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Compositional and nutritional value of lupin cultivars: Identifying high-protein seeds for enhanced protein isolate production and phytochemical valorisation

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ABSTRACT

The rising global demand for sustainable protein sources has increased the interest in legumes, particularly lupin, due to its nutritional and technological advantages. Beyond the high protein content, some phytochemicals in lupin seeds such as quinolizidine alkaloids (QAs), saponins, phytic acid, and total phenolic compounds (TPC) warrant further exploration due to their potential for valorisation following a seed protein isolation process, offering opportunities for their use in functional foods, nutraceuticals, or other high-value applications. This work evaluated eight cultivars from different cultivated lupin species —*L. albus* (Estoril, Celina, and Frieda), *L. angustifolius* (Giribita and Carabor), and *L. luteus* (Acos, Cardiga, and a commercial mixture)— in Europe as alternative protein sources. Each cultivar was assessed in terms of proximate composition, phytochemical content, and protein extraction yield. The findings revealed significant inter- and intra-species variability in protein, total dietary fibre, and fat content among the studied cultivars. Lupin protein profile was found to be particularly rich in essential amino acids, including leucine and lysine, as well as beneficial unsaturated fatty acids across all evaluated cultivars. The studied phytochemicals, especially quinolizidine alkaloids, vary significantly among species, while cultivars within the same species showed a more consistent profile. The wet extraction process yielded high-purity protein isolates with favourable extraction efficiency. These findings highlight the potential of lupin for diverse applications in the food and industrial sectors. Cultivar selection is essential to enhance protein extraction yields and colour, as well as to consider lupin as a source of various phytochemicals for valorisation.

1. Introduction

Global protein demand is projected to rise steadily in the coming years due to the growing world population and shifts in consumer dietary patterns. Legumes have attracted increasing attention as a sustainable protein source, offering a viable alternative to animal-derived proteins due to their lower production costs [1] and reduced environmental impact [2]. Among legumes, lupin, a member of the Fabaceae family, is notable for its technological and nutraceutical potential [3]. Lupin has been cultivated for centuries across various regions [4]. The four main cultivated lupin species are white lupin (*L. albus*), yellow lupin (*L. luteus*), narrow-leafed lupin (*L. angustifolius*), and Andean lupin (*L. mutabilis*) [4]. In Europe, the commercially cultivated species are *L. albus, L. luteus*, and *L. angustifolius*, which are used for improving soil fertility, human consumption and livestock feed, while *L. mutabilis* cultivation is restricted to the Andean region [4,5]. While lupins are successful protein crops in Australia, supporting a significant industry that utilizes lupin protein and other valuable components, the cropping area in Europe and its utilization remains modest [4].

Due to its high protein and low-fat content, lupin flour is considered an excellent raw material to produce protein isolates (PI). This favourable protein-to-fat ratio simplifies extraction, reducing the need for costly and time-consuming defatting stages and yielding a high-purity PI that can enrich various food products [6]. However, other seed

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components are often overlooked. Several procedures have been described for protein extraction [7,8]. Despite that, protein isolate extraction typically relies on a wet extraction process, including the solubilization of proteins under alkaline conditions, followed by their precipitation at the isoelectric point [9]. This process leaves behind various phytochemicals such as quinolizidine alkaloids (QAs), saponins, total phenolic compounds (TPC), and phytic acid in the aqueous side stream produced after protein precipitation. The current trend in food production advocates for better utilization of the entire chemical composition of raw material. In this context, it is essential to explore not only the protein fraction but also other phytochemicals, including alkaloids, saponins, phytic acid, and phenolic compounds. These phytochemicals can have significant nutraceutical applications and have an influence, either positive or negative, on the techno-functional properties of food ingredients [10–17].

A distinctive feature of lupins is their content of QAs, which serve as a nitrogen reserve and as a defence mechanism against pathogens and predators [18]. QAs accumulation in seeds depends on genotype as well as biotic (e.g., pathogens) and abiotic factors (e.g., climatic, environmental conditions and agricultural practices) [19,20]. In humans, most of the information on QAs toxicity is derived from sparteine, due to its therapeutic use in the past as an antiarrhythmic and oxytocic drug [5]. However, over 170 different QA structures have been identified across various lupin species [18]. Given the structural diversity and potential toxicity of QAs, the use of advanced analytical techniques such as HPLC-MS is crucial to accurately identify and quantify these alkaloids, ensuring food safety and regulatory compliance. Maximum levels for QAs in food products of 200 mg kg^{-1} dry weight have been implemented by some national food authorities [21,22]. Due to QAs toxicity and strong bitter taste, a debittering process is needed when whole seeds are intended for human consumption. Currently, aqueous treatment is the only commercially applied debittering method, particularly for whole-seed snack [23]. This process, however, requires substantial amounts of water and time and leads to material loss. Various strategies have been explored to recover QAs from the leaching waters during debittering [24,25].

Lupin crops intended for high-protein products in human and animal nutrition are selected based on multiple criteria, including crop yield potential, environmental adaptability, and seed traits such as protein content and phytochemical composition. A parallel valorisation strategy for both proteins and phytochemicals involves evaluating the protein extraction yield, protein isolate purity and the recovery of phytochemicals –QAs, saponins, phytic acid and TPC– after the wet extraction process. Considering these aspects in different cultivars is crucial for promoting lupin in food, pharmaceutical, and industrial applications.

The aim of this work was to enhance the understanding of the protein content, extraction yield, and phytochemical composition of lupin cultivars for obtaining protein isolates and phytochemicals. The study assessed nine European lupin cultivars, including *L. albus* (Estoril, Celina, and Frieda), *L. angustifolius* (Giribita and Carabor), and *L. luteus* (Acos, Cardiga, and a commercial mixture, hereafter referred as CM), grown in Central Europe and Southwest Europe (*i.e.* Iberian Peninsula). The selection of multiple species and cultivars, grown under varying geographical and environmental conditions, is aimed to support a broader utilization of lupin as a food protein source in Europe, even from cultivars not traditionally classified for human consumption. By providing these insights, the study supports the scientific community, food industry, and farmers in making informed decisions regarding the utilization of lupin cultivars.

2. Material and methods

2.1. Plant material

This study examined three commercial cultivars of *Lupinus albus* (Estoril, Celina and Frieda), two of *Lupinus angustifolius* (Giribita and Carabor) and three of *Lupinus luteus* (Acos, Cardiga and CM). The Celina, Frieda and Carabor cultivars were kindly provided by Deutsche Saatveredelung (Lippstadt, Germany). Estoril, Cardiga, Acos, and Giribita cultivars were kindly provided by the Instituto Nacional de Investigación Agraria y Veterinaria de Portugal (Oeiras, Portugal). CM, which is used for soil fertilization and foraging, was purchased from Semillas Batlle (Molins de Rei, Spain). Hulled seeds from each cultivar were ground using a Moulinex Grinder (Model AR110830, Écully, France).

