

Lupin-based high-protein extrudates: Physicochemical, nutritional and techno-functional properties

Paola Navarro-Vozmediano^a, José V. García-Pérez^b, Amparo Quiles^c, Ricard Bou^d,
Rosa Jorba-Martín^e, José Benedito^{b,*}

^a Programa de Doctorado en Ciencia, Tecnología y Gestión Alimentaria, Grupo ASPA, Instituto Universitario de Ingeniería de Alimentos - FoodUPV, Universitat Politècnica de València, Camí de Vera s/n, E46022 València, Spain

^b Grupo ASPA, Instituto Universitario de Ingeniería de Alimentos - FoodUPV, Universitat Politècnica de València, Camí de Vera s/n, E46022 València, Spain

^c Food Chemistry and Microstructure Research Group, Instituto Universitario de Ingeniería de Alimentos - FoodUPV, Universitat Politècnica de València, Camí de Vera s/n, E46022 València, Spain

^d Food Safety and Functionality program, IRTA, Finca Camps i Armet s/n, E17121 Monells, Spain

^e Institut d'Investigació Sanitària Pere Virgili (IISPV), C. Dr Mallafré Guasch 4, Edifici D, E43005 Tarragona, Spain

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ABSTRACT

Lupin is an underutilized legume with high protein content (30–40 %) and strong potential for sustainable cultivation, but its use in the food industry is limited by the presence of anti-nutritional (saponins, alkaloids, polyphenols) and anti-technological factors, as well as suboptimal techno-functional properties. Low-moisture extrusion (LME) offers a promising strategy to overcome these limitations while producing high-protein texturized ingredients. In this study, five formulations combining lupin flour with laboratory or commercial lupin protein isolates were subjected to LME to evaluate effects on anti-nutritional compounds, techno-functional properties, and textural attributes. Extrusion reduced polyphenols (avg. 42 %), antioxidant activity (avg. 17 %) and saponins (avg. 47 %), while alkaloids remained stable. Water and fat absorption capacities were largely maintained (avg. 1.26 g water/g dm and 1.66 g oil/g dm, respectively), compared to powder formulations (avg. 1.31 g water/g dm and 2.25 g oil/g dm, respectively). Microstructural observations, along with bulk density (avg. 1.09 g/cm³) and texture analysis (average hardness of 346 N) confirmed the formation of compact high-protein extrudates (up to 88.6 % protein) and showed the effects of protein incorporation on matrix organization and textural properties, supporting their suitability as functional ingredients. Overall, this work demonstrates that LME can enhance the properties of lupin flour and isolates, producing high-protein extrudates with improved nutritional, functional, and structural qualities.

1. Introduction

The growing demand for sustainable protein sources has accelerated the use of plant proteins in the food industry. Legumes are particularly attractive due to their high protein content and lower environmental footprint compared to animal-based proteins (Pam Ismail et al., 2020). Among legumes, lupin has gained attention not only for its high protein content (around 40 %) but also for its contribution to environmental sustainability, as its capacity for nitrogen fixation reduces the need for external fertilizers and pesticide applications (Lo et al., 2021; Szczepański et al., 2022). However, its incorporation into food systems is often limited by the presence of anti-nutritional factors (ANF) such as

polyphenols, saponins and alkaloids, which can reduce digestibility and nutrient bioavailability and by anti-technological factors (ATF), including polyphenols and lipids, which may interfere with protein extraction and processing and subsequent applications in food formulations (Chéreau et al., 2016; Rahate et al., 2021). Although most ANF and ATF are reduced during alkaline protein extraction, some may persist or be concentrated in the resulting isolates, depending on the specific processing conditions applied (Navarro-Vozmediano et al., 2025). In addition, plant proteins frequently exhibit limited techno-functional properties such as solubility, water and fat absorption, emulsification and foaming capacity, which are critical for their suitability in food formulations (De Carvalho Oliveira et al., 2025;

* Corresponding author at: Instituto de Ingeniería de Alimentos, Food-UPV, Universitat Politècnica de València, Camí de Vera s/n, E46022 València, Spain.
E-mail address: jjbenedi@tal.upv.es (J. Benedito).

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Venkateswara-Rao et al., 2023). Moreover, plant proteins often require some degree of texturization to improve their application in food product development by simulating the structure of meat fibers.

Therefore, various strategies can be employed to overcome these limitations of plant-based proteins, including the ANF and ATF presence, their limited techno-functional properties and the lack of a fiber-like texture. Additional processing steps, including baking, cooking and extrusion, have been reported to partially reduce the concentration of various ANF and ATF in lentil, pea and bean flours (Sánchez-Velázquez et al., 2021). Among these approaches, extrusion has emerged as one of the most effective strategies to overcome these limitations. During this process, proteins are subjected to high temperature, pressure and mechanical deformation, leading to structural protein changes that may enhance digestibility, improve techno-functional and textural properties, while also reducing ANF and ATF content (Han et al., 2025; Schmid et al., 2022). Depending on the target application, extrusion can be operated under different moisture content. High-moisture extrusion (HME), with moisture levels above 50 %, enables the development of fibrous, meat-like texturized proteins through protein alignment and crosslinking (Bou et al., 2022). In contrast, low-moisture extrusion (LME), typically conducted at moisture levels under 35 %, is widely used for the production of texturized proteins with a sponge-like structure and a firm, porous texture (Akharume et al., 2021; Dekkers et al., 2018).

Previous research on LME texturization has mainly focused on flours or flour blends, including soybean and rice flour (Park et al., 2024), wheat flour (Chai et al., 2025), rice, pea and carob flour blend (Arribas et al., 2017) or chickpea, sorghum and maize flours (Wang et al., 2019). However, very few studies have addressed the extrusion of protein isolates or formulations with very high protein content, such as pea protein isolate (Webb et al., 2023) or soybean protein isolate (Xiao et al., 2023).

Regarding lupin, research has predominantly focused on HME (Elhordoy et al., 2025; Palanisamy et al., 2019). In contrast, LME studies have primarily examined lupin-based blends with other ingredients, such as rice and pumpkin Alefew et al. (2024), whereas the application of extrusion to lupin as a sole raw material has been largely restricted to improving digestibility in animal feed (Kasanova et al., 2025; Mendowski et al., 2019; Prandini et al., 2005). In this context, the application of LME to high-protein lupin-based formulations represents a promising yet underexplored approach, as it enables the production of texturized, high-protein matrices with a strong potential for incorporation into protein-rich foods, including meat analogues such as dry-cured sausages or hamburgers.

Additionally, as mentioned above, extrusion can also contribute to the reduction of ANF and ATF while modifying techno-functional properties. For instance, Han et al. (2025) found that LME (10 % and 20 % moisture levels) effectively reduced the levels of polyphenols, saponins and tannins of oilseed formulations. Likewise, reductions in saponin content due to extrusion have been reported in faba bean (Saldanha do Carmo et al., 2025) and pea and pinto beans

(Sánchez-Velázquez et al., 2021). Moreover, several studies have also demonstrated the ability of extrusion process to modify the water and fat absorption indexes of different plant proteins (Cagnin et al., 2024; Espinosa-Ramírez et al., 2021).

Despite these findings, limited information is available on the application of low-moisture extrusion to high-protein lupin-based systems. Therefore, the objective of this study was to develop high-protein lupin-based extrudates using LME and to evaluate the effects of extrusion on the reduction of ANF and ATF, the enhancement of techno-functional properties and the resulting textural characteristics.

2. Materials and methods

2.1. Raw material and formulations. particle size assessment

Lupin flour (LF) (Semillas Batlle S.A, Barcelona, Spain) was selected as the reference raw material for extrusion. However, since the main objective of the study was to maximize the protein content of the extrudates, LF was blended with a laboratory-prepared lupin protein isolate (LPI) obtained via alkaline extraction according to Navarro-Vozmediano et al. (2025) at different ratios (100:0, 20:80 and 0:100 w/w), allowing the evaluation of extrusion effects in systems with progressively increasing protein content. In addition, LF was blended with a commercial lupin protein isolate (LPI.C) (Prolupin GmbH, Grimm, Germany) at 80:20 and 60:40 (w/w) ratios to evaluate the feasibility of extrusion using available commercial lupin protein isolates. Blends with higher proportions of LPI.C could not be extruded due to process limitations. Consequently, five different powder formulations were extruded: (i) LF 100:0 LPI, (ii) LF 20:80 LPI, (iii) LF 0:100 LPI, (iv) LF 80:20 LPI.C and (v) LF 60:40 LPI.C. The chemical characterization of the raw materials (determined as explained in Section 2.3.1) is presented in Table 1.

2.1.1. Particle size assessment

Particle size distributions of the raw materials (LF, LPI and LPI.C) were determined using a laser diffraction analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK) equipped with a dry dispersion unit (Scirocco 2000, Malvern Instruments Ltd., Malvern, UK). The volume-weighted mean diameter (D[4.3]) and percentile diameters d(0.1), d(0.5) and d(0.9) were recorded (Table 1).

2.2. Low-moisture extrusion (LME) processing

Before extrusion, each formulation was hydrated to reach a water content of 30 g water/100 g. The experimental setup for lupin extrusion is illustrated in Fig. 1. Extrusion was carried out using a single-screw extruder (KE 19/25, Brabender, Germany) equipped with a 3 mm diameter die, operating at a compression ratio of 3:1 and 150 rpm extruder screw speed. Samples were fed into the extruder at 1.34 kg/h

Table 1
Chemical characterization and particle size assessment of raw materials.

		LF	LPI	LPI.C
Chemical characterization	Protein content (g protein/100 g dm)	42.4 ± 0.8	88.6 ± 0.3	83.9 ± 1.6
	FC (g/100 g dm)	3.75 ± 0.10	10.33 ± 0.60	4.20 ± 0.21
	TPC (mg gallic acid/g dm)	2.72 ± 0.11	1.56 ± 0.17	1.57 ± 0.36
	AA	5.71 ± 0.56	5.99 ± 0.50	2.57 ± 0.49
	(µM Trolox/ g dm)			
	TSC (g oleoanic/100 g dm)	1.25 ± 0.08	2.04 ± 0.17	0.89 ± 0.09
	AC (g lupinine/100 g dm)	0.44 ± 0.02	nd	nd
Particle size	d(0.1) (µm)	65 ± 7	66 ± 2	14 ± 1
	d(0.5) (µm)	687 ± 27	224 ± 2	44 ± 2
	d(0.9) (µm)	1361 ± 22	603 ± 21	96 ± 2
	D[4.3] (µm)	714 ± 23	296 ± 7	50 ± 2

LF, lupin flour; LPI, lupin protein isolate; LPI.C, commercial lupin protein isolate; FC, fat content; TPC, total polyphenol content; AA, antioxidant activity; TSC, total saponin content; AC, alkaloid content; d(0.1), 10 % standard percentile; d(0.5), 50 % standard percentile; d(0.9) 90 % standard percentile; D[4,3], volume-weight mean diameter. Values are presented as average ± SD. nd: non-detectable.

