.01^{Ca}
	10 % moisture extrusion	357.1 ± 15.0^{Eb}	4.61 ± 0.12^{Bb}	91.8 ± 6.2^{Db}	675.1 ± 38.9^{Bb}	2.06 ± 0.21^{Bb}

Different capital (among different formulations) and small (with/without extrusion) letters within the same column indicate significant differences ($p < 0.05$). HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w).

condensed tannins after extrusion with 25 % moisture content was found in pea seeds (96 % Renata, 82.5 % Salara, and 89.3 % Ballet), faba beans (54.4 %) and kidney beans (83.8 %) (Alonso et al., 1998). Increasing the moisture content from 10 % to 20 % resulted in a further reduction of condensed tannins in HP and PHP, which was similar to the findings reported by Abd El-Hady and Habiba (2003). Increasing the moisture from 18 % to 22 % resulted in lower tannins in peas (− 6.97 % and − 5.76 %), chickpeas (−7.31 % and − 13.85 %) and kidney beans (−7.30 % and − 4.29 %) at 140 °C and 180 °C, respectively. Similarly, condensed tannins in lentil seed decreased after the moisture content increased from 14 % (0.065, 0.040, and 0.020 mg/100 g) to 22 % (0.059, 0.035 and 0.011 mg/100 g) at 140, 160 and 180 °C, respectively (Rathod & Annapure, 2016).

3.2.4. Saponins

Saponins inhibit the activity of digestive and metabolic enzymes and bind to mineral nutrients (iron, zinc, and vitamin E) reducing their absorption. This anti-nutritional factor also contributes to a bitter taste in plant proteins. Peas were well-documented as one of the predominant sources of dietary saponins (Singh et al., 2017), 80–250 mg/100 g saponins were found in 13 different pea cultivars (Heng et al., 2006). Surprisingly, saponin content in hemp blends was significantly higher than that in pea blends, which indicates a higher saponin content in hemp. Moreover, RHP contains the highest saponin levels (973.3 mg/100 g) among all blends. On the contrary, Russo and Reggiani (2015) and Russo and Reggiani (2013) reported really low saponin level in hemp, with 47.0–70.0 mg/100 g and 5.9–8.1 mg/100 g, respectively. Meanwhile, Nzotta and Onabanjo (2021) also reported a much lower saponin content in flaxseed (390 mg/100 g). Despite much higher saponin levels were detected in oilseed blends, they still showed a lower saponin level compared with the other two common plant proteins, quinoa (1.5 g/100 g) and soybean (1.3 g/100 g) (Herrera et al., 2019).

A slight reduction (ranging between −6.3 % and −19.73 %) in saponin content was found in oilseed blends after 10 % moisture extrusion. However, Gui et al. (2012) reported that the saponin in red ginseng increased from 5.5 % to 6.7 %–7.2 % after extrusion with different processing variables (20–30 % moisture, 200–250 rpm and 115–130 °C). Sánchez-Velázquez et al. (2021) reported a similar reduction of saponin in green split peas (17 %) and Pinto bean (13 %), while an increase of saponin level was found in other pulses (*e.g.*, red lentil and red kidney bean) with the same treatment (flour feed rate 36 kg/h, moisture addition rate 0.8 kg/h, 650 rpm, and 30–120 °C). The only change of saponin content was found in RHP (+6.3 %) after increasing the moisture from 10 % to 20 % during extrusion. Similarly, Gui et al. (2012) did not detect any significant difference of saponin content in red ginseng with 20 % and 30 % moisture extrusion. In addition, Kowalski et al. (2016) indicated a positive linear correlation between moisture (15 %–25 %) and saponin content in quinoa flour. In this study, shear force and thermal treatment could degrade the original structure of saponin in oilseed blends, which explained why extrusion slightly decreases the saponin levels (Kowalski et al., 2016).

3.2.5. Trypsin inhibitors

Trypsin inhibitors reduce the biological activity of trypsin, leading to a reduction in absorption and digestion of dietary proteins. Duque-Estrada et al. (2023) reported that pea protein exhibits higher trypsin inhibitor activity (7.6 TIU/mg sample, dry basis, and 15.7 TIU/mg protein) compared to hemp protein (5.3 TIU/mg sample, dry basis, and 10.6 TIU/mg protein). However, in the present study, oilseed blends with hemp protein showed higher TIU than those blended with pea protein. Meanwhile, Russo and Reggiani (2015) similarly reported a high average trypsin inhibitor value in six hemp varieties (22.7 ± 2.6 TIU/mg). In contrast, flaxseeds are known to contain relatively low levels of trypsin inhibitors, compared to soybean and canola seeds (Kajla et al., 2015). Similarly with these findings, this study demonstrated that flaxseed blends, especially FP (3.90 TUI/mg), had the lowest trypsin inhibitor activity among the rapeseed and flaxseeds blends analysed.

Among all protein blends, trypsin inhibitors activity was largely reduced (−57.95 % to −92.35 %) after 10 % moisture extrusion. Similarly, 79.7 % and 69.4 % decrease in trypsin inhibitor was found in carioca bean and black bean after extrusion, respectively (Batista et al., 2010). Meanwhile, a sharp decrease (74 %–79 %) in trypsin inhibitor activity was reported in three plant blends (pea/faba beans, faba beans/pea/quinoa and pea/faba bean/hemp) after extrusion (Duque-Estrada et al., 2023). Moreover, Osuna-Gallardo et al. (2023) reported a complete loss (100 % reduction) of trypsin inhibitor activity in ayocote bean flours after extrusion, compared to an initial activity of 15.86 TIU/mg in the pre-extruded flours. A slight change of trypsin inhibitor activity was found in HP (−1.04 TIU/mg), HHP (+0.83TIU/mg), and RP (−1.31 TIU/mg) after the moisture content increased from 10 % and 20 %. This finding agreed with the ones reported by Kaur et al. (2015). Weak increase or even no significant effect of moisture content (14, 17 and 20 %)

on trypsin inhibitor activity was found in wheat, rice, barley, and oat. This reflects that the inactivation of trypsin inhibitors is mainly induced by high extrusion temperature.