2.2. Proximal composition

Dry matter and ash contents were measured according to the AOAC methods 950.46 and 920.153, respectively [26,27]. Total nitrogen was quantified through the Kjeldahl method (AOAC 928.08) [28]. Protein nitrogen was determined following of Licitra et al. [29] with minor modifications. Briefly, 2 g of the sample was mixed with 20 mL of deionized water and homogenized. Then, 20 mL of 24 % trichloroacetic acid was added and centrifuged at 13,000 *g* for 10 min (Eppendorf, Hamburg, Germany). The resulting supernatant (20 mL) containing the non-protein nitrogen (NPN) fraction was subjected to digestion, distillation, and titration as described for total nitrogen determination method. Protein nitrogen content was calculated as the difference between total nitrogen and NPN content, and pure protein content was obtained by multiplying the protein nitrogen content by 6.25 (AOAC 928.08) [28]. Total fat content was quantified through Soxhlet extraction method after acid hydrolysis (AOAC 991.36) [30]. Total dietary fibre was analysed following the AOAC method 985.29 [31]. All analyses were conducted in duplicate.

2.3. Phytochemical quantification

2.3.1. Total phenolic compounds (TPC)

Phenolic compounds were extracted following Villacrés et al. [32] with slight modifications. Each flour sample (0.5 g) was dispersed in 5 mL of a 0.2 M hydrochloric acid in methanol solution, stirred, and sonicated at 40 kHz during 15 min at room temperature (3000512 model, J.P. Selecta S.A., Barcelona, Spain). The sample was collected and centrifuged at 14,000 *g* for 10 min at 4 °C. A calibration curve was prepared using gallic acid (0–100 mg L^{-1}), and quantification was performed using the Folin-Ciocalteu reagent measuring the absorbance at 760 nm using a UV–vis spectrophotometer (UV-1800 Shimadzu, Kyoto, Japan). Results were expressed in milligrams of gallic acid equivalent per gram of dry weight of flour, with analyses conducted in triplicate.

2.3.2. Phytic acid content

Phytic acid content was determined following McKie & McCleary [33]. Phosphorus quantification was performed using an enzymatic method (K-PHYT, Megazyme, Bray, County Wicklow, Ireland), with a calibration curve of KH_2PO_4 (0–6 mg L^{-1}). The absorbance was measured at 655 nm with the spectrophotometer and results expressed in milligrams per gram of dry weight of flour. All analyses were conducted in triplicate.

2.3.3. Saponin content

Total saponin content was measured using the spectrophotometric method of Navarro del Hierro et al. [34], with minor modifications. Briefly, 1 g of lupin flour was extracted in 20 mL of water, stirred for 30 min, and centrifuged at 6000 *g* for 10 min. Aliquots of 25 μL were mixed with 100 μL of 10 % vanillin solution in absolute ethanol and 1 mL of 50 % sulfuric acid. A calibration curve was prepared using oleanolic acid standard solutions (0–0.8 mg mL^{-1}). Samples were heated at 60 °C for 10 min, cooled at room temperature and then the absorbance was measured at 540 nm using the spectrophotometer. Results were expressed as milligrams of oleanolic acid per gram of dry weight of flour, with analyses conducted in triplicate.

2.3.4. Alkaloid content by UHPLC-MS/MS

Alkaloids were extracted and quantified following Khedr et al. [35], with some modifications. Briefly, 1 g of lupin flour was mixed with 50 μL of 100 mg L^{-1} scopolamine (used as an internal standard) and allowed to rest for 30 min. Subsequently, 10 mL of distilled water was added to the mixture. After 15 min, 10 mL of acetonitrile was added in the previous solution. The resulting mixture was vortexed for 1 min and then sonicated at 40 kHz during 10 min at room temperature. The QUECHERS method (1 g NaCl and 4 g $MgSO_4$) was used for the extraction process. After the addition of salts, the mixture was vortexed for 30 s, and the pH was adjusted to 10.5 with 50 % NaOH. The sample was then vortexed for an additional 1 min and centrifuged at 2200 *g* for 5 min. The supernatant was filtered through a nylon syringe membrane with a pore diameter of 0.2 μm and diluted 10-fold (100 μL in 1 mL) with ultrapure water for subsequent UHPLC-MS/MS analysis. Samples with high alkaloid content were further diluted with ultrapure water containing 1 % acetonitrile to ensure they felt within the linear range of the calibration curve.

Chromatographic separation was performed using an Acquity Premier UHPLC system coupled with a Xevo-TQ-S micro tandem quadrupole mass spectrometer detector (Waters Corporation, Milford, USA). The separation was performed on an Acquity Premier HSS T3 column (1.8 μm, 2.1 × 100 mm, Waters Corporation, Milford, USA), at a flow rate of 0.3 mL min^{-1} and a column temperature of 40 °C. The aqueous mobile phase consisted of 50 mM ammonium formate buffer (pH 4.6) and the organic phase was acetonitrile. The gradient was as follows: initial, 90 % A; 0.5 min, 90 % A; 5.0 min, 60 % A; 7.0 min, 60 % A; 7.1 min, 90 % A; 10.0 min, 90 % A. The autosampler was maintained at 5 °C, with an injection volume of 5.0 μL. Mass spectrometric detection was performed using ElectroSpray Ionization in positive mode with Multiple Reaction Monitoring. The conditions were: the capillary voltage at 0.5 kV, source temperature of 150 °C, desolvation temperature at 500 °C, desolvation gas (nitrogen) flow at 1,000 L h^{-1}, and cone gas flow of 150 L h^{-1}. Argon was employed as the collision gas. The two most abundant ions were selected for detection: one for quantification and the other for confirmation. The selected transitions, along with their optimal conditions, are described in Table 1.

A blank soybean flour sample (Borchers, Oyten, Germany), devoid of alkaloids, was used as a representative matrix for method validation [18, 35]. The matrix was spiked with each analyte at concentrations of 1, 5, and 10 mg L^{-1}. Quantification was performed using matrix-matched calibration, with scopolamine as the internal standard. Concentrations were calculated based on the recovery rates determined during the method validation process.