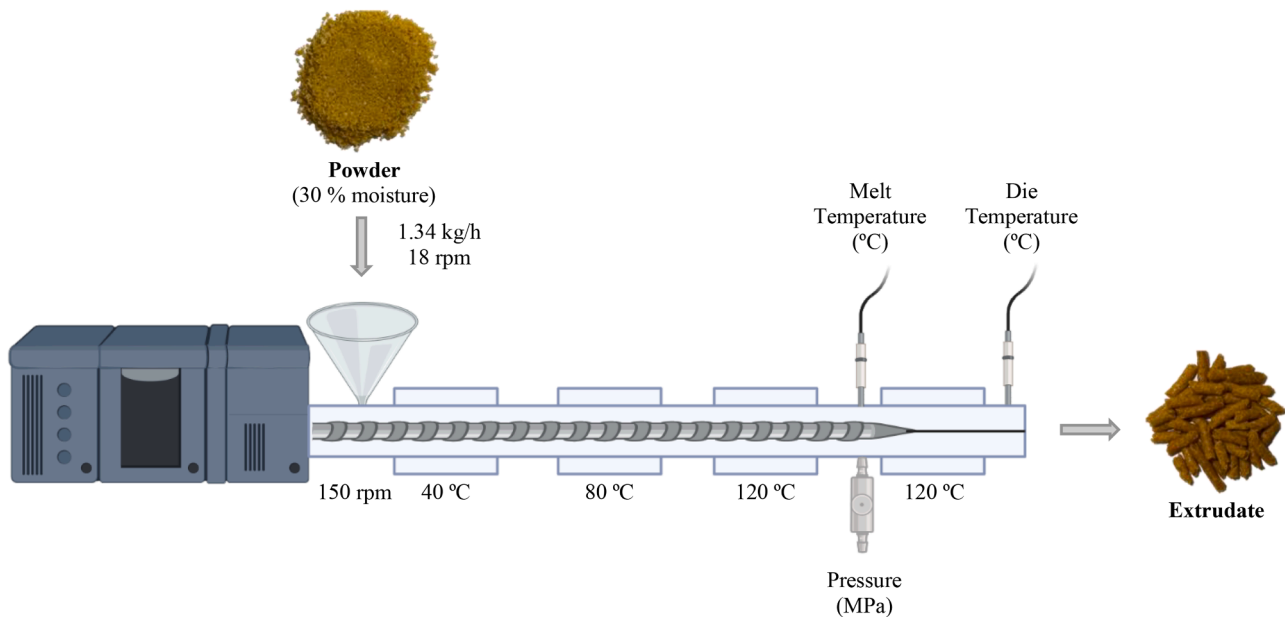


Fig. 1. Scheme of extrusion process.

and 18 rpm speed. Barrel temperatures (Fig. 1) were set at 40, 80, 120 °C (melt and die temperature) and were monitored together with the pressure during extrusion.

After extrusion, samples were dried at 65 °C in a convective drier (Binder FD 260, Binder, Tuttlingen, Germany) to reach a final moisture content of 8 g water/100 g to avoid mold growth. The water loss after extrusion and drying was calculated using Eq. (1).

$$\text{Water loss(\%)} = \frac{M_1 - M_2}{M_1} \times 100 \quad (1)$$

where, M_1 (%) is the moisture content before the extrusion/drying and M_2 is the moisture content after the extrusion/drying.

Specific mechanical energy (SME) was calculated using the Eq. (2) (Fang et al., 2014).

$$\text{SME} \left(\frac{\text{kJ}}{\text{kg}} \right) = \frac{2 \times \pi \times n \times T}{m_f} \quad (2)$$

where, n (rpm) is the screw speed, T (Nm) is the torque and m_f (g/min) is the mass flow rate (Gulati et al., 2016).

2.3. Characterization of powders and extrudates

For powders, protein, fat, alkaloid, total polyphenol and total saponin content and antioxidant activity were determined. For extrudates, the same parameters were assessed, along with techno-functional properties, including water and fat absorption indexes, bulk density, texture, color and microstructure.

2.3.1. Anti-nutritional and anti-technological factors and techno-functional properties

2.3.1.1. Protein content. Determination of protein was conducted using Kjeldahl method 2.062 (AOAC, 1984), considering a protein factor of $N \times 6.25$.

2.3.1.2. Fat content. Determination of fat content (FC) was conducted using Soxhlet extraction method 991.36 (AOAC, 1996).

2.3.1.3. Total polyphenol content and antioxidant activity. Methanolic extracts for total polyphenol content and antioxidant activity

quantification were obtained following the method described by Navarro-Vozmediano et al. (2025) without modifications.

Quantitative analysis of total polyphenol content (TPC) was carried out following the Folin-Ciocalteu method (Singleton et al., 1999), with slight modifications as described in Navarro-Vozmediano et al.(2025). While, antioxidant activity (AA) was determined by the ferric reducing antioxidant power assay (FRAP) (Benzie & Strain, 1996), with slight modifications as described in Navarro-Vozmediano et al.(2025), to adapt it to microplates.

2.3.1.4. Total saponin content. The total saponin content (TSC) was measured using the spectrophotometric method described by Navarro del Hierro et al.(2020).

2.3.1.5. Alkaloid content. Alkaloid extracts were prepared according to the protocol described in Ganzera et al.(2010), and their content was quantified using a modified version of the titration method by Ruiz (1977), as adapted by Navarro-Vozmediano et al.(2025).

2.3.1.6. Water and fat absorption indexes. Water and fat absorption indexes (WAI and FAI) were evaluated following the method proposed by Lian et al.(2020), with minor modifications as described by Navarro-Vozmediano et al.(2025).

2.3.1.7. Instrumental color. The color was measured using a Konica Minolta CM-2600D colorimeter (Tokyo, Japan), previously calibrated with illuminant D65 and a 10° visual angle. CIELab parameters L^* (lightness), a^* (green–red), and b^* (blue–yellow) were recorded, and h^* (hue angle), C^* (chroma), and ΔE_1 and ΔE_2 (total color difference between powders and extrudates and total color differences between LF 100:0 LPI extrudate and other extrudates, respectively) were calculated (Sakai et al., 2022).

2.3.1.8. Bulk density. After drying, the diameter and length of extrudates were measured using a caliper. At least twenty measurements were taken for each formulation and bulk density (BD) was calculated using the Eq. (3), assuming cylindrical shape (O'Shea et al., 2014).

$$\text{BD} \left(\frac{\text{g}}{\text{cm}^3} \right) = \frac{4 \times m}{\pi \times D^2 \times L} \quad (3)$$

where, m (g) is the mass of the extrudate, D (cm) is its diameter and L (cm) is its length.

2.3.1.9. Textural analysis. Textural properties of the extrudates were evaluated by means of a compression-extrusion test using a TA.XT2i texture analyser (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell and a 4.5 cm diameter cylindrical methacrylate probe. 20 g of extrudates were placed in a methacrylate cylinder (5 cm diameter and 6 cm length) to ensure a sample height of approximately 1.9 cm. Samples were compressed to 15 % strain at a test speed of 2 mm/s. Ten measurements were carried out for each formulation. The experimental data was recorded and subsequently analyzed using Exponent Lite 6.1.4.0 software (Stable Micro System, Surrey, UK). Hardness (N) was determined from the force-time curves obtained as the maximum compression force.

2.3.2. Microstructure

The extrudates were examined using Field Emission Scanning Electron Microscopy (FESEM). The samples were fixed onto stubs with carbon paste and coated with a thin gold layer by sputtering. Observations were carried out using a Zeiss Ultra 55 FESEM (Oberkochen, Germany) at an accelerating voltage of 4 kV and a working distance of 12 mm.

2.4. Statistical analysis

Experimental results were reported as average \pm SD and one-way analysis of variance (ANOVA) with 95 % confidence ($p < 0.05$) was carried out to identify significant differences between samples. For each formulation, comparisons were made between the powder and its corresponding extrudate to evaluate the effect of extrusion, while differences between formulations were assessed separately for powders and extrudates. ANF, ATF and techno-functional properties were compared using LSD intervals (Least Significant Difference). All statistical analysis was performed using the Statgraphics Centurion XVIII software (Statpoint Technologies, VA, USA).

3. Results and discussion

3.1. Water loss and extrusion parameters

The five feed formulations used (Table 2) had an initial moisture content of 30 g water/100 g dm. During extrusion, water evaporated due to the high temperatures involved, resulting in an average moisture loss of 48.4 % (Table 2) and yielding extrudates with an average moisture

Table 2

Extrudate water loss and process parameters for the extrusion of powder formulations prepared by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios.

	WLE (%)	WLD (%)	Melt T (°C)	Die T (°C)	P (MPa)	SME (kJ/kg)
LF 0:100	48.4 \pm 0.2 ^b	48.3 \pm 0.3 ^b	123.1 \pm 0.1 ^a	124.9 \pm 0.3 ^a	2.9 \pm 0.9 ^d	977.2 \pm 8.3 ^c
LPI	44.3 \pm 0.6 ^c	52.1 \pm 0.5 ^a	123.2 \pm 0.6 ^a	124.1 \pm 0.5 ^a	2.9 \pm 0.5 ^d	871.5 \pm 5.1 ^d
LF 100:0	47.6 \pm 1.1 ^b	49.1 \pm 1.0 ^b	123.1 \pm 0.8 ^a	123.9 \pm 0.6 ^a	3.6 \pm 0.8 ^c	509.1 \pm 7.6 ^e
LPI.C	48.6 \pm 2.6 ^b	48.1 \pm 2.7 ^b	123.4 \pm 0.3 ^a	124.4 \pm 0.5 ^a	7.8 \pm 0.3 ^b	1048.3 \pm 9.5 ^b
LF 60:40	53.1 \pm 1.8 ^a	43.0 \pm 2.2 ^c	122.6 \pm 0.1 ^a	124.9 \pm 0.2 ^a	10.9 \pm 1.2 ^a	1178.1 \pm 10.1 ^a
LPI.C						

WLE, water loss after extrusion; WLD, water loss after drying; T, temperature; P, melt pressure; SME, specific mechanical energy.

Values are presented as average \pm SD. Different lowercase letters in columns indicate significant differences ($p < 0.05$).

content of 15 g water/100 g. The highest water loss was observed in LF 60:40 LPI.C (53.1 %, Table 2), whereas LF 20:80 LPI showed the lowest (44.3 %, Table 2). After the subsequent drying process, an average additional water loss of 48.1 % was observed, reducing the moisture content to a final value of 8 g water/100 g which would likely prevent mold growth and ensure the stability and safety of the final product.

Regarding extrusion parameters, melt and die temperatures were very similar to each other, averaging 123.1 °C and 124.4 °C, respectively, with no significant ($p > 0.05$) differences across formulations (Table 2). Melt temperature was consistently maintained at the set values for all formulations, indicating effective temperature control by the extruder and stable high-temperature processing conditions. In contrast, melt pressure exhibited a different pattern: it remained relatively constant for the formulations containing LF and LF+LPI but significantly ($p < 0.05$) increased with the addition of LPI.C. Thus, the formulation with the highest proportion of the commercial isolate (LF 60:40 LPI.C) achieved the largest melt pressure (up to 10.9 MPa, Table 2). Among other factors, this increase could be linked to the very fine particle size in LPI.C (D[4.3] 50 μ m, d(0.1) 14 μ m, d(0.5) 44 μ m and d(0.9) 96 μ m, Table 1). Very fine particles exhibit higher interparticle cohesion and reduced void spaces, which, upon wetting, promote surface gel formation that limits moisture penetration, increases material cohesiveness, and consequently might raise flow resistance and pressure during extrusion (Jiang et al., 2025). Accordingly, formulations containing the commercial isolate, LF 80:20 LPI.C and LF 60:40 LPI.C, led to the highest SME figures (Table 2). Similar to LPI.C formulations, SME showed a significant ($p < 0.05$) increasing trend with LPI proportion, likely due to the lower particle size observed for LPI compared to LF (D [4.3] 296 μ m, d(0.1) 66 μ m, d(0.5) 224 μ m and d(0.9) 603 μ m and D [4.3] 714 μ m, d(0.1) 65 μ m, d(0.5) 687 μ m and d(0.9) 1361 μ m, respectively, Table 1). The smaller particle size, associated with a greater surface area, increases interparticle friction, thereby enhancing resistance to flow and increasing energy requirements (McGuire et al., 2022). These observations are consistent with previous reports for maize (Sharifi et al., 2021) and soybean (Singh & Koxsel, 2021). Furthermore, extrusion behavior may also be affected by fat content, which improves flow and reduces the SME (Majzoobi & Farahnaky, 2010), as observed in formulations containing LPI (FC of 10.33 g/100 g dm, Table 1) compared to LPI.C (FC of 4.2 g/100 g dm, Table 1).