Taken together, extrusion effectively reduced TPC, condensed tannins, and trypsin inhibitors. However, its efficacy in reducing phytic acid and saponins was relatively limited. Therefore, additional pre-treatments may be required for further reducing their contents. Masud et al. (2007) reported significant reductions in phytic acid among seven wheat varieties after soaking (24 h, −22.5 % to −25.0 %), germination (48 h, − 36 % to −39 %), and heating (80 °C for 1 h, −27 % to −32 %). Similarly, Luo et al. (2009) observed reductions in phytic acid in faba beans of 50.96 %, 68.28 %, and 83.97 % following soaking (1 day), germination (120h), and accelerated fermentation, respectively. Moreover, Sinha and Kawatra (2003) noted that soaking cowpea for 18 h reduced phytic acid by 20.0 %, while germination for 72 h led to 47.8 % reduction. Regarding saponins, Sharma and Sehgal (1992) reported a 77 % reduction after sprouting faba bean for 48 h. Ramli et al. (2021) found that soaking kacang koro seed for 36 h reduced saponin content by 33.60 %, and subsequent fermentation led to additional 32.8 % reduction (in total 66.40 %). Furthermore, Sharma et al. (2022) found that moist heating methods, including boiling and autoclaving, reduced saponin levels in quinoa by 14–64 %. These findings suggest that combination of extrusion with such pre-treatments may enhance the reduction of phytic acid and saponin content.

3.3. *In vitro protein digestibility*

As shown in Fig. 2, oilseed blends with pea protein showed higher protein digestibility than hemp protein, which was also observed by Manus et al. (2021). It should be noticed that IVPD reached over 85 % after defatted oilseeds meals were mixed with pea protein. This is due not only to the good digestibility of pea protein, but also to the fact that these formulations have balanced amino acids profile, which results in high protein nutritional quality, and potentially improved the protein digestibility. Compared with raw protein blends, extrusion significantly promoted IVPD in all formulations ($p < 0.05$). This tendency is in agreement with the findings of Alonso et al. (2000), who reported that extrusion cooking increased the protein digestion in faba beans and kidney beans by 23.5 % and 21.9 %, respectively. Rathod and Annapure (2017) also reported that the digestibility of rice and lentil blends increased from 31.6 % to 48.8 %–68.24 % after extrusion at different temperatures (70, 95 and 120 °C) and feed moistures (16 %, 20 % and 24 %). In addition, increasing moisture content from 10 to 20 % could not further enhance the IVPD of protein blends, even causing a decrease in IVPD of HHP (from 86.5 % to 82.6 %) and PHP (89.2–87.0 %). Similarly, Wang et al. (2008) claimed that the maximum IVPD of flaxseed was obtained by extrusion with 10 % moisture after using surface response methodology to optimize moisture content (4–28 %) and other controlled variables.

3.4. *Protein-protein interactions*

Protein-protein interactions have been extensively studied through protein solubility assays (Liu & Hsieh, 2008; Osen et al., 2015). Phosphate buffer is commonly used to quantify water-soluble proteins in their native state, as it does not disrupt chemical bonds (Chiang, 2007). Conversely, certain solvents are well-documented for their ability to disrupt specific chemical interactions between proteins. For instance, DTT cleaves disulfide bonds, SDS interferes with hydrophobic and ionic interactions, and urea disrupts hydrogen bonds and hydrophobic interactions (Tanger et al., 2021). Therefore, examining changes in protein solubility of oilseed blends using buffers containing these reagents can provide valuable insights into the types and extent of molecular interactions affected by extrusion. As shown in Fig. 3, these solvents have been applied individually or in combination to elucidate protein-protein interaction mechanisms.

The solubility of protein under native state measured by two different kits were almost the same, with slight differences found in RHP

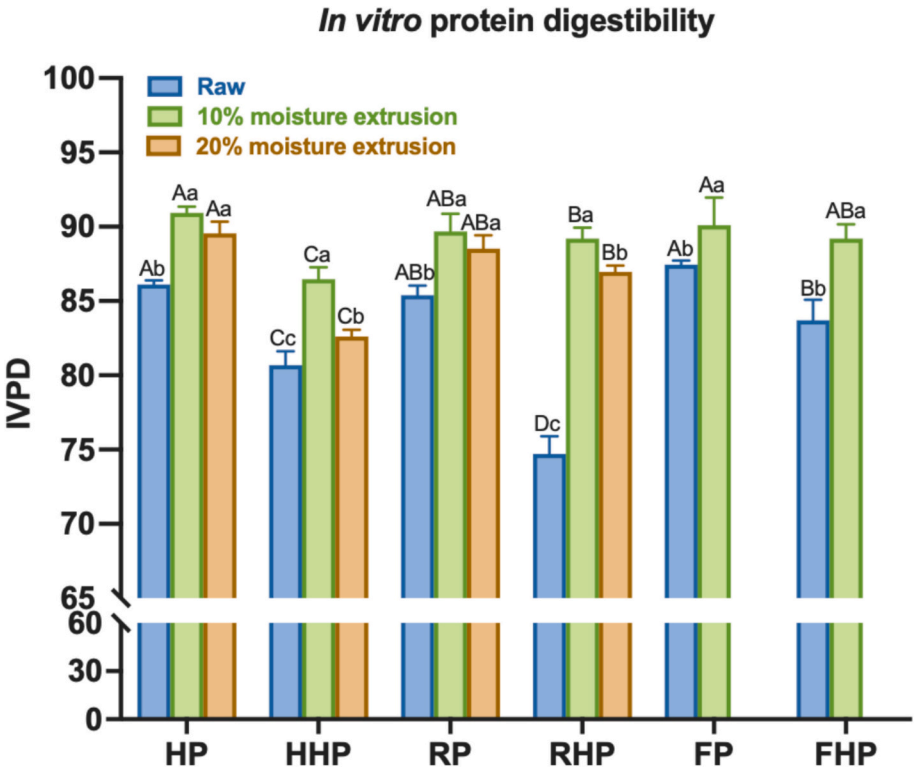

Fig. 2. *In vitro* protein digestibility of oilseed blends before and after extrusion. Different capital (among different formulations) and small (with/without extrusion) letters within the same column indicate significant differences ($p < 0.05$).