2.4. In vitro protein digestibility (IVPD)

The *in vitro* protein digestibility (IVPD) of lupin protein flours was determined following the multienzyme technique (3-enzyme method) based on Hsu et al. [36] with some modifications. Samples were prepared to contain 62.5 mg of protein, mixed with 10 mL of distilled water at 37 °C, and homogenized using an UltraTurrax T25 model disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) at 12,000 rpm for 15 s. The mixture was adjusted to pH 8 with NaOH 1M before a multienzyme solution addition. The multienzyme solution was prepared containing 14 mg/mL trypsin (1634 BAEE U/mg), 3.2 mg/mL of chymotrypsin (57 U/mg), and 65 μg/mL protease (774 U/mg) solution. All enzymes were obtained from Sigma-Aldrich (Madrid, Spain). Subsequently, the multienzyme solution was adjusted to pH 8 and 1 mL was added to the protein suspension, mixed and kept under agitation at 37 °C. The pH drop was recorded over 10 min using a pH meter. The multienzyme solution was freshly prepared before each series of tests.

IVPD (%) was calculated as the percentage of digestible protein using the following equation [37]:

$$IVPD = 65.66 + 18.10 \cdot \Delta pH10\,min \quad (3)$$

where ΔpH10min represents the change in pH after 10 min.

2.5. Amino acid profile

Samples (100 mg) were digested using a Milestone Ethos One microwave system (Milestone Srl, Sorisole, Italy) at 170 °C for 15 min with 30 mL of 6 M hydrochloric acid. For tryptophan determination, a separate digestion method was used: 200 mg of sample were treated with 15 mL of 5 M sodium hydroxide at 170 °C for 40 min. Each digestion was performed in duplicate. After digestion, samples were diluted in either 0.1 M hydrochloric acid or water (for tryptophan determination) and analysed using an Agilent 110 series HPLC system (Agilent Technologies Inc., Santa Clara, USA), following Agilent application note 5994-2189 E N [38].

Separation was performed using a Zorbax Eclipse AAA column (4.6 × 150 mm, 3.5 μm; Agilent Technologies Inc., Santa Clara, USA), with the oven temperature set to 40 °C. The mobile phase A consisted of a phosphate-borate buffer (10 mM sodium hydrogen phosphate and 10 mM sodium tetraborate at pH 8.2), while mobile phase B was a mixture of acetonitrile/methanol/water (45:45:10 v/v/v). Samples were derivatized using o-phthalaldehyde and 9-fluorenyl methyl chloroformate Agilent P/N 5061–3335 and 5061–3337, respectively; Agilent Technologies Inc., Santa Clara, USA) with an automated Agilent 1260 Infinity II model vial sampler (Agilent Technologies Inc., Santa Clara, USA).

Table 1
UHPLC-MS/MS data of quinolizidine alkaloids analysis with mass spectrometry transitions and optimised conditions.

Alkaloid name	Retention time (min)	Precursor ion ($m{\cdot}z^{-1}$)	Product ion ($m{\cdot}z^{-1}$)	Dwell time (s)	Cone Voltage (V)	Collision energy (eV)
Lupinine	1.33	170.16	136	0.025	20	34
			152		35	19
			124		35	23
			96		4	32
Albine	2.02	233	112.033	0.025	2	24
			138.129		2	18
			120.037		2	16
Angustifoline	2.54	235.22	193	0.025	14	18
			112		32	30
Sparteine	5.23	235.22	98	0.025	40	30
			233		40	26
			84		68	34
Lupanine	2.01	249.16	136	0.025	46	26
			114		50	26
			84		46	42
13-hidroxilupanine	1.49	265.16	114	0.025	20	26
			152		20	26
			112		20	26

Quantification was performed using fluorescence detector with excitation/emission wavelengths of 345/455 and 265/315 with all amino acids, except cysteine, which was quantified using a diode-array detector set to 382 nm and 262 nm (Agilent Series 1200; Agilent Technologies Inc., Santa Clara, USA). Peak identification was conducted by comparing retention time and spectral information with pure standard solutions (Agilent P/N 5061–3330, Agilent Technologies Inc., Santa Clara, USA), and quantification was achieved using internal standards (norvaline and sarcosine amino acids).

The essential amino acid index (EAAI) [39] and the *in vitro* digestible indispensable amino acid score (DIAAS) [40] were employed to evaluate the protein quality of the analysed cultivars. EAAI were calculated using the following equations (Equation (1)):

$$\text{EAAI}\ (\%) = \sqrt[n]{\frac{a1}{a1R}\ \text{x}\ldots\ldots\text{x}\frac{an}{anR}} \quad (1)$$

In these formulas, *a* represents the amount of essential amino acids (mg) per g of the tested protein, *aR* denotes the corresponding amount of the amino acid (mg) per g of the reference protein, and *n* is the total number of amino acids considered in the calculation (nine, as methionine-cysteine and tyrosine-phenylalanine were paired). The reference protein used was based on the amino acid pattern recommended by the World Health Organization and the Food and Agriculture Organization (FAO/WHO/UNU) [41] for adult humans.

In vitro DIAAR was calculated according to FAO [41] and the *proxy* approach as described by Sousa et al. [42] which uses the total protein digestibility instead of the individual amino acid digestibility. Contrary to Sousa et al. [42], who used the INFOGEST static protocol of *in vitro* digestion, we used the simpler 3-enzyme method (as described in section 2.4), which showed a good correlation with *in vivo* studies for the determination of protein digestibility of legumes [40]. Therefore, the *in vitro* DIAAR of each indispensable amino acid was calculated following equation (2). *In vitro* DIAAS corresponded to the limiting indispensable amino acid (LIAA), i.e., with the lowest DIAAR value.

$$\textit{in vitro}\ \text{DIAAR}\ (\%) = \frac{(\text{mg AA/g protein of the test ingredient})}{\text{mg AA/g reference protein}} \times \textit{IVPD}\ (\%) \quad (2)$$

2.6. Fatty acids determination

The lipid fraction was extracted using the Folch et al. [43] method with chloroform-methanol mixture. The extracted lipids were converted to fatty acid methyl esters using NaOH/methanol and BF_3, following the ISO 5509–1978 (E) [44] standard procedure. FAMEs were analysed by gas chromatography using an Agilent 8860 GC-FID system (Agilent Technologies Inc., Santa Clara, USA) equipped with a Zebron ZB-FAME capillary column (30 m, 0.25 mm i. d., 0.20 μm; Phenomenex Inc., Torrance, USA). The results were expressed as the percentage of total fatty acids.