3.2. Changes in protein, ANF and ATF content after extrusion

3.2.1. Protein content

The experimental results revealed significant ($p < 0.05$) differences in protein content between the powder formulations (Table 3). As expected, formulations with higher LPI content, whether commercial or lab-prepared, showed the highest protein content. The same trend was observed for the extrudates (Table 3). However, the extrudates exhibited slightly but significantly ($p < 0.05$) lower protein content compared with their corresponding powder formulations. Similarly, Espinosa-Ramírez et al. (2021), Wang et al. (2019), Ai et al. (2016), Robutti et al. (2002) and Escobedo-Avellaneda et al. (2025) reported that extrudates of amaranth, chickpea, bean, maize and soybean flours contained significantly ($p < 0.05$) lower protein content than the corresponding flours. In contrast, Marzo et al. (2002) and Solanas et al. (2005) observed that extrusion led to a significant ($p < 0.05$) increase in protein content for pinto bean and soybean. However, some authors, including Escobedo-Avellaneda et al. (2025) and Espinosa-Ramírez et al. (2021) have suggested that protein should be largely unaffected by the extrusion process as plant proteins tend to be more resistant. Thereby, the reductions observed in protein content in the present work may be primarily linked to structural modifications caused by denaturation, aggregation or Maillard reactions, which could potentially affect its solubility and extractability during Kjeldahl analysis. This may lead to an apparent decrease in measured protein content rather than a true loss of protein Arribas et al. (2017); Robutti et al. (2002).

Table 3

Protein content, anti-nutritional and anti-technological factors of powder formulations prepared by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios and their corresponding extrudates.

	Formulation	Protein content (g protein/100 g dm)	FC (g/100 g dm)	TPC (mg gallic acid/g dm)	AA (μ M Trolox/ g dm)	TSC (g oleanolic/100 g dm)	AC (g lupinine/100 g dm)
Powder	LF 0:100 LPI	88.6 \pm 0.3 ^{Aa}	10.33 \pm 0.60 ^{Aa}	1.56 \pm 0.17 ^{Da}	5.99 \pm 0.50 ^{Aa}	2.04 \pm 0.17 ^{Aa}	nd
	LF 20:80 LPI	78.6 \pm 1.1 ^{Ba}	8.08 \pm 0.63 ^{Ba}	1.82 \pm 0.04 ^{Ca}	5.84 \pm 0.03 ^{Aba}	1.91 \pm 0.10 ^{Aa}	0.08 \pm 0.01 ^{Da}
	LF 100:0 LPI	42.4 \pm 0.8 ^{Ba}	3.75 \pm 0.10 ^{Ca}	2.72 \pm 0.11 ^{Aa}	5.71 \pm 0.56 ^{Ba}	1.25 \pm 0.08 ^{Ba}	0.44 \pm 0.02 ^{Aa}
	LF 80:20 LPI.C	48.5 \pm 0.2 ^{Da}	3.87 \pm 0.12 ^{Ca}	2.65 \pm 0.05 ^{Aa}	5.12 \pm 0.11 ^{Ca}	1.21 \pm 0.15 ^{Ba}	0.33 \pm 0.04 ^{Ba}
	LF 60:40 LPI.C	59.4 \pm 0.7 ^{Ca}	4.28 \pm 0.22 ^{Ca}	2.41 \pm 0.05 ^{Ba}	4.46 \pm 0.11 ^{Da}	1.11 \pm 0.05 ^{Ba}	0.27 \pm 0.02 ^{Ca}
Extrudate	LF 0:100 LPI	84.2 \pm 0.4 ^{Ab}	8.96 \pm 0.10 ^{Ab}	1.20 \pm 0.08 ^{Ab}	5.09 \pm 0.35 ^{Ab}	0.97 \pm 0.08 ^{Ab}	nd
	LF 20:80 LPI	74.2 \pm 0.4 ^{Bb}	6.49 \pm 0.35 ^{Bb}	1.21 \pm 0.12 ^{Ab}	5.26 \pm 0.35 ^{Ab}	0.83 \pm 0.02 ^{Bb}	0.06 \pm 0.01 ^{Ca}
	LF 100:0 LPI	39.9 \pm 0.7 ^{Bb}	3.41 \pm 0.12 ^{Bb}	1.26 \pm 0.12 ^{Ab}	4.87 \pm 0.30 ^{Bb}	0.80 \pm 0.05 ^{Bb}	0.35 \pm 0.03 ^{Aa}
	LF 80:20 LPI.C	45.4 \pm 0.9 ^{Bb}	3.45 \pm 0.21 ^{Bb}	1.20 \pm 0.09 ^{Ab}	3.80 \pm 0.30 ^{Cb}	0.65 \pm 0.04 ^{Cb}	0.24 \pm 0.04 ^{Ba}
	LF 60:40 LPI.C	55.1 \pm 0.7 ^{Cb}	4.02 \pm 0.23 ^{Cb}	1.29 \pm 0.09 ^{Ab}	3.63 \pm 0.24 ^{Cb}	0.58 \pm 0.02 ^{Cb}	0.21 \pm 0.04 ^{Ba}

FC, fat content; TPC, total polyphenol content; AA, antioxidant activity; TSC, total saponin content; AC, alkaloid content.

Values are presented as average \pm SD. Different capital letters in columns indicate significant differences ($p < 0.05$) between formulations of powders and extrudates. Different lowercase letters in columns indicate significant differences ($p < 0.05$) between powder and its extrudate. nd: non-detectable.

3.2.2. Fat content

Variations in fat content (FC) were observed depending on the formulation (Table 3). Due to the higher fat content of the isolates compared to LF, formulations with a greater proportion of protein isolate, either LPI or LPI.C, exhibited significantly ($p < 0.05$) higher FC compared to those richer in LF for both powders and extrudates. Interestingly, the extrudates showed a significant ($p < 0.05$) reduction in FC compared to their corresponding powder formulation. The highest relative loss of fat (around 20 %), between powders and extrudates, was found in the formulation LF 20:80 LPI (Fig. 2). Espinosa-Ramírez et al. (2021) found that FC of different flours (rice, oat, amaranth, chickpea, sorghum, quinoa, lentil, black bean and pinto bean) significantly ($p < 0.05$) decreased after extrusion. Similarly, Wang et al. (2019) observed comparable reductions in chickpea, sorghum and maize flour extrusion. Several mechanisms may explain this reduction, including the formation of lipid-starch complexes under high temperature and pressure, which could favor the retention of the lipid fraction (De Pilli et al., 2008), oil dripping observed at the nozzle (Van Hoan et al., 2010) and the formation of other lipid complexes, which could be not extractable using petroleum ether (Arribas et al., 2017; De Pilli et al., 2011). Therefore, given the absence of oil dripping and the minimal starch content in lupin flour and isolates (0.2 g/100 g dm; Arzami et al., 2022), the lower FC observed in extrudates is most likely attributable to the formation of

lipid complexes unextractable by Soxhlet method without acid hydrolysis using petroleum ether.

Overall, FC was higher in LPI-rich extrudates, while those containing LPI.C showed values similar to LF. Noteworthy, does not represent a drawback, as it may enhance lipid-binding capacity and thus support the use of these extrudates in protein-rich food applications.

3.2.3. Total polyphenol content and antioxidant activity

Among the powder formulations, LF (LF100:0 LPI) exhibited the highest total polyphenol content (TPC) (2.72 mg gallic acid/g dm, Table 3). Consequently, formulations with a higher proportion of LF showed significantly ($p < 0.05$) higher TPC compared to those richer in LPI (1.56 mg gallic acid/g dm, Table 1) or LPI.C (1.57 mg gallic acid/g dm, Table 1). Similarly, as also observed in Table 1, the highest antioxidant activity (AA) was found in LPI (5.99 μ M Trolox/ g dm,) and LF (5.71 μ M Trolox/ g dm), which may explain the significantly ($p < 0.05$) higher values observed in powder formulations containing greater proportions of these components (Table 3).

A significant ($p < 0.05$) decrease in TPC was observed in all extrudates compared to their corresponding powder formulations (Fig. 2). The relative loss of TPC ranged from 23 to 55 %, with the greatest reduction found in LF 100:0 LPI and LF 80:20 LPI.C. Similarly, AA significantly decreased ($p < 0.05$) after extrusion in all formulations and

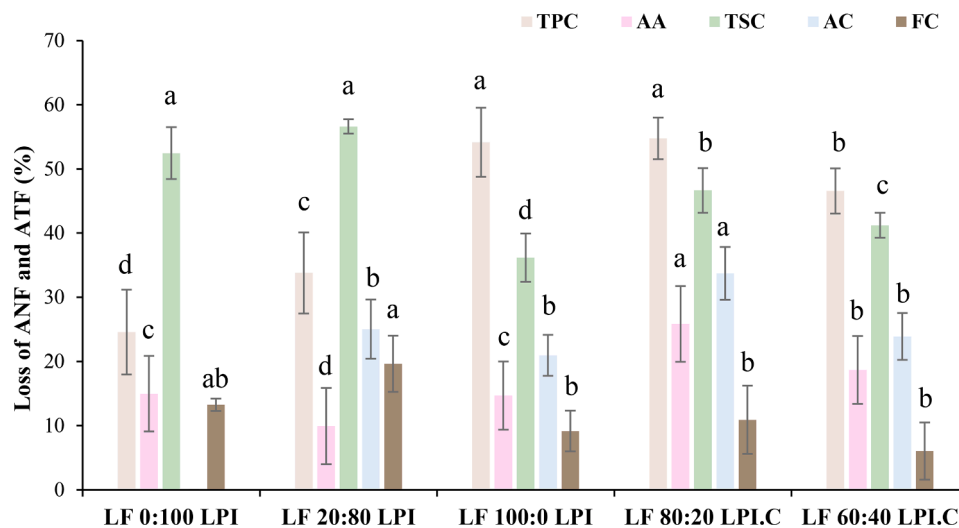


Fig. 2. Relative loss of total polyphenol content (TPC), antioxidant activity (AA), total saponin content (TSC), alkaloid content (AC) and fat content (FC) between powder formulations obtained by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios and their corresponding extrudates. Relative loss was calculated as the ratio between the change of ANF or ATF content between extrudates and powders. Different lowercase letters indicate significant differences ($p < 0.05$) between samples for each ANF and ATF.

relative losses ranged from 10 to 26 %, with the largest reductions found in LF 80:20 LPI.C. No significant ($p > 0.05$) differences in TPC were observed between the extrudates (Table 3). In contrast, extrudates with higher proportions of LPI or LF exhibited the highest AA, following the same trend observed for the powders (Table 3). Overall, extrusion had a stronger impact on TPC than on AA, as indicated by the greater relative losses observed. Consistent with these findings, previous studies have reported reductions in TPC following extrusion in various food matrices, including lentil (Rathod & Annapure, 2016), pea (Alonso et al., 2000), sunflower (Vidal et al., 2022) and flaxseed-pea mixture (Han et al., 2025). This reduction in TPC may be explained by either to the thermal degradation of phenolic compounds during extrusion or to structural modifications that reduce their chemical reactivity and decrease their extractability through polymerization (Lund, 2021). Consequently, extrusion could also affect the AA, due to its close association with phenolic compounds. However, the thermal treatment can also promote the formation of Maillard reaction products with antioxidant properties, which may partially compensate for phenolic losses (Rufián-Henares & Delgado-Andrade, 2009; Sharma et al., 2012). Therefore, the comparatively lower losses of AA relative to TPC observed in the extrudates (Fig. 2) may be attributed, at least in part, to the formation of these compounds.