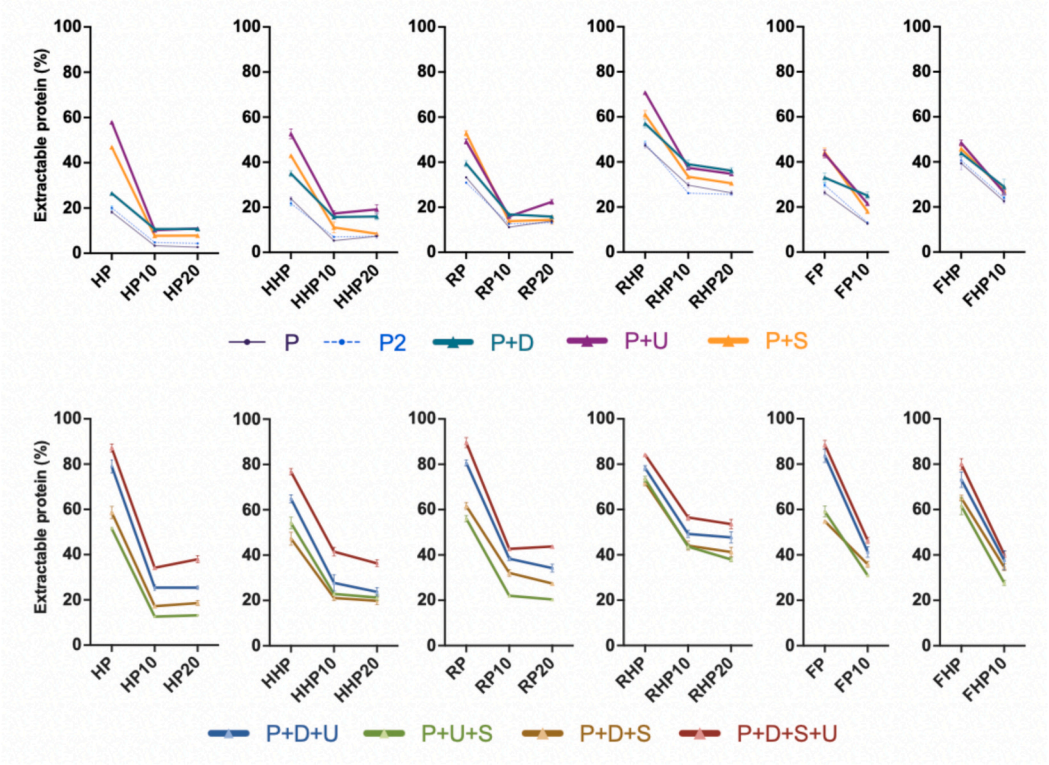

Fig. 3. Extractable protein of raw and extruded oilseed blends with different extracting buffer. P, sodium phosphate buffer, measured by BCA kit; P2, sodium phosphate buffer, measured by Bio-Rad Protein Assay Kit; D, dithiothreitol; U, urea; S, sodium dodecyl-sulphate.

(29.7 % *vs* 26.2 %) and FP (26.3 % *vs* 29.7 %). It should be highlighted that rapeseed blends represented the highest extractable protein (%), which contributed to the high soluble protein in rapeseeds (over 60 %) (Kalaydzhiev et al., 2020; Quinn & Jones, 1976). However, moderate protein solubility (< 60 %) and low protein solubility (~17 %) were reported by Flores et al. (2006) and Malomo et al. (2014), respectively. Pea protein isolate was reported to have good protein solubility at pH 7, whereas Jiménez-Munoz, Brodkorb, et al. (2021) reported a low protein solubility (~13 %) of pea protein isolate, which is similar to the value reported in this study.

A significant increase in extractable protein (%) was found after adding urea, which indicated that abundant hydrogen bonds were present in oilseed blends. To the contrary, DTT showed the least exactable protein (%), which might be caused by less sulphur amino acid residues in plant protein resulting in small amount of sulphide bonds. Combination of different solvents always indicate a significant increase of protein solubility, but it should be highlighted that the one dissolved in P + U/P + S was higher than for P + D. This behaviour was observed in Osen et al. (2015)' s work, protein solubility of pea protein isolate was around 100 % in P + U, while only approximately 30 % in P + D. Among all chemical reagents, oilseed blends in P + U showed the highest solubility, with up to 70 % protein dissolved in buffer. Further dissolution may be limited by the interaction between proteins and starch.

After 10 % moisture extrusion, the extractable protein (%) was largely reduced. This is due to the formation of isopeptides at extrusion temperature, which led to protein aggregation (Pietsch et al., 2019; Verfaillie et al., 2024). Although the extractable protein was reduced after extrusion, for all treatments, it should be highlighted that the effect of extrusion on oilseed blends in P + D was less severe, because the disulphide bonds are thermally stable. As a result, although a considerable extractable protein content can be found in P + S + D, protein extractability dramatically decreases, and a similar behaviour was observed in the SDS-PAGE data. Overall, after extrusion, hydrophobic bonds were largely reduced, to a greater extent than disulphide bonds,

Table 3
Surface hydrophobicity modifications of oilseed blends before and after 10 % moisture extrusion and 20 % moisture extrusion.

Samples	Raw	10 % moisture extrusion	20 % moisture extrusion
HP	$48,468 \pm 3,466^{Ba}$	$12,657 \pm 1,199^{Bc}$	$27,242 \pm 574^{Ab}$
HHP	$27,047 \pm 1,605^{Da}$	$16,308 \pm 1,231^{Db}$	$17,294 \pm 862^{Cb}$
RP	$34,517 \pm 1,701^{Ca}$	$18,370 \pm 1,277^{Cc}$	$27,726 \pm 1,784^{Ab}$
RHP	$25,737 \pm 1,784^{Da}$	$19,899 \pm 1,259^{Cc}$	$21,877 \pm 1,729^{Bb}$
FP	$51,329 \pm 2,337^{Ba}$	$32,852 \pm 848^{Ab}$	NP
FHP	$30,518 \pm 2,242^{Ca}$	$20,107 \pm 1,169^{Cb}$	NP
BSA	$578,892 \pm 11,182^{A}$		

Different capital (among different formulations) and small (with/without extrusion) letters within the same column indicate significant differences ($p < 0.05$). HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w). NP – not produced.

but hydrogen bonds remain mainly responsible for protein-protein interactions. Increasing moisture content from 10 % to 20 % showed slight effect on protein extractability, with positive effects observed in HP, but negative effects in HHP (apart from P + U) and RHP. It is observed that the solubility of RP in Urea increased significantly, where the overall extractable protein slightly increased.

3.5. *Surface hydrophobicity measurement*

Hydrophobic interactions are the main force for maintaining the tertiary structure of proteins. This force is affected by the shape and size

of proteins, type of amino acid residues (mainly non-polar amino acids), and the cross-link between protein-protein or proteins with other molecules (Aider et al., 2012). Therefore, surface hydrophobicity is an important parameter indicating the changes in protein conformation and functional properties.