2.7. Preparation of lupin protein isolates (LPI)

LPI were prepared using an alkaline extraction followed by isoelectric point precipitation procedure. Briefly, 8 g of ground lupin seeds were suspended in distilled water at a 1:10 w:v ratio. The suspension was homogenized using a DI 25 Basic Homogenizer (IKA, Staufen, Germany) for 1 min at 12,000 rpm. The pH of the mixture was then adjusted to 10 by adding 1N NaOH to solubilize the proteins. The resulting suspension was stirred at room temperature for 30 min and then centrifuged at 4,700 *g* for 15 min. The supernatant was collected, and the remaining pellet (Fibre Fraction; FF) was washed with distilled water at a 1:10 w:w ratio and centrifuged again at 4,700 *g* for 15 min. FF was dried in an oven at 100 °C oven for 24 h and weighed. The pH of the supernatant was then adjusted to 4.5 using 1 N HCl to precipitate the proteins. The suspension was left to stand for 1 h at 4 °C. Afterwards, the suspension was centrifuged at 4,700 *g* for 15 min. The resulting LPI was washed with distilled water at a 1:10 w:w ratio, centrifuged again at 4,700 *g* for 15 min, and dried in an oven at 100 °C for 24 h and weighed. The protein content of both LPI and FF was determined using the Dumas method, employing a FP828P carbon/nitrogen analyser (LECO, St. Joseph, MI, USA). A conversion factor of 6.25 was used to calculate protein content from the measured nitrogen content. Protein balance of LPI and FF fractions were calculated in duplicate for each lupin cultivar as a percentage of the protein content of seeds using the following equation:

$$\text{Protein recovery LPI or FF}\ (\%) = \frac{\text{weight of LPI or FF (g d.m.) x protein content LPI or FF (\% d.m.)}}{\text{weight of the flour (g d.m.) x protein content of flour (\% d.m.)}} \quad (4)$$

Weights were expressed as dry matter (d.m.).

2.8. Colour of LPI

The colour of the LPI was measured at four random points of the powder samples using a Minolta Colorimeter CR400/410 (Konica Minolta, Tokyo, Japan). The colorimeter was calibrated with a blank reference before measurements. The colour results were obtained under a D65 standard illumination with a 2° observer angle and results were expressed according to the CIE L × a*b × colour space.

2.9. Statistical analysis

All data were obtained from at least two independent replicates and recorded as mean ± standard deviations. Statistical analyses were conducted using JMP software version 16.2.0 (SAS Institute Inc., Cary, NC, USA). Series of one-way analysis of variance (ANOVA) were performed considering all cultivars regardless of the species and for each lupin species (*L. albus, L. luteus* and *L. angustifolius*) as independent variables to assess significant differences in the studied parameters. Tukey's test followed each ANOVA analysis. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Proximate composition and phytochemical content

The chemical composition of eight lupin cultivars is presented in Table 2. The protein content of the lupin cultivars ranged from 292.8 to 416.5 g kg^{-1}, which is comparable to that of soybean and higher than that of other legumes, such as faba bean, pea, chickpea, yellow pea and lentil (250–300, 219, 226, 232–252 and 269 g kg^{-1}, respectively) [6,12,14,45]. The high protein content in *Lupinus* spp, compared with other legumes, is attributed to their low starch content, which is replaced by fat as the main seed energy source [46]. The protein content is influenced by genetic factors and growing conditions [47], explaining the observed variability among different lupin species and cultivars. Despite this variability, the average protein concentration of cultivars from the same species agrees with previous research [39,48–50]. Worth noting

Table 2
Proximate composition and bioactive compounds content (mean ± SD) of the studied cultivars.

	Moisture (g·kg^{-1})	Crude protein (g·kg^{-1} d.m.)	Dietary fibre (g·kg^{-1} d.m.)	Fat (g·kg^{-1} d.m.)	Ash (g·kg^{-1} d.m.)	NPN (g·kg^{-1} d.m.)	TPC (mg·g d.m.)	Phytic acid (mg·g d.m.)	Saponins (mg·g d.m.)
Lupinus albus									
cv. Celina	106.0 ± 1.2	416.5 ± 1.6$^{a, A}$	336.7 ± 4.3$^{a, C}$	80.0 ± 0.2$^{b, B}$	39.0 ± 0.1$^{c, CD}$	4.2 ± 0.1$^{c, C}$	1.88 ± 0.07$^{a, CD}$	7.35 ± 0.25$^{b, D}$	42.49 ± 0.67$^{a, A}$
cv. Estoril	79.0 ± 0.1	367.7 ± 7.2$^{b, BC}$	331.7 ± 6.9$^{a, CD}$	91.9 ± 1.4$^{a, A}$	43.2 ± 0.2$^{a, B}$	6.5 ± 0.3$^{a, A}$	1.83 ± 0.11$^{a, CD}$	11.22 ± 0.87$^{a, B}$	36.34 ± 1.97$^{b, ABC}$
cv. Frieda	102.2 ± 1.4	358.7 ± 4.1$^{b, BCD}$	344.7 ± 9.7$^{a, C}$	96.3 ± 3.7$^{a, A}$	39.9 ± 0.1$^{b, C}$	5.4 ± 0.4$^{b, ABC}$	1.64 ± 0.15$^{a, D}$	7.91 ± 0.38$^{b, D}$	34.85 ± 2.77$^{b, BC}$
Mean *L. albus*	**95.7 ± 13.1**	**381.0 ± 28.1**	**337.7 ± 8.2**	**89.4 ± 7.7**	**40.7 ± 2.0**	**5.4 ± 1.2**	**1.78 ± 0.15**	**8.83 ± 1.87**	**37.89 ± 3.91**
Lupinus angustifolius									
cv. Carabor	109.1 ± 0.1	341.3 ± 3.1$^{a, D}$	310.4 ± 8.7$^{b, D}$	59.3 ± 2.6$^{a, D}$	39.3 ± 0.1$^{a, CD}$	4.6 ± 0.1$^{a, BC}$	2.11 ± 0.25$^{a, BCD}$	8.28 ± 0.45$^{a, CD}$	32.06 ± 1.54$^{a, BCD}$
cv. Giribita	86.8 ± 0.8	292.8 ± 0.7$^{b, E}$	501.0 ± 1.9$^{a, A}$	59.1 ± 1.0$^{a, D}$	37.6 ± 0.4$^{b, D}$	6.5 ± 0.6$^{a, A}$	1.98 ± 0.05$^{a, BCD}$	8.09 ± 0.33$^{a, CD}$	26.68 ± 2.32$^{b, D}$
Mean *L. angustifolius*	**97.9 ± 12.9**	**317.1 ± 28.1**	**405.7 ± 110.2**	**59.2 ± 1.6**	**38.4 ± 1.0**	**5.6 ± 1.1**	**2.05 ± 0.18**	**8.19 ± 0.37**	**29.37 ± 3.43**
Lupinus luteus									
cv. Acos	84.4 ± 0.5	376.5 ± 0.9$^{a, B}$	399.2 ± 1.0$^{a, B}$	55.8 ± 1.9$^{b, D}$	47.9 ± 1.1$^{a, A}$	5.9 ± 0.2$^{a, AB}$	2.50 ± 0.27$^{b, B}$	13.91 ± 0.99$^{a, A}$	37.68 ± 2.84$^{a, AB}$
cv. Cardiga	80.1 ± 0.1	349.2 ± 12.6$^{a, CD}$	407.6 ± 7.7$^{a, B}$	71.4 ± 0.7$^{a, C}$	48.6 ± 0.1$^{a, A}$	5.1 ± 0.3$^{a, ABC}$	3.32 ± 0.23$^{a, A}$	10.30 ± 1.14$^{b, BC}$	35.55 ± 2.16$^{ab, BC}$
CM	79.7 ± 0.2	359.7 ± 3.2$^{a, BCD}$	332.0 ± 2.4$^{b, CD}$	55.9 ± 0.1$^{b, D}$	40.1 ± 1.0$^{b, C}$	5.6 ± 0.7$^{a, ABC}$	2.27 ± 0.32$^{b, BC}$	10.55 ± 1.20$^{b, B}$	30.86 ± 2.66$^{b, CD}$
Mean *L. luteus*	**81.4 ± 2.4**	**361.8 ± 13.6**	**379.6 ± 37.3**	**61.0 ± 8.1**	**45.5 ± 4.2**	**5.5 ± 0.5**	**2.66 ± 0.49**	**11.59 ± 2.00**	**34.70 ± 3.75**