Overall, protein incorporation maintained similar TPC to LF and although AA increased with LPI addition, this could be considered beneficial, as it may enhance the nutritional value and oxidative stability of meat analogues produced with these high-protein extrudates.

3.2.4. Total saponin content

As reported in Table 3, powder formulations containing a greater proportion of LPI showed significantly ($p < 0.05$) higher total saponin content (TSC) compared to the other formulations and the same trend was observed for extrudates. A significant ($p < 0.05$) decrease in TSC was observed in all extrudates compared to their corresponding powders, with reductions ranging from 36 to 57 % and the highest relative reduction observed in LF 0:100 LPI and LF 20:80 LPI (Table 3 and Fig. 2). Similar results have been reported in previous studies, including reductions of TSC to trace levels in acha and soybean flours after extrusion (Anuonye et al., 2010) and an 18 % decrease in TSC in a flaxseed-pea mixture (Han et al., 2025). Additionally, Sánchez-Velázquez et al. (2021) found a 17 and 13 % reduction of TSC after extrusion in pea and pinto bean, respectively. This decrease might be attributed to hydrolytic effects during extrusion, which degrade the native saponin structures (Han et al., 2025; Xu et al., 2025).

Overall, the inclusion of LPI.C reduced the TSC of extrudates, while those containing LPI, although higher than LF, remained below 1 g oleanolic acid/100 g dm, indicating that these protein-rich formulations are suitable for use in meat analogue products.

3.2.5. Alkaloid content

Alkaloid content (AC) varied across formulations (Table 3). Both LF 0:100 LPI powder and its extrudate exhibited non-detectable levels of alkaloids. Therefore, formulations with a greater proportion of LPI exhibited significantly ($p < 0.05$) lower AC compared to those richer in LF. This likely reflects the high water solubility of alkaloids, which facilitates their removal during protein extraction and minimizes potential toxicity concerns (Aguilar-Acosta et al., 2020; El-Adawy et al., 2001; Schryvers et al., 2023; Taberner-Pibernat et al., 2026).

No significant ($p > 0.05$, Table 3) differences in AC were observed between powders and their corresponding extrudates. Although extrudates generally showed slightly lower AC values (Table 3), this changes could be likely due to experimental variability as quinolizidine alkaloids present in lupin are highly thermally stable (Schryvers et al., 2023). Moreover, these reductions were insufficient to reach levels considered safe for human consumption (0.02 g lupinine/100 g dm) (ACNFP, 1996). Similarly, Chamone et al. (2023) reported that extrusion treatment was unable to effectively reduce AC in white lupin flour. Consequently, only

LF 0:100 LPI extrudates could be considered suitable for consumption and inclusion in meat analogue products without a prior debittering process. Future research may explore the use of sweet lupin varieties as a strategy to achieve lower AC and enhance the overall quality of the extrudates (Bou et al., 2022).

3.3. Impact of extrusion on techno-functional properties

3.3.1. Water and fat absorption indexes

The water absorption index (WAI) is affected by several factors, including the levels of hydrophilic amino acids, the presence of non-protein constituents and both the structure and composition of proteins (Sathe & Salunkhe, 1981). On the other hand, the fat absorption index (FAI) describes the ability of proteins to interact with lipids via capillary action, a process influenced by electrostatic attractions, hydrophobic effects and hydrogen bonding (Lawal, 2004). The WAI and FAI values were formulation-dependent (Table 4), likely reflecting compositional differences. In particular, LF 100:0 LPI powder showed the highest WAI (1.43 g water/g dm). Thus, powder formulations with a greater proportion of LF exhibited significantly ($p < 0.05$) higher WAI compared to those richer in protein isolate, either LPI or LPI.C and this trend was also observed for extrudates. The higher WAI of LF rich formulations may result from their potentially greater carbohydrate fraction compared to the isolates, as carbohydrates can interact with water through hydrogen bonding, allowing them to retain water within the matrix and increase the overall WAI (Kaur et al., 2013; Zhang et al., 2022). This pattern was consistent with observations by Tas et al. (2022), who reported that navy bean, which had a higher carbohydrate content than chickpea, also exhibited greater WAI. Notably, extrusion had no significant effect on WAI ($p > 0.05$), as powders and extrudates displayed comparable values (Table 4).

As for the FAI, the highest value was found in LF 0:100 LPI powder (Table 4). Thus, formulations with a greater proportion of LPI led to significantly ($p < 0.05$) higher FAI in both powders and extrudates. In contrast to the trend observed for WAI, the extrusion significantly ($p < 0.05$) reduced the FAI of extrudates, with an average loss of 25 % compared to their corresponding powder formulation. Similarly, Vidal et al. (2022) found that extrusion did not significantly ($p > 0.05$) affect the WAI of canola, soybean and sunflower cakes, while FAI was significantly ($p < 0.05$) decreased. In contrast, Qiu et al. (2020) reported that extrusion significantly ($p < 0.05$) improved WAI for oat, rye and black beans flours. Espinosa-Ramírez et al. (2021) demonstrated that extrusion significantly ($p < 0.05$) reduced FAI in oat, amaranth, quinoa and chickpea flours, while significantly ($p < 0.05$) increasing it in rice, sorghum, lentil, black bean and pinto bean flours. A similar trend was

Table 4

Water and fat absorption indexes of powder formulations obtained by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios and their corresponding extrudates.

	Formulation	WAI (g water/g dm)	FAI (g oil/g dm)
Powder	LF 0:100 LPI	1.24 ± 0.04 ^{Ca}	2.63 ± 0.08 ^{Aa}
	LF 20:80 LPI	1.33 ± 0.06 ^{Ba}	2.50 ± 0.15 ^{ABa}
	LF 100:0 LPI	1.43 ± 0.04 ^{Aa}	1.70 ± 0.06 ^{Da}
	LF 80:20 LPI.C	1.32 ± 0.02 ^{Ba}	2.10 ± 0.05 ^{Ca}
	LF 60:40 LPI.C	1.25 ± 0.03 ^{Ca}	2.32 ± 0.16 ^{Ba}
	Extrudate	LF 0:100 LPI	1.15 ± 0.05 ^{Ca}
LF 20:80 LPI		1.28 ± 0.06 ^{Ba}	1.70 ± 0.13 ^{ABb}
LF 100:0 LPI		1.38 ± 0.05 ^{Aa}	1.49 ± 0.06 ^{Cb}
LF 80:20 LPI.C		1.30 ± 0.03 ^{ABa}	1.72 ± 0.13 ^{ABb}
LF 60:40 LPI.C		1.18 ± 0.04 ^{Ca}	1.80 ± 0.03 ^{Ab}

WAI, water absorption index; FAI, fat absorption index.

Values are presented as average ± SD. Different capital letters in columns indicate significant differences ($p < 0.05$) between formulations for powders and extrudates. Different lowercase letters in columns indicate significant differences ($p < 0.05$) between powder and its extrudate.

reported by Wang et al. (2019), who observed an increase in FAI for sorghum flour but a decrease for chickpea and maize flours. A plausible explanation for the lower FAI of extrudates compared to powders in the present study could be the structural changes that limit protein-lipid interactions or the reduced ability of the low-expanded extrudate structures to retain oil (Espinosa-Ramírez et al., 2021). Differences in FAI between formulations may be related to higher proportion of LPI or LPI.C, as greater protein content provides more hydrophobic amino acid residues and binding sites that interact with oil, enhancing retention (Sánchez-Mendoza et al., 2017). This effect is consistent for both powders and extrudates, as shown in Tables 3 and 4.

Extrusion has been suggested to generate strong shear forces capable of disrupting macromolecules and inducing protein denaturation and aggregation, leading to alterations in the exposure of hydrophilic and hydrophobic regions and potentially affecting water and oil absorption (Marzec & Lewicki, 2006). Given the variability in published results, the impact of extrusion on WAI and FAI appears to depend on several factors, including the type of raw material, extrusion conditions and the degree of expansion. Overall, extrusion allowed the production of high-protein extrudates, with LPI formulations averaging 1.22 g water/g dm and 1.66 g oil/g dm and LPI.C formulations averaging 1.24 g water/g dm and 1.76 g oil/g dm, for WAI and FAI, respectively. These results highlight their suitability as ingredients for the development of meat analogue products.

3.3.2. Color properties

Significant ($p < 0.05$) differences in color parameters were observed among formulations for both powders and extrudates (Table 5 and Fig. 3). In powder formulations, L^* values decreased, while a^* and b^* increased as the proportion of LPI increased, resulting in darker and redder and yellower formulations compared to LF 100:0 LPI. These color changes may be advantageous for their use as an ingredient in meat analogues and could potentially reduce the use of colorants. Formulations containing LPI.C showed L^* , a^* and b^* values more similar to those of LF 100:0 LPI, likely due to the lighter color of LPI.C (Fig. 3). According to the manufacturer's technical documentation, the LPI.C was produced from previously defatted flour and dried by spray drying, which could explain the color differences compared to the LPI. Domínguez-Valencia et al. (2025) also found that prior defatting of LF using supercritical CO_2 resulted in lighter and whiter LPI.

Extrusion caused a significant ($p < 0.05$) decrease of L^* and a significant ($p < 0.05$) increase of a^* and b^* color parameters compared to powders (Table 5), resulting in a darker appearance with redder and yellower tones. These changes were perceptible to the human eye, as the total color differences (ΔE_1 , Fig. 3) exceeded 5 units (Mikulec et al., 2019). Additionally, to assess the effect of increased protein content on color parameters, the remaining extrudates with the LF 100:0 LPI extrudate (ΔE_2 , Fig. 3). Total color differences remained above the

perceptible threshold, except for the LF 80:20 LPI.C and LF 60:40 LPI.C extrudates, likely due to the high LF proportion and the similar color of LPI.C and LF. Moreover, no significant ($p > 0.05$) differences in ΔE_2 were observed between LF 0:100 LPI and LF 20:80 LPI (Fig. 3). These color differences may be attributed to several factors. Carotenoids, lipid-soluble pigments closely linked to the FC of food matrices are known to affect the a^* (redness) and b^* (yellowness) parameters (Bou et al., 2022; Siger et al., 2023). In the present study, formulations with the lowest FC values also showed reduced a^* and b^* values, along with significantly ($p < 0.05$) lower AA (Tables 3 and 5). Moreover color differences between powders and their extrudates may also result from Maillard reactions during extrusion (Espinosa-Ramírez et al., 2021; Jebalia et al., 2019; Nayak et al., 2011) and from protein structural modifications induced by the high temperatures (Llave et al., 2018).