Surface hydrophobicity of six oilseed blends before and after extrusion are shown in Table 3. Formulation with pea presented higher surface hydrophobicity than the formulation with hemp protein. Shahbal et al. (2023) also reported pea having higher surface hydrophobicity than hemp at 5 % and 10 % (w/w) solution. On the contrary, Nasrollahzadeh et al. (2022) reported a much higher surface hydrophobicity for hemp (83,533–188,786) when compared to pea (12,991), which might be because protein enriching processing resulted in protein denaturation and unfolding (more exposed non-polar and hydrophobic amino acids). In this study, pea proteins were isolated by wet extraction, while hemp proteins were obtained by dry fractionation with 49 % protein content. Flaxseed blends tend to present a higher surface hydrophobicity, which is due to their higher amount of non-polar amino acids (Sharma & Saini, 2022).

The surface hydrophobicity of protein blends showed a sharp reduction after extrusion with water level of 10 % (−16.3 % to −48.7 %) and 20 % (−15.2 % to −44.1 %). Gao et al. (2023) also applied 10 % moisture extrusion to wheat germ protein and detected a maximum reduction of 65.1 % in surface hydrophobicity. The reduction in surface hydrophobicity was related to protein aggregation (folding) and stretching of protein caused by shear force, high temperature, and high pressure. Consequently, the hydrophobic sites of protein were located inside the aggregates and therefore resulted in reduced interaction with ANS.

Apart from HHP, an increase in surface hydrophobicity was found in protein blends, after the water level increased from 10 % to 20 %. This finding is in agreement with Yanqing et al. (2022), who reported an increase in surface hydrophobicity in alkaline protease hydrolysed soy protein after moisture increased from 18 to 26 %. Meanwhile, Brishti et al. (2021) found that the surface hydrophobicity of extruded mung bean protein increased from 596 to 748, then to 891, after the water level was adjusted to 30 %, 49.3 % and 60 %, respectively. High levels of moisture led to more hydrophobic domains being exposed to ANS by denaturing the proteins as well as limiting protein aggregation (Vajda & Perczel, 2014).

3.6. ATR-FTIR

In proteins, secondary structure refers to local segments of a protein/polypeptide chain, which are stabilised by hydrogen bonds. ATR-FTIR is widely applied to determine the secondary structure of proteins. Amide *I* band (1600–1700 cm^{-1}) was utilised to determine β-sheet, α-helix, random coil, and β-turn, which are the common types of secondary structure.

The main secondary structure of oilseed blends is β-sheet (Fig. 4) (31.0 %–38.3 %). This is because plant proteins always have higher β-sheet levels and low α-helix levels, compared to animal proteins (Berrazaga et al., 2019). It is noticeable that hemp blends presented higher α-helix levels than the pea blends. Liu et al. (2022) claimed that ~25 % of α-helix were found in hemp protein, which can explain a relatively higher α-helix content in hemp blends. Pea proteins were reported to have a higher percentage of random coils as secondary structure (*e.g.*, 74.0 %) (Shen et al., 2022), which was also reflected in this study. Flaxseed blends presented lower β-sheet levels than other oilseeds. On the contrary, Pham et al. (2020) found that 58.4 % of secondary structure in flaxseed was β-sheet. Kaushik et al. (2015) reported similar β-sheet levels (29.4 %) in flaxseed.

Apart from HP, a slight increase in β-sheet content was also found in oilseed blends after extrusion, together with a little reduction in β-turn. More α-helix contents were found in HHP, RP and RHP, while random coil content had more fluctuation with the formulation and moisture content. This finding agrees with the one of Beck et al. (2017), who reported a significant increase in β-sheet content in soybean after extrusion (37.7 %–42.3 %) and a decrease in β-turn (23.0 %–16.2 %). Conversely, Meng et al. (2022) applied low moisture extrusion (26–35 %) to pea protein and observed a decrease in β-sheet content when the screw speed was below 675 rpm and the α-helix content was reduced for all conditions. This might be because the temperature, screw speed and moisture content all largely affected the secondary structures in the final product (Xiao et al., 2022). Besides random coil in HHP and RP, no significant difference was found after increasing moisture content to 20 %, which is similar to the findings of Gao et al. (2022), who did not observe any difference in α-helix, β-sheet and β-turn of extruded rice protein at 25 %, 30 %, 35 % and 40 % moisture content.

3.7. FPLC

FPLC technology was used to analyse the molecular weight (M_w) distribution of the raw and extruded oilseed blends without denaturing treatment (Fig. 5). The protein profiles varied among all the oilseed blends. Low M_w fractions (Albumin) has exerted a higher signal compared to high M_w fractions (globulin) in HP (8.1 kDa and 14.3 kDa, compared with 58.1 kDa), HHP (7.4 kDa, compared with 98.4 kDa) and RP (12.6 kDa, compared with 130 kDa). Conversely, FHP has more high M_w fractions signal (109.3 kDa). Two groups of protein profiles presented similar peaks intensity in the remaining samples. Wang et al. (2023) suggested that protein enrichment led to a reduction in the peaks observed, representing the globulin fraction due to aggregation. Similarly, oilseeds mixed with highly processed peas showed a lower peak intensity for high M_w fractions compared with those blends with less processed hemp flour.

Ten % moisture extrusion showed a pronounced negative effect on protein fractions, with the area under the curve which was found to be significantly reduced. The only exception was the peak representing 3.3 kDa in RP10 which was larger than the one observed for the raw

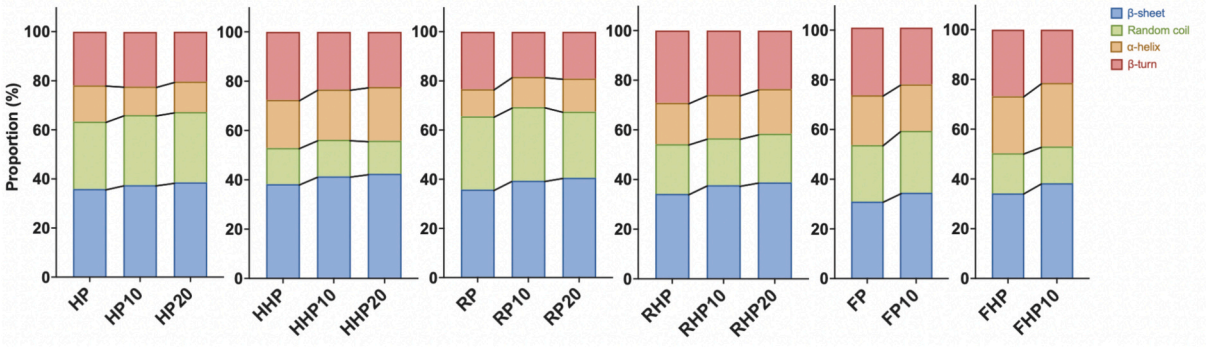

Fig. 4. Secondary structure of protein blends before and after 10 % or 20 % moisture extrusion.