CM, commercial mixture; d.m., dry matter; NPN, non-protein nitrogen; TPC, total phenolic compounds.
[a-c] denotes statistical differences within species ($p < 0.05$).
A-D denotes statistical differences between cultivars ($p < 0.05$).

that among the four major lupin species worldwide, including the *L. albus*, *L. angustifolius*, *L. luteus*, and *L. mutabilis*, the latter species typical from the Andean region stands out with the highest protein content, showing a range from 320 to 526 g kg^{-1}, attributed to genetic diversity and agronomic factors [23,51,52], although this species is not present in Europe on a commercial scale [4]. Significant differences were found among studied cultivars within the same species. Within L. *albus,* cultivar Celina had the highest protein content at 416.5 g kg^{-1}. Within L. *angustifolius* cultivars, it stood out Carabor (341.3 g kg^{-1}). Although no differences were observed in *L. luteus*, Acos cultivar and CM stood out (376.5 and 359.7 g kg^{-1} respectively). Overall, the high protein content of lupin species and cultivars makes them very promising as an alternative to soy for the obtainment of PI.

The total dietary fibre (TDF) content ranged from 310.4 to 501.0 g kg^{-1}, which is considerably higher than other legumes such as yellow peas, faba bean and soybeans (91–189, 110–180 and 136–236 g kg^{-1}, respectively) [6,14,45]. The observed intraspecies variability help to explain there were no differences between species. Fibre content data is consistent with previous research [3,6,23,53–56]. Within L. *albus* cultivars, no significant differences were found, whereas within *L. angustifolius*, a notable difference was observed between Carabor (310.4 g kg^{-1}) and Giribita (501.0 g kg^{-1}). Among L. *luteus* cultivars, CM had the lowest content (332.0 g kg^{-1}) whereas Acos and Cardiga had similar contents (399.2 and 407.6 g kg^{-1}, respectively). In the different lupin cultivars, a certain complementarity can be observed between the protein content and the fibre content, particularly when comparing *L. albus* Celina and L. *albus* Giribita cultivars.

Fat content is also an important factor to consider in the development of PI as it may affect sensory properties and stability negatively [57]. The fat content of the studied cultivars ranged from 59.1 to 96.3 g kg^{-1}, which is, on average, 24 % lower than that of soybeans [58]. Despite not being classified as an oilseed crop, some lupin species have a relatively high oil content compared to other legumes [59]. In this regard, *L. albus* cultivars showed the highest fat content among the three studied species, which agrees with other studies [6,14,16,39,45], whereas *L. mutabilis* cultivars exhibited a higher fat content (from 130 to 246 g kg^{-1}) [23,51,52]. Within L. *albus* cultivars, Estoril and Frieda had the highest fat content, 91.9 and 96.3 g kg^{-1}, respectively. No significant differences were found among *L. angustifolius* cultivars whereas Cardiga had the highest fat content (71.4 g kg^{-1}) among *L. luteus* cultivars. Lupin seeds' moderate fat content facilitates protein extraction by eliminating the need for defatting steps. The variation in fat content among the studied lupin cultivars can be attributed, at least in part, to genetic and environmental factors [19,20,23]. For instance, water stress conditions have been shown to reduce seed fat content, while the duration that late-maturating varieties remain in the field during the maturation stage influences lipid accumulation, as extended maturation periods provide plants with additional time to convert seed carbohydrates into lipids [23].

The ash content across the lupin cultivars ranged from 39.9 to 48.6 g kg^{-1}, in line with other studies [6,14,15,23]. The ash content and variability within species was similar to that found between cultivars, thus resulting in no significant differences between species. Similar NPN values, ranging from 4.2 to 6.5 g kg^{-1}, were observed among cultivars agreeing with other studies [60].

The antioxidant and antimicrobial properties of phenolic compounds are well-known [61]. Phenolic compounds can interact with proteins and influence protein characteristics like secondary structure, surface hydrophobicity and thermal stability. These interactions can either enhance or reduce the functional and nutritional properties of proteins [32,62,63]. The Folin Ciocalteu method is widely used for the quantification of TPC in food and is recognized as a reference method [64], even though some substances such as ascorbic acid and other non-phenolic antioxidants can interfere in the measurement. The TPC of the analysed lupin species ranged from 1.64 mg g^{-1} to 3.32 mg g^{-1} (Table 2). These findings align with other studies [12,65], who reported substantial differences in TPC among different lupin species. Due to high intraspecies differences, no significant differences were observed

between the studied lupin species. However, *L. luteus* cultivars showed a tendency towards higher TPC levels than other evaluated cultivars. Among L. *luteus* cultivars, Cardiga had the highest TPC concentration at 3.32 mg g^{-1}, whereas no significant differences were observed within *L. albus* and *L. angustifolius* studied cultivars.