3.4. Bulk density, textural properties and microstructure

Bulk density (BD) reflects the degree of expansion achieved during the extrusion process (Rathod & Annapure, 2016). Formulations containing LF combined with either LPI or LPI.C exhibited the highest BD values, with no significant ($p > 0.05$) differences between them (Table 6). The average BD (1.10 g/cm^3) was considerably higher than those reported in previous studies. For example, Wang et al. (2019) recorded 0.17 g/cm^3 for sorghum, 0.29 g/cm^3 for chickpea and 0.10 g/cm^3 for maize. Similarly, Gutkoski and El-Dash (1999) reported 0.45 g/cm^3 for oats and Banki et al. (2021) reported 0.12 g/cm^3 for rice-pigeon pea (85:25 w/w) blend extrudates.

Lower BD is generally associated with greater expansion during extrusion, as the formation of pores reduces the overall density (Pitts et al., 2014). This expansion is largely dependent on starch content, which provides the structural basis for puffing (Chien et al., 2022). The elevated BD observed in this study may be explained by the formation of a homogeneous dense protein matrix, with low starch content (0.2 g/100 g dm , Arzami et al., 2022), consisting of compact layers with minimal or absent air cavities (Rathod & Annapure, 2016), which limited expansion during extrusion. Similarly, Wang et al. (2019) reported that higher protein and FC combined with lower starch or carbohydrate levels tend to produce denser extrudates with reduced puffing and expansion. Therefore, if the aim is to obtain a porous, directly consumable product, future formulations should consider the incorporation of starch or other expansion-promoting components to reduce BD. In contrast, the development of a dense, structured protein matrix with limited expansion may be advantageous for applications where a texturized high-protein ingredient is required.

Fig. 3 illustrates the appearance of the powder formulations and its extrudates. Extrudates displayed fibrous structures, characteristic of texturized plant-based proteins (Akharume et al., 2021; Dekkers et al., 2018). Texture is a critical attribute of extrudates and a key determinant

Table 5

Color parameters of powder formulations obtained by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios and their corresponding extrudates.

	Formulation	L^*	a^*	b^*	C^*	h^*
Powder	LF 0:100 LPI	62.2 ± 0.4^{Da}	7.9 ± 0.3^{Ab}	65.4 ± 0.9^{Ab}	65.8 ± 0.9^{Ab}	83.1 ± 0.2^{Ca}
	LF 20:80 LPI	63.2 ± 0.4^{Ca}	7.6 ± 0.3^{Bb}	56.9 ± 1.2^{Bb}	57.4 ± 1.2^{Bb}	82.4 ± 0.2^{Da}
	LF 100:0 LPI	68.8 ± 0.5^{Aa}	2.7 ± 0.3^{Db}	29.8 ± 1.0^{Eb}	29.9 ± 1.0^{Eb}	84.9 ± 0.5^{Aa}
	LF 80:20 LPI.C	67.3 ± 0.6^{Ba}	3.8 ± 0.3^{Cb}	32.3 ± 1.1^{Db}	32.5 ± 1.1^{Db}	83.3 ± 0.6^{Ca}
	LF 60:40 LPI.C	68.6 ± 0.7^{Aa}	3.7 ± 0.3^{Cb}	34.7 ± 1.1^{Cb}	34.9 ± 1.1^{Cb}	83.9 ± 0.5^{Ba}
	LF 0:100 LPI	55.6 ± 0.4^{Cb}	11.2 ± 0.3^{Ba}	69.9 ± 1.7^{Aa}	70.7 ± 1.7^{Aa}	80.9 ± 0.2^{Db}
Extrudate	LF 20:80 LPI	51.9 ± 0.2^{Eb}	11.8 ± 0.3^{Aa}	70.4 ± 1.6^{Aa}	71.4 ± 1.6^{Aa}	80.5 ± 0.3^{Db}
	LF 100:0 LPI	56.1 ± 0.6^{Bb}	4.9 ± 0.6^{Da}	48.8 ± 1.8^{Ba}	49.0 ± 1.8^{Ba}	84.2 ± 0.5^{Aa}
	LF 80:20 LPI.C	57.1 ± 0.4^{Ab}	5.5 ± 0.5^{Ca}	44.3 ± 1.4^{Ca}	44.6 ± 1.4^{Ca}	82.9 ± 0.5^{Cb}
	LF 60:40 LPI.C	55.0 ± 0.4^{Db}	5.6 ± 0.6^{Ca}	49.4 ± 1.8^{Ba}	49.8 ± 1.9^{Ba}	83.6 ± 0.6^{Ba}

L^* , lightness value; a^* , green-red; b^* , blue-yellow; C^* , chroma; h^* , hue angle.

Values are presented as average \pm SD. Different capital letters in columns indicate significant differences ($p < 0.05$) between formulations for powders and extrudates. Different lowercase letters in columns indicate significant differences ($p < 0.05$) between powder and their extrudate.












Formulation	Powder	Extrudate	ΔE_1	ΔE_2
LF 0:100 LPI			8.7 ± 1.1^e	21.9 ± 1.6^a
LF 20:80 LPI			18.2 ± 1.2^c	23.1 ± 1.5^a
LF 100:0 LPI			23.0 ± 1.5^a	
LF 80:20 LPI.C			15.4 ± 1.1^d	4.7 ± 1.3^b
LF 60:40 LPI.C			20.2 ± 1.2^b	2.3 ± 0.6^c
LPI.C				

Fig. 3. Appearance of powder formulations obtained by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios and their extrudates. Appearance of LPI.C powder. ΔE_1 , total color differences between powders and extrudates; ΔE_2 , total color differences between LF 100:0 LPI extrudate and other extrudates. Values are presented as average \pm SD. Different lowercase letters in columns indicate significant differences between samples ($p < 0.05$).

of consumer acceptance in formulated products that incorporate them as ingredients. As shown in Table 6, the lupin extrudates exhibited an average maximum force of 346 N. Taking into account the probe surface area (4.5 cm), the average maximum stress (0.22 N/mm²) was higher compared to other sources such as amaranth (avg. 0.08 N/mm²) (Zapotoczny et al., 2006) or wheat (0.03 N/mm²) (Azzollini et al., 2018), which were also tested using a compression-extrusion test with a similar configuration to the one used in the present work. Moreover, compared to amaranth (avg. 68 N-mm, Zapotoczny et al., 2006) and

wheat (5 N-mm, Azzollini et al., 2018), the lupin-based extrudates required higher energy to break, with an average area of 498 N-mm (Table 6). These results are in agreement with Chanvrier et al. (2013), who reported that higher protein content further increased hardness. The highest hardness and compression energy were observed for LF 80:20 LPI.C (Table 6). Texture results are consistent with the elevated BD previously discussed, indicating a compact and rigid structure, associated with the low starch content. However, despite the lowest BD was observed for LF 0:100 LPI, this was not clearly reflected in the

Table 6

Bulk density (BD) and texture parameters of extrudates from different powder formulations obtained by mixing lupin flour (LF) and laboratory (LPI) and commercial (LPI.C) protein isolates at different ratios.

Formulation	BD (g/cm ³)	Hardness (N)	Peak time (s)	Slope (N/mm)	Area (N·mm)
LF 0:100 LPI	0.94 ± 0.09 ^c	338 ± 17 ^c	1.59 ± 0.02 ^a	127 ± 11 ^d	519 ± 38 ^b
LF 20:80 LPI	1.18 ± 0.04 ^a	273 ± 22 ^d	1.56 ± 0.05 ^a	105 ± 13 ^e	406 ± 31 ^c
LF 100:0 LPI	1.02 ± 0.11 ^b	372 ± 23 ^b	1.45 ± 0.04 ^b	164 ± 7 ^b	514 ± 41 ^b
LF 80:20 LPI.C	1.16 ± 0.03 ^a	432 ± 30 ^a	1.45 ± 0.02 ^b	183 ± 17 ^a	623 ± 49 ^a
LF 60:40 LPI.C	1.17 ± 0.03 ^a	315 ± 41 ^c	1.36 ± 0.05 ^c	142 ± 16 ^c	426 ± 62 ^c

Values are presented as average ± SD. Different lowercase letters in columns indicate significant differences (p < 0.05).

texture parameters. Furthermore, although other studies have not evaluated these aspects, the extrudates led to an average peak time of 1.48 s and an average slope of 144 N/mm. As peak time and slope were inversely related, lower peak time values generally corresponded to higher slope values (Table 6). Nevertheless, no clear effect of formulation was observed. In general terms, the addition of LPI led to reduced hardness and slope and increased peak time compared to LF 100:0 LPI, while the required compression energy remained similar (Table 6).

Overall, these results could be closely related to the microstructural features observed (Fig. 4), as microstructure has been shown to strongly influence textural properties in plant-based proteins (Bhuiyan & Ngadi, 2024). LF 100:0 LPI exhibited a highly irregular structure composed of aggregates of small rounded particles along with larger isolated particles, likely associated with its broad particle size distribution (D[4.3] 714 µm, d(0.1) 65 µm, d(0.5) 687 µm and d(0.9) 1361 µm) (Fig. 4A and Table 1). A laminar fibrillar structure derived from plant cell wall components was also observed, where compounds such as lipids and

partially gelatinized starch appeared to interact and partially fuse with the fibers, although complete fusion was not achieved (Fig. 4B). This incomplete fusion may be associated with the low BD of this formulation where numerous small but shallow pores were present throughout the matrix (Table 6). In contrast, LF 0:100 LPI displayed elongated fibrous structures that progressively aggregated to form smooth-surfaced lamellae (Fig. 4C). At higher magnification, the smooth surface was densely populated with micropores, which may explain the lower density observed in this sample (Fig. 4D and Table 6) compared to the other formulations. LF 20:80 LPI showed a higher degree of fusion among the different extrudate components compared to LF 100:0 LPI. A continuous protein network was established, characterized by fibrous protein structures that encapsulated LF-derived aggregates (Fig. 4E). In some regions, these aggregates were more extensively fused, whereas in others they retained the less integrated morphology observed in LF 100:0 LPI (Fig. 4F). Consequently, a discontinuous matrix containing embedded aggregates was formed. The interactions between LF and LPI components may have allowed the structure to collapse more easily under compression, leading to low hardness despite its high density (Table 6).