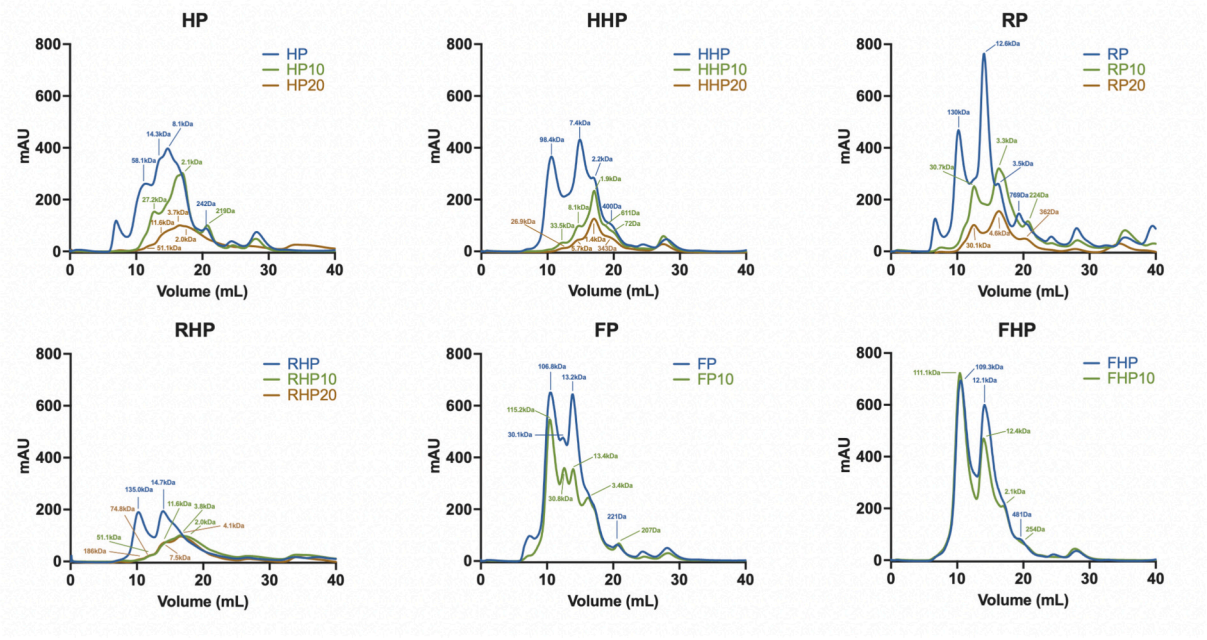

Fig. 5. FPLC gel filtration analysis of raw and extruded oilseed blends.

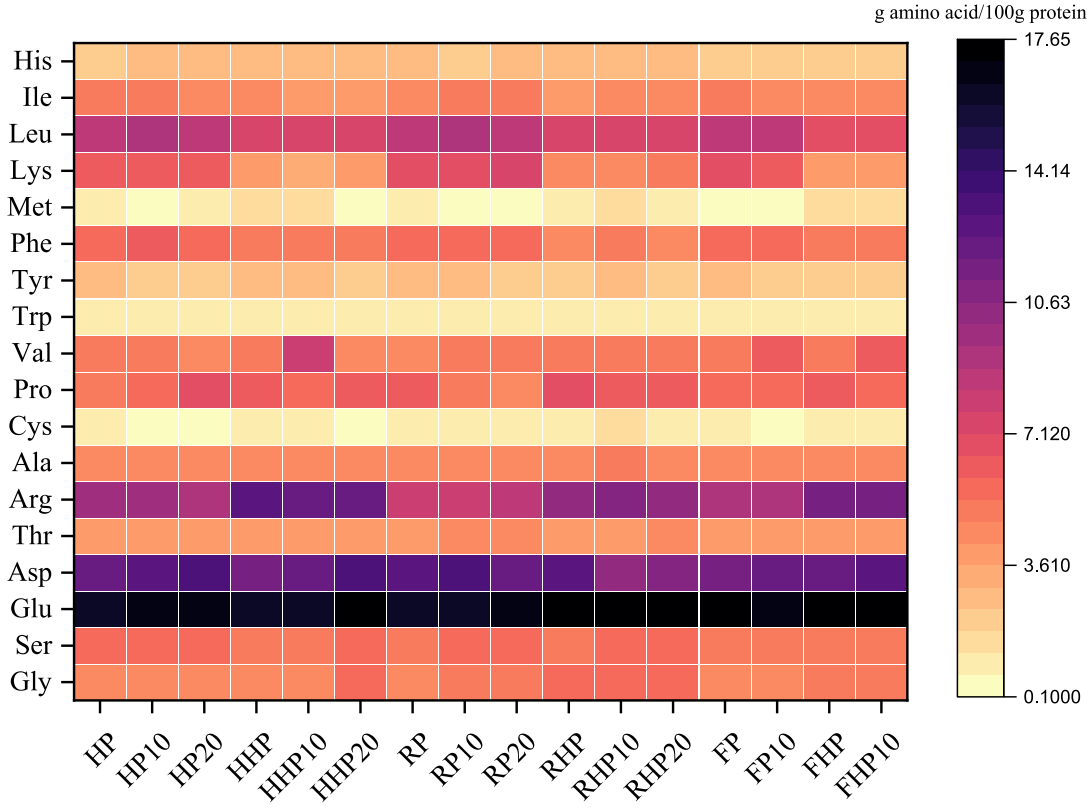

Fig. 6. Amino acid profiles of the raw and extruded oilseed blends (g amino acid/100 g protein).

ingredient. In HP, HHP, RP and RHP, extrusion greatly diminished the signal of high M_W protein fractions. However, a limited effect was found in the peaks representing $M_W < 3$ kDa. This might be because the aggregation and denaturation of high M_W proteins during extrusion cooking made them insoluble and thus undetectable by FPLC. Regarding flaxseed blends, the high M_W fractions were relativity stable after extrusion. A drop in the area under the curve was observed in the peak representing the medium M_W protein profiles (~30 kDa). In addition, when the feed moisture was increased from 10 % to 20 %, a further drop in the area under the curve was found in all the oilseed blends. Interestingly, high M_W protein profiles in HP (51.1 kDa) and RHP (74.8 kDa) were detectable after 20 % moisture extrusion, but completely disappeared in blends extruded at 10 % moisture.