In humans, phytic acid can interfere in the absorption of minerals such as zinc, iron and calcium, potentially leading to deficiencies, particularly in individuals highly reliant on plant-based diets [66]. In PI, phytic acid can affect protein functionality by forming complexes that impact on solubility, emulsification and gelling properties [10,67]. Phytate is mainly concentrated in seed cotyledons, resulting in a relative increase in phytate content in dehulled seeds [10]. In the hulled lupin seeds analysed in this study, phytic acid content ranged from 7.35 mg g^{-1} to 13.91 mg g^{-1} (Table 2). These findings agree with previous research ranging from 4 to 12 mg g^{-1} [65]. *L. mutabilis* cultivars presented, on average, a higher phytic acid content of 27.4 mg g^{-1} [23], although caution must be taken when comparing phytochemical contents from different works due to possible methodological differences. Notable differences were observed between the studied cultivars, with *L. luteus* cultivars exhibiting a higher mean phytate concentrations compared to *L. angustifolius* and *L. albus*. The highest concentration of phytic acid among *L. albus* cultivars corresponded to Estoril with 11.22 mg g^{-1}, slightly lower than previously reported values for this species [68,69]. No significant differences were found among *L. angustifolius* cultivars. Among L. *luteus* cultivars, Acos stood out with the highest phytic acid content at 13.91 mg g^{-1}.

Saponins, which act as plant defence compounds [70] and growth regulators [71], also impart bitterness and possess antimicrobial properties [11,47]. In animals, they exhibit various biological effects, including the hemolysis of erythrocytes and the modulation of nutrient absorption [12,70]. In the food industry, saponins are valued for their foaming and emulsifying properties [61]. The saponin content among the studied cultivars ranged from 26.68 mg g^{-1} to 42.49 mg g^{-1} (Table 2). The limited data and methodological differences in the literature makes difficult the comparison with other studies.

Lupins primarily contain QAs, which serve as secondary metabolites for defence against pathogens and predators [5]. Regarding the concentration of QAs, marked differences were observed both within and between the studied species (Table 3). These differences, caused by genetic, biotic and abiotic agents [19,20], have important implications for agricultural practices, but also for potential valorisation for therapeutic applications [13]. Studied L. *luteus* cultivars exhibited the highest QAs, levels ranging from 4,651 mg kg^{-1} to 6,824 mg kg^{-1}. Evaluated L. *albus* and *L. angustifolius* cultivars had substantially lower alkaloid contents (Table 3). The total QAs concentrations observed in the studied cultivars from different species were consistent with the values reported in previous studies [23,72]. *L. mutabilis* cultivars exhibit the highest QA content, averaging 28,000 mg kg^{-1} [23]. It is considered that bitter lupin species typically contain 5,000 to 60,000 mg kg^{-1} of alkaloids in flour, while sweet species contain less than 200 mg kg^{-1} [65]. Recent studies have shown that the *L. albus* sweet cultivars carry a natural mutation, known as pauper locus, which decreases QA levels below the established safe consumption threshold of 200 mg·kg^{-1} [22]. However, the presence of the pauper locus does not necessarily determines the low alkaloid content, since other critical alleles might be involved in QA accumulation together with environmental conditions [73].

Regarding the QA profile, six alkaloids were identified and quantified: lupinine, albine, angustifoline, sparteine, lupanine and 13α-hydroxylupanine. Lupinine was the predominant QA in *L. luteus* studied cultivars, ranging from 2,867 mg kg^{-1} to 5,573 mg kg^{-1}, accounting for 61–87 % of the total alkaloids. *L. albus* Estoril also contained lupinin, but in a much lower concentration (0.434 mg kg^{-1}) and relative abundance (0.1 %) than *L. luteus* cultivars. This species-specific pattern was also reported elsewhere [5]. Albine, instead, was exclusively detected in *L. albus* studied cultivars, with Estoril showing the highest concentration at 69.4 mg kg^{-1}. Lupanine, 13α-hydroxylupanine and angustifolin were only found in *L. albus* and *L. angustifolius* studied cultivars as also reported by other authors [74,75]. Lupanine concentration ranged from 17.8 mg kg^{-1} to 924 mg kg^{-1}, making up the major QA fraction in *L. albus* Celina and Freida cultivars. 13α-hydroxylupanine concentration ranged from 83.5 mg kg^{-1} to 1341 mg kg^{-1}, predominantly present in *L. angustifolius* cultivars comprising 73–77 % of the total QA. Angustifolin concentration ranged from 7.8 mg kg^{-1} to 164 mg kg^{-1}, not dominating in any studied cultivar but being higher in *L. angustifolius* as reported in other studies [75]. Sparteine was found in all cultivars, ranging from 0.18 mg kg^{-1} to 1784 mg kg^{-1}, with the highest levels found in *L. luteus* cultivars (12 %–38 %).

Although the QA concentration ranges observed in this study align with some reported in previous research [5,23], direct comparisons remain challenging due to differences in environmental and agronomic conditions under which crops were grown [19,23,76]. For example, in the work of Zafeiriou et al. [73] Celina exhibited the lowest alkaloid content among other *L. albus* cultivars and landraces, while in the present work, Celina was the *L. albus* cultivar with highest alkaloid content.

Table 3
Quinolizidine alkaloids content (mean ± SD) in lupin cultivars and relative content within species (%).

	Lupinine (mg·kg^{-1} d.m)	Albine (mg·kg^{-1} d.m)	Lupanine (mg·kg^{-1} d.m)	13α-Hydroxylupanine (mg·kg^{-1} d.m)	Angustifoline (mg·kg^{-1} d.m)	Sparteine (mg·kg^{-1} d.m)	Total alkaloids (mg·Kg^{-1} d.m)
Lupinus albus							
cv. Celina	ND	ND	$924 \pm 16^{a, A}$	$196 \pm 8.4^{b, BC}$	$14.5 \pm 0.5^{b, BC}$	$29.8 \pm 2.3^{a, D}$	$1165 \pm 25^{a, D}$
cv. Estoril	0.434 ± 0.045^{D}	$69.4 \pm 1.8^{a, A}$	$68.1 \pm 1.2^{c, D}$	$162 \pm 6.1^{b, CD}$	$14.5 \pm 0.3^{b, BC}$	$0.24 \pm 0.08^{c, D}$	$314 \pm 6^{c, E}$
cv. Frieda	ND	$25.9 \pm 2.4^{b, B}$	$489 \pm 69^{b, B}$	$371 \pm 58^{a, B}$	$24.5 \pm 3.3^{a, B}$	$14.5 \pm 2.2^{b, D}$	$925 \pm 72^{b, D}$
Relative content	0.1 %	2 %–22 %	20 %–80 %	16 %–51 %	1 %–5 %	0.1 %–3 %	
Lupinus angustifolius							
cv. Carabor	ND	ND	$346 \pm 34^{a, C}$	$1341 \pm 170^{a, A}$	$164 \pm 12^{a, A}$	$2.74 \pm 0.03^{a, D}$	$1855 \pm 218^{a, C}$
cv. Giribita	ND	ND	$17.8 \pm 2.5^{b, D}$	$83.5 \pm 9.6^{b, CD}$	$7.8 \pm 0.7^{b, CD}$	$0.180 \pm 0.008^{b, D}$	$109 \pm 12^{b, E}$
Relative content	–	–	16 %–19 %	73 %–77 %	6 %–9 %	0.1 %–0.2 %	
Lupinus luteus							
cv. Acos	$2867 \pm 100^{c, C}$	ND	ND	ND	ND	$1784 \pm 86^{a, A}$	$4651 \pm 189^{b, B}$
cv. Cardiga	$5573 \pm 240^{a, A}$	ND	ND	ND	ND	$1250 \pm 22^{b, B}$	$6824 \pm 247^{a, A}$
CM	$4227 \pm 70^{b, B}$	ND	ND	ND	ND	$703 \pm 79^{c, C}$	$4930 \pm 130^{b, B}$
Relative content	61 %–87 %	–	–	–	–	12 %–38 %	