Regarding the incorporation of the commercial protein isolate, LF 80:20 LPI.C exhibited higher hardness and BD values (Table 6), which may be related to the particle size of LPI.C (D[4.3] 50 µm, d(0.1) 14 µm, d(0.5) 44 µm and d(0.9) 96 µm, Table 1), promoting greater fusion between protein fibers and LF components (Fig. 4G and H). Conversely, LF 60:40 LPI.C, due to its higher protein content, likely developed a more irregular matrix in which protein fibers remained more independent from LF-derived aggregates, resulting in reduced hardness (Fig. 4I and J and Table 6). Overall, the incorporation of lupin protein influenced the microstructural organization of the extrudates, enhancing component fusion and matrix continuity, which was reflected in changes in BD and textural properties depending on protein type (LPI or LPI.C) and its proportion. Further quantitative analyses, such as fractal dimension and surface porosity evaluation (Bhuiyan et al., 2026), would be valuable to

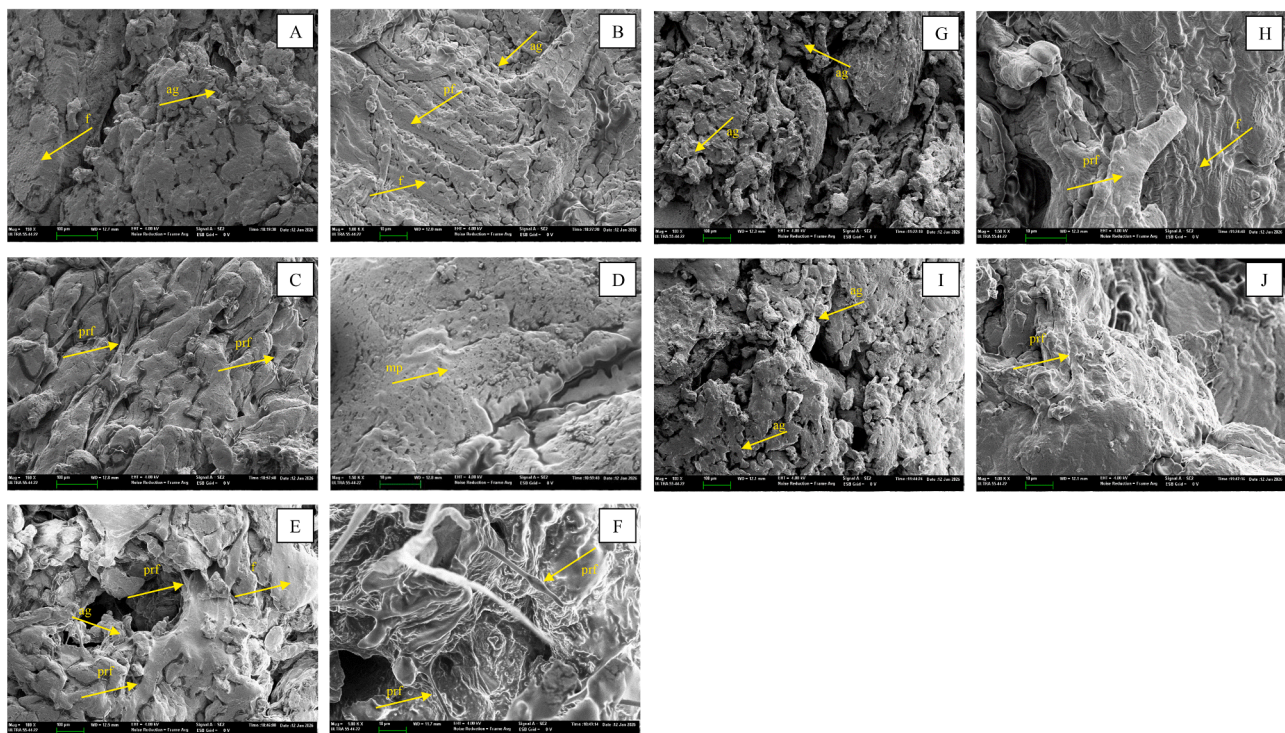


Fig. 4. Microstructure of extrudates observed by FESEM. LF 100:0 LPI (A-B), LF 0:100 LPI (C-D), LF 20:80 LPI (E-F), LF 80:20 LPI.C (G-H), LF 60:40 LPI.C (I-J). LF, lupin flour; LPI, lupin protein isolate; LPI.C, commercial lupin protein isolate. Arrows indicate: ag, aggregates; f, fusion of components; mp, micropores; pf, plant fibers; prf, protein fibers.

more comprehensively characterize the microstructure and should be addressed in future work.

4. Conclusions

The present study demonstrates that high-protein lupin-based formulations can be efficiently processed by extrusion, yielding stable extrudates with a uniform structure. Although slight apparent reductions in measured protein and fat were observed after extrusion, these might be more likely due to limited extractability caused by structural modifications under the combined effects of high temperature and pressure. Polyphenols, saponins and antioxidant constituents were substantially reduced during extrusion, probably due to hydrolysis and thermal effects, whereas heat-stable compounds like alkaloids were unaffected. Overall, the incorporation of protein isolates into lupin formulations resulted in extrudates with lower anti-nutritional and anti-technological factors, compared with extrudates from lupin flour alone. Regarding techno-functional properties, water absorption index remained high after extrusion, while fat absorption (FAI) decreased, likely due to structural compaction and altered protein-lipid interactions. Compared to the lupin flour formulation, the incorporation of protein isolates improved the FAI of the extrudates while maintaining high WAI values. As expected, the extrudates exhibited a hard and dense texture, attributed to the low starch content of lupin flour and isolates. Therefore, if the objective were to produce a porous, ready-to-eat product, future formulations should incorporate starch or other expansion-promoting components to reduce bulk density. However, maintaining a compact protein matrix may be advantageous for applications requiring a texturized high-protein ingredient. Microstructural analysis confirmed the formation of dense, cohesive protein networks, providing a structural basis for the observed bulk density and textural properties.

Collectively, extrusion proved to be an effective strategy to obtain high-protein texturized lupin ingredients suitable for meat analogue applications, with the additional benefit of reducing anti-nutritional and anti-technological factors. Further pilot-scale studies are required to confirm its industrial feasibility and future research should explore alternative formulations aimed at increasing expansion and optimizing textural properties.

Ethical statement - studies in humans and animals

No studies involving humans or animals were conducted in this research.

CRedit authorship contribution statement

Paola Navarro-Vozmediano: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José V. García-Pérez:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Amparo Quiles:** Writing – review & editing, Methodology, Conceptualization. **Ricard Bou:** Writing – review & editing, Investigation, Conceptualization. **Rosa Jorba-Martín:** Writing – review & editing, Formal analysis. **José Benedito:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, 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

Data will be made available on request.

References

- ACNFP. (1996). *Report on seeds from narrow leafed lupin* (p. 107). MAFF Publications.
- Aguilar-Acosta, L. A., Serna-Saldivar, S. O., Rodríguez-Rodríguez, J., Escalante-Aburto, A., & Chuck-Hernández, C. (2020). Effect of ultrasound application on protein yield and fate of alkaloids during lupin alkaline extraction process. *Biomolecules*, 10(2). <https://doi.org/10.3390/biom10020292>
- Ai, Y., Cichy, K. A., Harte, J. B., Kelly, J. D., & Ng, P. K. W. (2016). Effects of extrusion cooking on the chemical composition and functional properties of dry common bean powders. *Food Chemistry*, 211, 538–545. <https://doi.org/10.1016/j.foodchem.2016.05.095>
- Akharume, F. U., Aluko, R. E., & Adedeji, A. A. (2021). Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 198–224. <https://doi.org/10.1111/1541-4337.12688>
- Aleff, Y. D., Tiruneh, A. T., & Yehuala, T. F. (2024). Optimization of extrusion conditions for development of high quality rice-lupin-pumpkin based extruded snack food. *Heliyon*, 10(24), Article 40913. <https://doi.org/10.1016/j.heliyon.2024.e40913>
- Alonso, R., Grant, G., Dewey, P., & Marzo, F. (2000). Nutritional assessment in vitro and in vivo of raw and extruded peas (*Pisum sativum* L.). *Journal of Agricultural and Food Chemistry*, 48(6), 2286–2290. <https://doi.org/10.1021/jf000095o>
- Anuonye, J. C., Onuh, J. O., Egwim, E., & Adeyemo, S. O. (2010). Nutrient and antinutrient composition of extruded acha/soybean blends. *Journal of Food Processing and Preservation*, 34, 680–691. <https://doi.org/10.1111/j.1745-4549.2009.00425.x>
- AOAC. (1984). *Official methods of analysis. kjeldahl method (2.062)*. Washington DC: Association of Official Analytical Chemists.
- AOAC. (1996). *Official methods of analysis. fat (Crude) in meat and meat products (991.36)*. Washington DC: Association of Official Analytical Chemists.
- Arribas, C., Cabellos, B., Sánchez, C., Cuadrado, C., Guillamón, E., & Pedrosa, M. M. (2017). The impact of extrusion on the nutritional composition, dietary fiber and in vitro digestibility of gluten-free snacks based on rice, pea and carob flour blends. *Food & Function*, 8(10), 3654–3663. <https://doi.org/10.1039/C7FO00910K>
- Arzami, A. N., Morais, D., Carvalho, D., Vilaplana, F., Stoddard, F. L., & Mikkonen, K. S. (2022). Narrow-leafed lupin (*Lupinus angustifolius* L.): Characterization of emulsification and fibre properties. *Future Foods*, 6, Article 100192. <https://doi.org/10.1016/j.fufo.2022.100192>
- Azzollini, D., Derossi, A., Fogliano, V., Lakemond, C. M. M., & Severini, C. (2018). Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. *Innovative Food Science & Emerging Technologies*, 45, 344–353. <https://doi.org/10.1016/j.ifset.2017.11.017>
- Banki, N. M., Salihi, A., Muhammad, A., & Bala, S. M. (2021). Optimization and characterization of rice-pigeon pea flour blend using extrusion cooking process. *Legume Science*, 3(1). <https://doi.org/10.1002/leg3.73>
- Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”. *The FRAP assay. Analytical Biochemistry*, 239, 70–76.
- Bhuiyan, M. H. R., & Ngadi, M. (2024). Multifractional characterization of fried foods pore microstructure. *Food Bioscience*, 60, Article 104518. <https://doi.org/10.1016/j.fbio.2024.104518>
- Bhuiyan, M. H. R., Yeasmen, N., & Orsat, V. (2026). Food surface characterization by scanning electron microscopy and fractal analysis: A review. *Food Chemistry*, 498, Article 147148. <https://doi.org/10.1016/j.foodchem.2025.147148>
- Bou, R., Navarro-Vozmediano, P., Domínguez, R., López-Gómez, M., Pinet, M., Ribas-Agustí, A., Benedito, J. J., Lorenzo, J. M., Terra, X., García-Pérez, J. V., Pateiro, M., Herrera-Cervera, J. A., & Jorba-Martín, R. (2022). Application of emerging technologies to obtain legume protein isolates with improved techno-functional properties and health effects. *Comprehensive Reviews in Food Science and Food Safety*, 21(3), 2200–2232. <https://doi.org/10.1111/1541-4337.12936>
- Cagnin, C., Morais, D. N., & Prudencio, S. H. (2024). Structural, physicochemical and technofunctional properties of corn gluten meal modified by extrusion. *Food Research International*, 196, Article 115067. <https://doi.org/10.1016/j.foodres.2024.115067>
- Chai, Y., Wang, R., Zhang, B., Tang, Y., Li, C., Guo, B., & Li, M. (2025). The importance of molecular structure for textural and physicochemical properties of extruded wheat flour. *Foods (Basel, Switzerland)*, 14(10), 1829. <https://doi.org/10.3390/foods14101829>