3.8. Amino acid composition and protein quality

Relatively low levels of Trp (0.31–0.48 g/100 g), Cys (0.41–0.55 g/100 g) and Met (0.41–0.47 g/100 g) were observed in the oilseed blends (Fig. 6). In contrast, these blends were abundant in Leu, Arg, Glu and Asp. The incorporation of pea protein ingredient into the formulations significantly enhanced the overall amino acid composition, attributed to the high protein content of the pea protein. Meanwhile, the three oilseed blends tend to present similar amino acid profiles.

The effect of extrusion on amino acid profiles was largely dependent on the formulation, moisture, and temperature. Lys, is an amino acid which was reported to be significantly affected by extrusion (Singh et al., 2007). However, in this study, the reduction in Lys was only found in FP (10 % moisture extrusion), HP and RP (20 % moisture extrusion). It should be highlighted that Lys levels in RP increased following 10 % moisture extrusion. This may be attributed to the extrusion temperature being significantly lower than the threshold required to trigger chemical reactions, such as Maillard reaction, which typically causes a significant reduction in available Lys (Pizzoferrato et al., 1998). Among the amino acids analysed, Trp emerged as the most stable, with no observed changes post-extrusion, followed by His, which only exhibited a reduction in RP after 10 % moisture extrusion. For other essential amino acids, including IIe, Leu and Phe, changes were formulation-dependent and exhibited consistent patterns. Specifically, reductions were observed in HP following 10 % moisture extrusion, with more pronounced decreases after 20 % moisture extrusion. In contrast, HHP showed reductions irrespective of feed moisture levels. Conversely, RP and RHP demonstrated increases in these amino acids following 10 % moisture extrusion, while no significant differences were observed after 20 % moisture extrusion compared to unextruded samples. Surprisingly, FP and FHP showed no differences in these amino acids post-extrusion. In addition, Met levels varied across formulations. Reductions were observed in HP (10 % moisture extrusion, with slightly higher retention at 20 % moisture extrusion), HHP (20 % moisture extrusion), RP (20 % moisture extrusion), and FP (10 % moisture extrusion). However, RHP showed an increase in Met levels after 10 % moisture extrusion. Furthermore, Thr levels decreased after 20 % moisture extrusion across samples but increased in RP and RHP following 10 % moisture extrusion.

Overall, apart from HP and FHP, extrusion at 10 % moisture content tended to change the amino acid profiles of the oilseed blends. In contrast, extrusion at 20 % moisture content generally resulted in relatively less observable changes when compared to the raw blends. This observation aligns with findings reported by Jiddere and Filli (2015), who noted that increasing feed moisture during extrusion processing improved the retention of essential amino acids in sorghum malt and Bambara groundnut. These findings highlight the complex interplay between extrusion conditions, feed moisture content, and the retention of essential amino acids, underscoring the importance of optimizing processing parameters to preserve protein quality in extruded oilseed blends.

Table 4 shows the calculated protein quality parameters for the studied oilseed blends. EAAI reflecting the content of essential amino acids relative to egg white protein as a reference, showed minor variations across the blends, ranging from 59.86 (FHP) to 72.34 (HP). Blends containing pea protein showed slightly higher EAAI values compared to those with hemp protein. Extrusion, particularly at 10 % moisture, reduced the EAAI in pea protein blends, while in hemp protein blends, a decrease was observed only in HHP after 20 % moisture extrusion (declining from 69.50 to 60.47). The limiting amino acids in blends were grouped into three categories: Trp (HP, HHP, and RHP), Met + Cys (RP, FP), and Lys (FHP). Extrusion altered limiting amino acid profiles in HP, changed to Met + Cys, and in HHP, changed to Lys and Met + Cys after 10 % and 20 % moisture extrusion, respectively.

The predicted PERs also shown in Table 4, provide an estimate of the efficiency of essential amino acid profiles for protein utilization, ranging from 2.31 to 3.80. Pea blends displayed higher PER values compared to hemp protein blends, attributed to the relatively enhancement of essential amino acid profiles when oilseeds were blended with pea protein. Extrusion led to minimal changes to PER values, suggesting that that the extrusion process has a limited impact on the overall amino acid profiles.

The IVPDCAAS, which incorporates both the *in vitro* protein digestibility and limiting amino acid present in samples, revealed notable trends. For reference, the PDCAAS of hemp seed meal was reported to be 57 % (House et al., 2010), aligning closely with HHP blends (61.32 %). Pea protein was previously reported to have a much higher PDCAAS of 0.79 (Jiménez-Munoz, Tavares, et al., 2021). However, in this study, the IVPDCAAS values of hemp and pea blends were similar. Raw hemp blends (61.14 %–61.32 %) and rapeseed blends (58.27 %–60.08 %) showed higher IVPDCAAS values compared to flaxseed blends (51.89 %–52.86 %). Extrusion at a 10 % moisture level improved IVPDCAAS of RHP and FHP to 66.89 % and 58.87 %, respectively. The highest IVPDCAAS value was observed in RHP following 20 % moisture

Table 4
Protein quality parameters of HP, HHP, RP, RHP, FP and FHP before and after 10 % or 20 % moisture extrusion.

Oilseed blends	Treatment	EAAI (%)	AAS	BV	PER_1	PER_2	PER_3	PER_4	PER_5	IVPD (%)	IVPDCAAS (%)
HP	Raw	72.34	0.71 (Trp)	67.15	3.11	3.26	3.54	2.79	3.06	86.1 ± 1.5^{Ab}	61.14
	10 % moisture extrusion	60.14	0.12 (Met + Cys)	53.85	3.11	3.33	3.65	2.71	2.99	91.0 ± 0.4^{Aa}	11.17
	20 % moisture extrusion	67.83	0.44 (Met + Cys)	62.23	2.81	3.12	3.80	2.61	2.85	89.6 ± 0.8^{Aa}	39.72
HHP	Raw	69.50	0.76 (Trp)	64.06	2.46	2.68	2.87	2.43	2.98	80.7 ± 0.9^{Cc}	61.32
	10 % moisture extrusion	70.22	0.61 (Lys)	64.84	2.38	2.58	2.83	2.56	3.04	86.5 ± 0.8^{Ca}	52.75
	20 % moisture extrusion	60.47	0.40 (Met + Cys)	54.21	2.33	2.59	2.53	2.21	2.76	82.6 ± 0.5^{Cb}	33.05
RP	Raw	68.18	0.67 (Met + Cys)	62.61	3.02	3.22	3.39	2.76	2.94	85.4 ± 0.7^{Bb}	60.08
	10 % moisture extrusion	71.84	0.37 (Met + Cys)	66.61	3.19	3.36	3.48	2.80	2.96	89.7 ± 1.1^{ABa}	31.59
	20 % moisture extrusion	70.03	0.48 (Met + Cys)	64.63	3.12	3.29	3.70	2.83	2.99	88.5 ± 0.9^{ABa}	42.49
RHP	Raw	61.86	0.78 (Trp)	55.72	2.31	2.61	2.99	2.39	2.76	74.7 ± 1.2^{Dc}	58.27
	10 % moisture extrusion	63.75	0.75 (Trp)	57.78	2.46	2.69	2.97	2.49	2.89	88.2 ± 1.0^{Ba}	66.89
	20 % moisture extrusion	63.34	0.81 (Trp)	57.34	2.48	2.72	3.01	2.49	2.87	87.0 ± 0.4^{Bb}	70.44
FP	Raw	61.47	0.60 (Met + Cys)	55.31	2.89	3.08	3.23	2.76	2.99	87.4 ± 0.3^{Ab}	52.86
	10 % moisture extrusion	56.00	0.20 (Met + Cys)	49.34	2.91	3.14	3.36	2.77	2.98	90.1 ± 1.8^{Aa}	18.17
FHP	Raw	59.86	0.62 (Lys)	53.55	2.20	2.45	2.71	2.34	2.80	83.7 ± 1.4^{Bb}	51.89
	10 % moisture extrusion	62.09	0.66 (Lys)	55.98	2.28	2.50	2.81	2.49	2.89	89.2 ± 1.1^{ABa}	58.87