CM, commercial mixture; d.m., dry matter; ND, not detected.
[a-c] Denotes statistical differences within species ($p < 0.05$).
A-D Denotes statistical differences between species ($p < 0.05$).

Therefore, environmental and agronomic factors influence alkaloid biosynthesis, making it difficult to determine the precise sources of variation in QA concentration and composition [19]. Beyond analytical technique differences, variability in alkaloid content and diversity can be attributed to *(i)* genetic factors, as species and varieties differ in their inherent genetic information and, thus, in the genomic expression due to adaptation to different microhabitats [23]; *(ii)* environmental conditions, where moisture availability tends to reduce alkaloid content, whereas soil nitrogen availability, sunlight intensity, and temperature fluctuations can significantly influence production and accumulation of QAs at seed [19,23]; and *(iii)* temporal fluctuations within the same plant, known as the "turnover" effect, where alkaloid levels vary depending on weather conditions and time of day [23]. Therefore, the significant variation in QA contents and profiles among the studied cultivars reflects differences in genetic, agronomic and environmental factors influencing QA biosynthesis and accumulation.

3.2. Amino acid and fatty acid profiles

The amino acid composition and proportion of proteins are critical metrics for assessing nutritional quality. Table 4 shows that the predominant amino acids in the analysed cultivars were glutamic acid (18.69–24.68 g · 16 g^{-1} N), arginine (8.14–12.15 g · 16 g^{-1} N) and aspartic acid (8.03–10.16 g · 16 g^{-1} N). Although significant differences in amino acid content were observed between cultivars, the amino acid profile remained consistent across species which agrees with previous

Table 4
Amino acid profile (mean ± SD) of lupin cultivars and their nutritional values (g · 16 g^{-1} N).