- Chamone, M. E. R., Ascheri, J. L. R., Vargas-Solórzano, J. W., Stephan, M. P., & Carvalho, C. W. P. (2023). Chemical characterization of white lupin (*Lupinus albus*) flour treated by extrusion cooking and aqueous debittering processes. *Plant Foods for Human Nutrition*, 78(2), 292–298. <https://doi.org/10.1007/s11130-023-01050-0>
- Chanvrier, H., Desbois, F., Perotti, F., Salzmann, C., Chassagne, S., Gumy, J. C., & Blank, I. (2013). Starch-based extruded cereals enriched in fibers: A behavior of composite solid foams. *Carbohydrate Polymers*, 98(1), 842–853. <https://doi.org/10.1016/j.carbpol.2013.07.005>
- Chéreau, D., Videcoq, P., Ruffieux, C., Pichon, L., Motte, J.-C., Belaid, S., Ventura, J., & Lopez, M. (2016). Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications. *Oilseeds and Fats, Crops and Lipids*, 23(4), 406. <https://doi.org/10.1051/oef/2016020>
- Chien, H., Tsai, Y., Wang, H. D., Dong, C., Huang, C., & Kuo, C. (2022). Extrusion puffing pretreated cereals for rapid production of high-maltose syrup. *Food Chemistry: X*, 15 (July), Article 100445. <https://doi.org/10.1016/j.fochx.2022.100445>
- De Carvalho Oliveira, L., Suemitsu, L. Y., dos Santos, F. H., Netto, F. M., Tavares, G. M., de Castro, R. J. S., Bakalis, S., & Cristianini, M. (2025). Advances in high-pressure homogenization for plant proteins: Enhancing techno-functional properties and bioactive peptide release. *Current Food Science and Technology Reports*, 3(1), 11. <https://doi.org/10.1007/s43555-025-00057-9>
- De Pilli, T., Derossi, A., Talja, R. A., Jouppila, K., & Severini, C. (2011). Study of starch-lipid complexes in model system and real food produced using extrusion-cooking technology. *Innovative Food Science & Emerging Technologies*, 12(4), 610–616. <https://doi.org/10.1016/j.ifset.2011.07.011>
- De Pilli, T., Jouppila, K., Ikonen, J., & Kansikas, J. (2008). Study on formation of starch-lipid complexes during extrusion-cooking of almond flour. *Journal of Food Engineering*, 87, 495–504. <https://doi.org/10.1016/j.jfoodeng.2007.12.028>
- Dekkers, B. L., Boom, R. M., & van der Goot, A. J. (2018). Structuring processes for meat analogues. *Trends in Food Science and Technology*, 81, 25–36. <https://doi.org/10.1016/j.tifs.2018.08.011>
- Domínguez-Valencia, R., Bermúdez, R., Pateiro, M., Purriños, L., Benedito, J., & Lorenzo, J. M. (2025). Impact of supercritical CO₂ treatment on lupin flour and lupin protein isolates. *Foods (Basel, Switzerland)*, 14(4), 675. <https://doi.org/10.3390/foods14040675>
- El-Adawy, T. A., Rahma, E. H., El-Bedaway, A. A., & Gafar, A. F. (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry*, 74(4), 455–462. [https://doi.org/10.1016/S0308-8146\(01\)00163-7](https://doi.org/10.1016/S0308-8146(01)00163-7)
- Elhordoy, M. R., Kadam, A., Vazquez, D., Medrano, A., & Koksels, F. (2025). Exploring the potential of lupin (*Lupinus angustifolius*) flour-based ingredients in developing high moisture meat analogues. *Journal of Food Science*, 90(7). <https://doi.org/10.1111/1750-3841.70407>
- Escobedo-Avellaneda, Z., Colín-Oviedo, Á., Buitimea-Cantúa, G. V., Pérez-Carillo, E., Chuck-Hernández, C., Espinosa-Ramírez, J., Castagnini, J. M., & Welti-Chanes, J. (2025). Extrusion effects on composition, protein digestibility, and functional properties of cold-pressed oilseed cakes. *CYTA - Journal of Food*, 23(1). <https://doi.org/10.1080/19476337.2025.2549373>
- Espinosa-Ramírez, J., Rodríguez, A., De la Rosa-Millán, J., Heredia-Olea, E., Pérez-Carrillo, E., & Serna-Saldívar, S. O. (2021). Shear-induced enhancement of techno-functional properties of whole grain flours through extrusion. *Food Hydrocolloids*, 111. <https://doi.org/10.1016/j.foodhyd.2020.106400>
- Fang, Y., Zhang, B., & Wei, Y. (2014). Effects of the specific mechanical energy on the physicochemical properties of texturized soy protein during high-moisture extrusion cooking. *Journal of Food Engineering*, 121(1), 32–38. <https://doi.org/10.1016/j.jfoodeng.2013.08.002>
- Ganzera, M., Krüger, A., & Wink, M. (2010). Determination of quinolizidine alkaloids in different *Lupinus* species by NACE using UV and MS detection. *Journal of Pharmaceutical and Biomedical Analysis*, 53(5), 1231–1235. <https://doi.org/10.1016/J.JPBA.2010.05.030>
- Gulati, P., Weier, S. A., Santra, D., Subbiah, J., & Rose, D. J. (2016). Effects of feed moisture and extruder screw speed and temperature on physical characteristics and antioxidant activity of extruded proso millet (*Panicum miliaceum*) flour. *International Journal of Food Science & Technology*, 51(1), 114–122. <https://doi.org/10.1111/ijfs.12974>
- Gutkoski, L. C., & El-Dash, A. A. (1999). Effect of extrusion process variables on physical and chemical properties of extruded oat products. *Plant Foods for Human Nutrition*, 54(4), 315–325. <https://doi.org/10.1023/A:1008101209353>
- Han, R., McDowell, R., Gaunt, S., Mondor, M., & Hernández-Álvarez, A. J. (2025). Transforming oilseed blends: The impact of low-moisture extrusion on antinutritional factors, protein structure, and nutritional value. *Food Chemistry*, 484, Article 144417. <https://doi.org/10.1016/j.foodchem.2025.144417>
- Jebalia, I., Maigret, J. E., Réguerre, A. L., Navales, B., Guessasma, S., Lourdin, D., Della Valle, G., & Kristiawan, M. (2019). Morphology and mechanical behaviour of pea-based starch-protein composites obtained by extrusion. *Carbohydrate Polymers*, 223, Article 115086. <https://doi.org/10.1016/j.carbpol.2019.115086>
- Jiang, H., Zhang, N., Xie, L., Li, G., Chen, L., & Liao, Z. (2025). A comprehensive review of the rehydration of instant powders: Mechanisms, influencing factors, and improvement strategies. *Foods (Basel, Switzerland)*, 14(16), 2883. <https://doi.org/10.3390/foods14162883>
- Kasanova, N., Gainullina, M., Prosvirnikov, D., Tuntsev, D., & Golovkova, E. (2025). Use of extruded lupin in feeding young quails. *BIO Web of Conferences*, 161, Article 00018. <https://doi.org/10.1051/bioconf/202516100018>
- Kaur, M., Kaushal, P., & Sandhu, K. S. (2013). Studies on physicochemical and pasting properties of Taro (*Colocasia esculenta* L.) flour in comparison with a cereal, tuber and legume flour. *Journal of Food Science and Technology*, 50(1), 94–100. <https://doi.org/10.1007/s13197-010-0227-6>
- Lawal, O. S. (2004). Functionality of African locust bean (*Parkia biglobosa*) protein isolate: Effects of pH, ionic strength and various protein concentrations. *Food Chemistry*, 86(3), 345–355. <https://doi.org/10.1016/j.foodchem.2003.09.036>
- Lian, H., Luo, K., Gong, Y., Zhang, S., & Serventi, L. (2020). Okara flours from chickpea and soy are thickeners: Increased dough viscosity and moisture content in gluten-free bread. *International Journal of Food Science and Technology*, 55(2), 805–812. <https://doi.org/10.1111/ijfs.14332>
- Llave, Y., Fukuda, S., Fukuoka, M., Shibata-Ishiwatari, N., & Sakai, N. (2018). Analysis of color changes in chicken egg yolks and whites based on degree of thermal protein denaturation during ohmic heating and water bath treatment. *Journal of Food Engineering*, 222, 151–161. <https://doi.org/10.1016/j.jfoodeng.2017.11.024>
- Lo, B., Kasapis, S., & Farahnaky, A. (2021). Lupin protein: Isolation and techno-functional properties, a review. *Food Hydrocolloids*, 112, Article 106318. <https://doi.org/10.1016/j.foodhyd.2020.106318>
- Lund, M. N. (2021). Reactions of plant polyphenols in foods: Impact of molecular structure. *Trends in Food Science & Technology*, 112, 241–251. <https://doi.org/10.1016/j.tifs.2021.03.056>
- Majzoobi, M., & Farahnaky, A. (2010). Comparison of the effects of extrusion cooking on some cereal starches. *International Journal of Food Engineering*, 6(3). <https://doi.org/10.2202/1556-3758.1456>
- Marzec, A., & Lewicki, P. P. (2006). Antiplasticization of cereal-based products by water. Part I. Extruded flat bread. *Journal of Food Engineering*, 73(1), 1–8. <https://doi.org/10.1016/j.jfoodeng.2004.12.002>
- Marzo, F., Alonso, R., Urdaneta, E., Arricibita, F. J., & Ibáñez, F. (2002). Nutritional quality of extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. In *J. anim. sci* (Vol. 80). <https://academic.oup.com/jas/article-abstract/80/4/875/4789380>
- McGuire, K., Silveru, K., Ambrose, K., & Alavi, S. (2022). Food powder flow in extrusion: Role of particle size and composition. *Processes*, 10(1). <https://doi.org/10.3390/pr10010178>
- Mendowski, S., Chapoutot, P., Chesneau, G., Ferlay, A., Enjalbert, F., Cantalapedra-Hijar, G., Germain, A., & Nozière, P. (2019). Effects of replacing soybean meal with raw or extruded blends containing faba bean or lupin seeds on nitrogen metabolism and performance of dairy cows. *Journal of Dairy Science*, 102(6), 5130–5147. <https://doi.org/10.3168/jds.2018-15416>
- Mikulec, A., Kowalski, S., Sabat, R., Skoczylas, L., Tabaszewska, M., & Wyrocka-Gurgul, A. (2019). Hemp flour as a valuable component for enriching physicochemical and antioxidant properties of wheat bread. *LWT - Food Science and Technology*, 102, 164–172. <https://doi.org/10.1016/j.lwt.2018.12.028>
- Navarro del Hierro, J., Reglero, G., & Martín, D. (2020). Chemical characterization and bioaccessibility of bioactive compounds from saponin-rich extracts and their acid-hydrolyses obtained from fenugreek and quinoa. *Foods (Basel, Switzerland)*, 9(9). <https://doi.org/10.3390/foods9091159>
- Navarro-Vozmediano, P., Bou, R., García-Pérez, J. V., Dalmau, E., & Benedito, J. J. (2025). Impact of seed germination on the physicochemical properties of lupin flours and the physicochemical and techno-functional properties of their protein isolates. *Food and Bioprocess Technology*, 18(2), 1614–1630. <https://doi.org/10.1007/s11947-024-03546-2>
- Nayak, B., Berrios, J. D. J., Powers, J. R., & Tang, J. (2011). Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *Journal of Food Science*, 76(6). <https://doi.org/10.1111/j.1750-3841.2011.02279.x>
- O'Shea, N., Arendt, E., & Gallagher, E. (2014). Enhancing an extruded puffed snack by optimising die head temperature, screw speed and apple pomace inclusion. *Food and Bioprocess Technology*, 7(6), 1767–1782. <https://doi.org/10.1007/s11947-013-1181-x>
- Palanisamy, M., Franke, K., Berger, R. G., Heinz, V., & Töpfl, S. (2019). High moisture extrusion of lupin protein: Influence of extrusion parameters on extruder responses and product properties. *Journal of the Science of Food and Agriculture*, 99(5), 2175–2185. <https://doi.org/10.1002/jsfa.9410>
- Pam Ismail, B., Senaratne-Lenagala, L., Stube, A., & Brackenridge, A. (2020). Protein demand: Review of plant and animal proteins used in alternative protein product development and production. *Animal Frontiers*, 10(4), 53–63. <https://doi.org/10.1093/af/vfaa040>
- Park, C. S., Seo, M. S., Jung, S. Y., Lee, S., Park, B., Park, S. Y., & Kim, Y. S. (2024). Analyzing quality characteristics of texturized vegetable protein using defatted soy flour with rice flour and rice starch. *Food Science and Preservation*, 31(4), 518–526. <https://doi.org/10.11002/ftp.2024.31.4.518>
- Pitts, K. F., Favaro, J., Austin, P., & Day, L. (2014). Co-effect of salt and sugar on extrusion processing, rheology, structure and fracture mechanical properties of wheat-corn blend. *Journal of Food Engineering*, 127, 58–66. <https://doi.org/10.1016/j.jfoodeng.2013.11.026>
- Prandini, A., Morlacchini, M., Moschini, M., Fusconi, G., Masoero, F., & Piva, G. (2005). Raw and extruded pea (*Pisum sativum*) and lupin (*Lupinus albus* var. Multitalia) seeds as protein sources in weaned piglets' diets: Effect on growth rate and blood parameters. *Italian Journal of Animal Science*, 4(4), 385–394. <https://doi.org/10.4081/ijas.2005.385>
- Qiu, T., Xiong, H., Zhu, X., & Sun, Y. (2020). Effect of drum drying and extrusion on physicochemical properties and storage stability of black grains. *Food Science*, 41, 73–83.
- Rahate, K. A., Madhumita, M., & Prabhakar, P. K. (2021). Nutritional composition, anti-nutritional factors, pretreatments-cum-processing impact and food formulation potential of faba bean (*Vicia faba* L.): A comprehensive review. In *LWT - Food Science and Technology*, 138. <https://doi.org/10.1016/j.lwt.2020.110796>