Different capital (among different formulations) and small (with/without extrusion) letters within the same column indicate significant differences ($p < 0.05$). EAAI – essential amino acid index; AAS – amino acid score; BV – Predicted biological value; PER_{1-5} – Protein efficiency ratio; IVPD (%) – *In vitro* protein digestibility (IVPD) and IVPDCAAS – *In vitro* protein digestibility-corrected amino acid score. HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w).

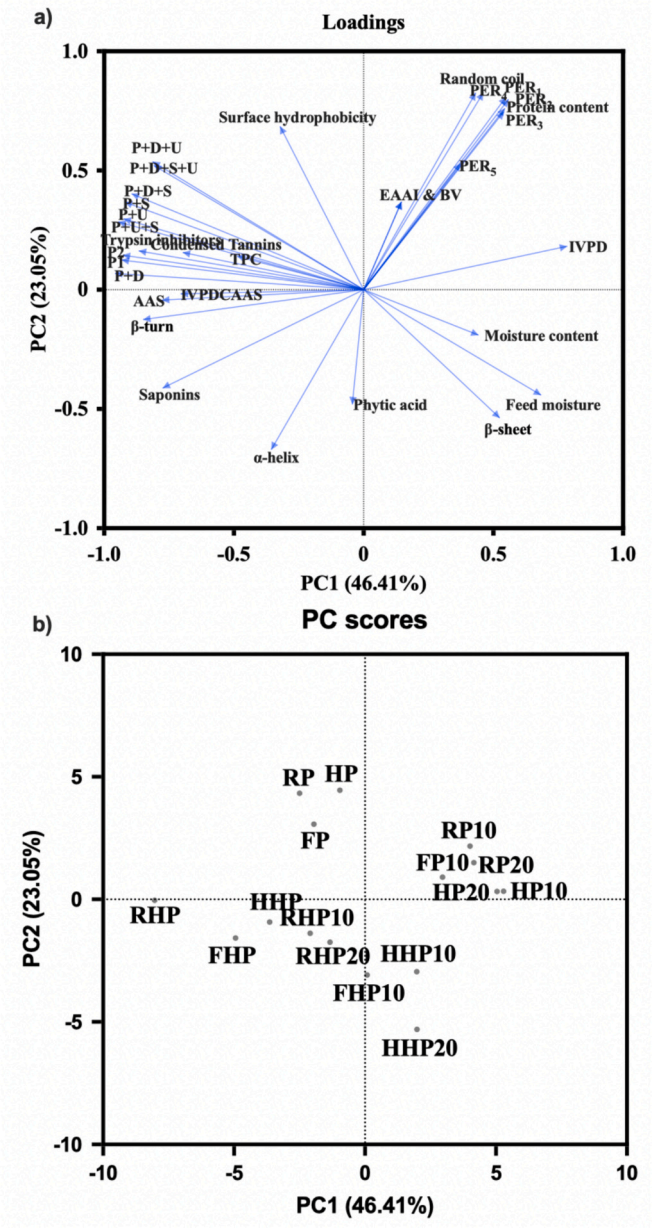

Fig. 7. Principal component analysis (PCA) plots of descriptive analysis for raw and extruded oilseed blends. P1, sodium phosphate buffer, measured by BCA kit; P2, sodium phosphate buffer, measured by Bio-Rad Protein Assay Kit; D, dithiothreitol; U, urea; S, sodium dodecyl-sulphate; EAAI – essential amino acid index; AAS – amino acid score; BV – Predicted biological value; PER_{1-5} – Protein efficiency ratio; IVPD (%) – *In vitro* protein digestibility (IVPD) and IVPDCAAS – *In vitro* protein digestibility-corrected amino acid score. HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w).

extrusion. Interestingly, apart from FHP and RHP, IVPDCAAS exhibited an inverse relationship with IVPD. A reduction in liming essential amino acids significantly impacted the final IVPDCAAS scores. For instance, the lowest IVPDCAAS score (11.17) was observed in HP following 10 % moisture extrusion. Despite HP exhibiting the highest IVPD (91.0 %) among all oilseed blends, the reduced IVPDCAAS score can be attributed to a significant decrease in the Met + Cys content, which declined from 0.75 to 0.12 g/100 g protein. This highlights the adverse effects of extrusion on the protein quality of oilseed blends, particularly due to its influence on the availability of limiting essential amino acids, underscoring the need for optimization in processing conditions to minimize these negative impacts.

3.9. Principal component analysis and Pearson correlation coefficient analysis

To evaluate the characteristics of oilseed blends before and after extrusion at 10 % and 20 % moisture levels, principal component analysis (PCA) was performed. The analysis including feeding moisture, moisture content, protein content, antinutritional factors, secondary structure, surface hydrophobicity, and protein quality parameters (Fig. 7) was performed. The first principal component (PC1) (Fig. 7a) accounted for 46.41 % of the total variation, while the second principal component (PC2) explained 23.05 %, resulting in a cumulative variance of 69.45 %. PC1 showed a strong positive correlation with IVPD (+0.78), feed moisture (+0.68), and PER_2 (+0.56), and a strong negative correlation with P + D (−0.96), P + U + S (−0.95), and P1 (−0.93) solubility.