- Rathod, R. P., & Annapure, U. S. (2016). Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. *LWT - Food Science and Technology*, 66, 114–123. <https://doi.org/10.1016/j.lwt.2015.10.028>
- Robutti, J., Borrás, F., González, R., Torres, R., & De Greef, D. (2002). Endosperm properties and extrusion cooking behavior of maize cultivars. *LWT - Food Science and Technology*, 35(8), 663–669. <https://doi.org/10.1006/fstl.2002.0926>
- Rufián-Henares, J. A., & Delgado-Andrade, C. (2009). Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Research International*, 42(3), 394–400. <https://doi.org/10.1016/j.foodres.2009.01.011>
- Ruiz, L. P. (1977). A rapid screening test for lupin alkaloids. *New Zealand Journal of Agricultural Research*, 20(1), 51–52. <https://doi.org/10.1080/00288233.1977.10427301>
- Sakai, K., Okada, M., & Yamaguchi, S. (2022). Decolorization and detoxication of plant-based proteins using hydrogen peroxide and catalase. *Scientific Reports*, 12(1), 1–10. <https://doi.org/10.1038/s41598-022-26883-8>
- Saldanha do Carmo, C., Mecha, E., Schmidt, G., Oliveira, J., Bronze, M. R., Knutsen, S. H., & Serra, A. T. (2025). Impact of low-moisture extrusion on carbohydrate constituents and antinutrients of a blend of a faba bean protein concentrate and a beta-glucan enriched oat fraction. *Food Research International*, 221, Article 117542. <https://doi.org/10.1016/j.foodres.2025.117542>
- Sánchez-Mendoza, N. A., Ruiz-Ruiz, J. C., Dávila-Ortiz, G., & Jiménez-Martínez, C. (2017). Propiedades tecnofuncionales y biológicas de harina, aislado y fracciones proteicas mayoritarias de semillas de Inga paterno. *CyTA - Journal of Food*, 15(3), 400–408. <https://doi.org/10.1080/19476337.2017.1286522>
- Sánchez-Velázquez, O. A., Ribéreau, S., Mondor, M., Cuevas-Rodríguez, E. O., Arcand, Y., & Hernández-Álvarez, A. J. (2021). Impact of processing on the in vitro protein quality, bioactive compounds, and antioxidant potential of 10 selected pulses. *Legume Science*, 3(2). <https://doi.org/10.1002/leg3.88>
- Sathe, S. K., & Salunkhe, D. K. (1981). Functional properties of the great northern bean (*Phaseolus vulgaris* L.) proteins: Emulsion, foaming, viscosity and gelation properties. *Journal of Food Science*, 46(1), 71–81. <https://doi.org/10.1111/j.1365-2621.1981.tb14533.x>
- Schmid, E., Farahnaky, A., Adhikari, B., & Torley, P. J. (2022). High moisture extrusion cooking of meat analogs: A review of mechanisms of protein texturization. *Comprehensive Reviews in Food Science and Food Safety*, 21(6), 4573–4609. <https://doi.org/10.1111/1541-4337.13030>
- Schryvers, S., Arinzechukwu, C., Miserez, B., Eeckhout, M., & Jacxsens, L. (2023). The fate of quinolizidine alkaloids during the processing of lupins (*Lupinus* spp.) for human consumption. *Food Chemistry*, 429, Article 136847. <https://doi.org/10.1016/j.foodchem.2023.136847>
- Sharifi, S., Majzoobi, M., & Farahnaky, A. (2021). Effects of particle size and moisture content of maize grits on physical properties of expanded snacks. *Journal of Texture Studies*, 52(1), 110–123. <https://doi.org/10.1111/jtxs.12565>
- Sharma, P., Gujral, H. S., & Singh, B. (2012). Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry*, 131(4), 1406–1413. <https://doi.org/10.1016/j.foodchem.2011.10.009>
- Siger, A., Grygier, A., & Czubinski, J. (2023). Comprehensive characteristic of lipid fraction as a distinguishing factor of three lupin seed species. *Journal of Food Composition and Analysis*, 115, Article 104945. <https://doi.org/10.1016/j.jfca.2022.104945>
- Singh, R., & Koksel, F. (2021). Effects of particle size distribution and processing conditions on the techno-functional properties of extruded soybean meal. *LWT - Food Science and Technology*, 152, Article 112321. <https://doi.org/10.1016/j.lwt.2021.112321>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0066-879\(99\)99017-1](https://doi.org/10.1016/S0066-879(99)99017-1)
- Solanas, E., Castrillo, C., Balcells, J., & Guada, J. A. (2005). In situ ruminal degradability and intestinal digestion of raw and extruded legume seeds and soya bean meal protein. *Journal of Animal Physiology and Animal Nutrition*, 89(3–6), 166–171. <https://doi.org/10.1111/j.1439-0396.2005.00555.x>
- Szczepański, A., Adamek-Urbańska, D., Kasprzak, R., Szudrowicz, H., Śliwiński, J., & Kamaszewski, M. (2022). Lupin: A promising alternative protein source for aquaculture feeds? *Aquaculture Reports*, 26. <https://doi.org/10.1016/j.aqrep.2022.101281>
- Taberner-Pibernat, P., Bou, R., Castellari, M., Benedito, J. J., García-Pérez, J. V., Trigka, C., & Ribas-Agustí, A. (2026). Enhancing lupin protein isolation: Modulation of extraction parameters to maximize protein and phytochemical recovery, and phytase treatment for improved functionality. *Food Bioscience*, 75, Article 108130. <https://doi.org/10.1016/j.fbio.2025.108130>
- Tas, O., Ertugrul, U., Grunin, L., & Oztop, M. H. (2022). An investigation of functional quality characteristics and water interactions of navy bean, chickpea, pea, and lentil flours. *Legume Science*, 4(1), 1–9. <https://doi.org/10.1002/leg3.136>
- Van Hoan, N., Mouquet-Rivier, C., & Treche, S. (2010). Effects of starch, lipid and moisture contents on extrusion behavior and extrudate characteristics of rice-based blends prepared with a very-low-cost extruder. *Journal of Food Process Engineering*, 33(3), 519–539. <https://doi.org/10.1111/j.1745-4530.2008.00288.x>
- Venkateswara-Rao, M., CK, S., Rawson, A., DV, C., & N, V. (2023). Modifying the plant proteins techno-functionalities by novel physical processing technologies: A review. *Critical Reviews in Food Science and Nutrition*, 63(19), 4070–4091. <https://doi.org/10.1080/10408398.2021.1997907>
- Vidal, N. P., Roman, L., Swaraj, V. J. S., Ragavan, K. V., Simsek, S., Rahimi, J., Kroetsch, B., & Martínez, M. M. (2022). Enhancing the nutritional value of cold-pressed oilseed cakes through extrusion cooking. *Innovative Food Science & Emerging Technologies*, 77, Article 102956. <https://doi.org/10.1016/j.ifset.2022.102956>
- Wang, S., Ai, Y., Hood-Niefer, S., & Nickerson, M. T. (2019). Effect of barrel temperature and feed moisture on the physical properties of chickpea, sorghum, and maize extrudates and the functionality of their resultant flours—Part 1. *Cereal Chemistry*, 96(4), 609–620.
- Webb, D., Dogan, H., Li, Y., & Alavi, S. (2023). Use of legume flours and fiber for tailoring structure and texture of pea protein-based extruded meat alternatives. *Journal of Food Science*, 88(1), 57–71. <https://doi.org/10.1111/1750-3841.16397>
- Xiao, T., Su, X., Jiang, R., Zhou, H., & Xie, T. (2023). Low moisture extrusion of soybean protein isolate: Effect of β -glucan on the physicochemical properties of the product. *LWT - Food Science and Technology*, 179, Article 114660. <https://doi.org/10.1016/j.lwt.2023.114660>
- Xu, Y., Jia, F., Wu, Y., Jiang, J., Zheng, T., Zheng, H., & Yang, Y. (2025). The impact of extrusion cooking on the physical properties, functional components, and pharmacological activities of natural medicinal and edible plants: A review. *Foods (Basel, Switzerland)*, 14(11), 1869. <https://doi.org/10.3390/foods14111869>
- Zapotoczny, P., Markowski, M., Majewska, K., Ratajski, A., & Konopko, H. (2006). Effect of temperature on the physical, functional, and mechanical characteristics of hot-air-puffed amaranth seeds. *Journal of Food Engineering*, 76(4), 469–476. <https://doi.org/10.1016/j.jfoodeng.2005.05.045>
- Zhang, T., Guo, Q., Xin, Y., & Liu, Y. (2022). Comprehensive review in moisture retention mechanism of polysaccharides from algae, plants, bacteria and fungus. In *Arabian journal of chemistry*, 15. Elsevier B.V.. <https://doi.org/10.1016/j.arabjc.2022.104163>