Table 5
Pearson correlation coefficients (r) among protein content, moisture content, antinutritional factors, structural properties, and protein quality of oilseed blends under extrusion processing at moisture levels of 10 % and 20 %.

	10 % moisture extrusion	20 % moisture extrusion
Protein content	−0.0068	0.0206
Moisture content	0.1925	0.9538***
Total polyphenols	−0.2982	−0.0367
Phytic acid	0.0315	−0.2100
Condensed tannins	−0.4798	−0.3819
Saponins	−0.3587	−0.2198
Trypsin inhibitors	−0.8325***	−0.8055***
Surface hydrophobicity	−0.6957*	−0.6031*
P1	−0.6705*	−0.6997*
P2	−0.6926*	−0.7153*
P + D	−0.6513*	−0.7017*
P + U	−0.8797***	−0.8744***
P + S	−0.8900***	−0.9095***
P + D + U	−0.9415***	−0.9421***
P + U + S	−0.8895***	−0.9119***
P + D + S	−0.8643***	−0.8907***
P + D + S + U	−0.9614***	−0.9644***
β-sheet	0.6038*	0.7920**
Random coil	0.0155	0.0292
α-helix	0.0207	−0.1345
β-turn	−0.6031*	−0.7145*
EAAI	−0.1477	−0.0133
AAS	−0.5645	−0.5565
BV	−0.1478	−0.0136
PER_1	0.0787	0.0291
PER_2	0.0743	0.0750
PER_3	0.0989	0.1676
PER_4	0.1748	−0.1024
PER_5	0.2117	−0.2622
IVPD	0.6908*	0.4594
IVPDCAAS	−0.5073	−0.4987

*, **, and *** means the difference are significant at the 0.05, 0.01, and 0.001 level respectively. P1, sodium phosphate buffer, measured by BCA kit; P2, sodium phosphate buffer, measured by Bio-Rad Protein Assay Kit; D, dithiothreitol; U, urea; S, sodium dodecyl-sulphate; EAAI – essential amino acid index; AAS – amino acid score; BV – Predicted biological value; PER_{1-5} – Protein efficiency ratio; IVPD (%) – *In vitro* protein digestibility (IVPD) and IVPDCAAS – *In vitro* protein digestibility-corrected amino acid score. HP – defatted hempseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); HHP – defatted hempseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); RP – defatted rapeseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w); RHP – defatted rapeseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w); FP – defatted flaxseed meal was mixed with pea protein isolate at a ratio of 1:1 (w/w), and FHP – defatted flaxseed meal was mixed with hemp protein concentrate at a ratio of 1:1 (w/w).

PC2 was positively associated with PER_4 (+0.83), random coil (+0.83), PER_1(−0.81), while negatively correlated with α-helix (−0.67), β-sheet (−0.54) and phytic acid (−0.48). As show in Fig. 7b, oilseeds blends containing pea protein isolate (RP, HP, and FP) clustered in a different quadrant compared to those containing hemp protein concentrate (RHP, HHP, and FHP), suggesting the addition of pea protein isolate or hemp protein concentrate significantly influenced the physicochemical properties of the blends. Extrusion at 10 % and 20 % moisture levels did not markedly affect most measured parameters. Interestingly, rapeseed-hemp blends (RHP, RHP10, and RHP20) remained in the same quadrant, indicating limited impact of extrusion at both moisture levels on this formulation.

Simultaneously, Pearson correlation analysis was conducted to access the influence of extrusion on measured variables. As shown in Table 5, trypsin inhibitors, surface hydrophobicity, protein-protein interactions, and β-turn content were negatively correlated following 10 % moisture extrusion. Conversely, IVPD and β-sheet content exhibited positive correlations. Increasing feed moisture from 10 % to 20 %, was positively correlated with final moisture content in extruded oilseed blends. However, the strong correlation between IVPD and extrusion at 10 % feed moisture level was no longer significant at 20 % fed moisture level.

Taken together, the effects of extrusion processing were formulation dependent. The incorporation of pea protein isolate, or hemp protein concentrate into defatted oilseed cakes strongly influenced blend characteristics and extrusion outcome. Extrusion induced limited changes in rapeseed-hemp blends but significantly affected other formulations. The structure modification observed, including changes in surface hydrophobicity, disruption of protein-protein interactions, and alterations in secondary structure, are consistent with findings from previous studies (Chen et al., 2011; Chiang, 2007; Li et al., 2023; Xiao et al., 2025). Overall differences between the 10 % and 20 % feed moisture conditions were minimal, the primary distinction was that higher feed moisture resulted in greater moisture retention in the final extrudates. In addition, extrusion at 10 % moisture improved IVPD, but neither moisture level was strongly correlated with improved protein quality after accounting for changes in amino acid composition.

4. Conclusion

This study elucidated the effects of low-moisture extrusion (10 % and 20 % moisture) on the protein characteristics, antinutritional factors, and amino acid profiles of six different oilseed blends. Protein content remained unchanged following extrusion, while the process effectively reduced TIU and significantly decreased polyphenols, condensed tannins, and saponins levels. Consequently, these reductions contributed to an increase in *in vitro* protein digestibility. Extrusion also significantly reduced protein solubility and disrupted hydrophobic interactions, although most disulphide bonds were retained in extruded blends. Moreover, extrusion influenced the secondary protein structure, with a notable increase in β-sheet (%) content and a decrease in β-turn content. Regarding amino acid profiles, extrusion has a limited impact overall, though a significant reduction in the limiting amino acid Met was observed. Comparatively, 10 % moisture extrusion had a greater impact on surface hydrophilicity and amino acid profiles than 20 % moisture extrusion. Improved protein quality was observed only in RHP and FHP, RHP extruded at 20 % feed moisture exhibited the highest protein quality, with an IVPDCAAS value of 70.44. Despite most amino acids were retained post-extrusion in other formulations, the IVPDCAAS was significantly reduced, largely due to the decrease in Met. Nonetheless, extrusion improved the overall quality of oilseed blends by reducing ANFs and enhancing protein digestibility, although some negative effects on limiting amino acids were observed. Further research is recommended to evaluate the health benefits, sensory attributes, and textural properties of these extruded oilseed blends. Such studies would provide more robust evidence supporting their potential as high-quality, palatable, and nutritious ingredients for plant-based meat products.

CRediT authorship contribution statement

Ruixian Han: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Rebecca McDowell:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Sarah Gaunt:** Writing – review & editing, Validation, Resources, Project administration, Investigation, Conceptualization. **Martin Mondor:** Writing – review & editing, Validation, Supervision. **Alan Javier Hernández-Álvarez:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

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

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