	L. albus				L. angustifolius			L. luteus			
	Celina	Estoril	Frieda	Mean	Carabor	Giribita	Mean	Acos	Cardiga	CM	Mean
Essential amino acid											
Histidine	1.95 ± 0.05$^{b, D}$	1.96 ± 0.04$^{b, CD}$	2.57 ± 0.02$^{a, A}$	**2.16 ± 0.32**	2.08 ± 0.08$^{a, BCD}$	2.21 ± 0.11$^{a, ABC}$	**2.20 ± 0.16**	2.42 ± 0.11$^{a, AB}$	2.36 ± 0.16$^{a, AB}$	2.56 ± 0.09$^{a, A}$	**2.45 ± 0.13**
Isoleucine	3.88 ± 0.11$^{a, ABC}$	4.04 ± 0.12$^{a, AB}$	4.23 ± 0.07$^{a, AB}$	**4.05 ± 0.18**	3.39 ± 0.08$^{b, C}$	4.21 ± 0.25^{a,A}	**3.9 ± 0.61**	3.64 ± 0.17$^{a, BC}$	3.64 ± 0.18$^{a, BC}$	3.85 ± 0.12$^{a, ABC}$	**3.71 ± 0.16**
Leucine	6.34 ± 0.22$^{a, BC}$	6.55 ± 0.10$^{a, AB}$	6.99 ± 0.13$^{a, AB}$	**6.63 ± 0.32**	5.55 ± 0.16$^{b, C}$	7.13 ± 0.40^{a,A}	**6.50 ± 1.13**	7.11 ± 0.27$^{a, AB}$	7.00 ± 0.33$^{a, AB}$	7.28 ± 0.26$^{a, AB}$	**7.13 ± 0.26**
Lysine	4.04 ± 0.13$^{a, C}$	3.93 ± 0.10$^{a, C}$	4.25 ± 0.03$^{a, BC}$	**4.07 ± 0.17**	3.88 ± 0.05$^{b, C}$	4.58 ± 0.28$^{a, AB}$	**4.33 ± 0.55**	4.30 ± 0.10$^{a, BC}$	4.44 ± 0.23$^{a, ABC}$	4.93 ± 0.15$^{a, A}$	**4.56 ± 0.32**
Methionine	0.35 ± 0.01$^{c, E}$	0.78 ± 0.03$^{b, C}$	0.92 ± 0.01$^{a, B}$	**0.68 ± 0.27**	0.32 ± 0.01$^{b, E}$	0.43 ± 0.02^{a,D}	**0.37 ± 0.07**	0.94 ± 0.02$^{a, B}$	1.01 ± 0.01$^{a, A}$	0.41 ± 0.02$^{b, D}$	**0.79 ± 0.79**
Cysteine	0.40 ± 0.01$^{a, B}$	0.40 ± 0.02$^{a, B}$	0.45 ± 0.01$^{a, AB}$	**0.42 ± 0.03**	0.37 ± 0.01$^{b, B}$	0.49 ± 0.01^{a,A}	**0.44 ± 0.08**	0.51 ± 0.02$^{a, A}$	0.48 ± 0.03$^{a, A}$	0.52 ± 0.03$^{a, A}$	**0.50 ± 0.03**
Phenylalanine	3.45 ± 0.08$^{ab, AB}$	3.42 ± 0.11$^{b, AB}$	3.75 ± 0.03$^{a, AB}$	**3.54 ± 0.18**	3.19 ± 0.11$^{a, B}$	3.89 ± 0.25^{a,A}	**3.54 ± 0.43**	3.48 ± 0.15$^{a, AB}$	3.56 ± 0.18$^{a, AB}$	3.86 ± 0.16$^{a, A}$	**3.63 ± 0.22**
Tyrosine	2.44 ± 0.31$^{a, AB}$	2.20 ± 0.13$^{a, B}$	1.85 ± 0.04$^{a, BC}$	**2.16 ± 0.31**	1.87 ± 0.20$^{b, BC}$	2.86 ± 0.06^{a,A}	**2.36 ± 0.58**	1.38 ± 0.10$^{b, C}$	1.55 ± 0.02$^{ab, C}$	1.93 ± 0.14$^{a, BC}$	**1.62 ± 0.26**
Threonine	2.80 ± 0.06$^{a, AB}$	2.79 ± 0.13$^{a, AB}$	2.87 ± 0.11$^{a, AB}$	**2.82 ± 0.09**	2.390 ± 0.12$^{a, B}$	3.09 ± 0.27^{a,A}	**2.81 ± 0.52**	2.59 ± 0.12$^{a, B}$	2.73 ± 0.02$^{a, AB}$	2.92 ± 0.17$^{a, AB}$	**2.75 ± 0.17**
Tryptophan	0.35 ± 0.02$^{a, A}$	0.19 ± 0.08$^{a, A}$	0.22 ± 0.01$^{a, A}$	**0.25 ± 0.08**	0.39 ± 0.09$^{a, A}$	0.40 ± 0.04^{a,A}	**0.39 ± 0.06**	0.30 ± 0.10$^{a, A}$	0.37 ± 0.17$^{a, A}$	0.45 ± 0.02$^{a, A}$	**0.37 ± 0.11**
Valine	3.35 ± 0.07$^{b, BC}$	3.57 ± 0.09$^{ab, AB}$	3.87 ± 0.05$^{a, A}$	**3.60 ± 0.24**	2.97 ± 0.11$^{b, C}$	3.71 ± 0.20^{a,A}	**3.43 ± 0.54**	3.37 ± 0.12$^{a, BC}$	3.45 ± 0.14$^{a, AB}$	3.45 ± 0.09$^{a, AB}$	**3.42 ± 0.10**
Non-essential amino acid											
Alanine	2.74 ± 0.16$^{b, C}$	3.19 ± 0.02$^{a, AB}$	3.58 ± 0.03$^{a, A}$	**3.17 ± 0.38**	2.66 ± 0.01$^{b, C}$	3.05 ± 0.12$^{a, AB}$	**2.93 ± 0.32**	3.26 ± 0.11$^{a, AB}$	3.39 ± 0.15$^{a, AB}$	3.03 ± 0.08$^{a, BC}$	**3.23 ± 0.18**
Arginine	9.90 ± 0.07$^{a, BC}$	8.81 ± 0.24$^{b, CD}$	9.86 ± 0.16$^{a, BC}$	**9.52 ± 0.57**	8.49 ± 0.42$^{b, CD}$	10.17 ± 0.42$^{a, AB}$	**9.57 ± 1.29**	9.10 ± 0.55$^{b, BCD}$	8.14 ± 0.45$^{b, D}$	12.15 ± 0.55$^{a, A}$	**9.80 ± 1.92**
Aspartic Acid	9.28 ± 0.25$^{b, B}$	9.66 ± 0.19$^{ab, AB}$	10.10 ± 0.03$^{a, AB}$	**9.68 ± 0.39**	8.03 ± 0.18$^{b, C}$	10.16 ± 0.41$^{a, A}$	**9.33 ± 1.53**	9.10 ± 0.38$^{a, BC}$	8.99 ± 0.40$^{a, BC}$	9.73 ± 0.32$^{a, AB}$	**9.28 ± 0.46**
Glutamic Acid	19.91 ± 0.77$^{b, CD}$	20.87 ± 0.31$^{b, BCD}$	24.68 ± 0.09$^{a, A}$	**21.82 ± 2.29**	18.69 ± 0.52$^{b, D}$	22.07 ± 1.02$^{a, ABC}$	**20.89 ± 2.63**	23.66 ± 1.28$^{a, AB}$	22.91 ± 1.21$^{a, ABC}$	23.79 ± 0.74$^{a, AB}$	**23.45 ± 0.95**
Glycine	3.42 ± 0.10$^{b, BC}$	3.51 ± 0.03$^{b, BC}$	4.08 ± 0.03$^{a, A}$	**3.67 ± 0.33**	3.35 ± 0.05$^{b, C}$	3.88 ± 0.12$^{a, A}$	**3.71 ± 0.42**	3.63 ± 0.14$^{a, BC}$	3.69 ± 0.17$^{a, ABC}$	3.82 ± 0.14a, ^{AB}	**3.71 ± 0.15**
Proline	3.62 ± 0.05$^{c, CD}$	3.95 ± 0.09$^{b, ABC}$	4.34 ± 0.01$^{a, A}$	**3.97 ± 0.33**	3.33 ± 0.08$^{b, D}$	4.07 ± 0.19$^{a, AB}$	**3.79 ± 0.55**	3.71 ± 0.06$^{a, CD}$	3.75 ± 0.07$^{a, C}$	3.89 ± 0.15$^{a, BC}$	**3.78 ± 0.11**
Serine	4.02 ± 0.02$^{b, BC}$	4.19 ± 0.12$^{b, BC}$	4.56 ± 0.08$^{a, AB}$	**4.26 ± 0.26**	3.66 ± 0.09$^{b, C}$	4.57 ± 0.24$^{a, A}$	**4.22 ± 0.66**	4.10 ± 0.18$^{a, BC}$	4.07 ± 0.14$^{a, BC}$	4.41 ± 0.19$^{a, AB}$	**4.19 ± 0.21**
Nutritional values											
Σ EAA (g · 16 g^{-1} N)	29.33 ± 0.30$^{b, BC}$	29.83 ± 0.80$^{ab, ABC}$	31.98 ± 0.38$^{a, AB}$	**30.38 ± 1.33**	26.39 ± 0.84$^{b, C}$	32.68 ± 1.81$^{a, A}$	**30.28 ± 4.64**	30.04 ± 1.29$^{a, ABC}$	30.59 ± 1.46$^{a, ABC}$	32.17 ± 1.24$^{a, AB}$	**30.94 ± 1.43**
EAAI (%)	87.96 ± 1.24$^{b, AB}$	86.53 ± 1.59$^{b, AB}$	96.12 ± 0.89$^{a, AB}$	**90.20 ± 5.17**	81.81 ± 0.41$^{b, B}$	103.13 ± 3.70$^{a, A}$	**92.47 ± 15.07**	93.56 ± 6.89$^{a, AB}$	97.32 ± 8.71$^{a, AB}$	99.88 ± 3.94$^{a, A}$	**96.92 ± 3.18**
IVPD (%)	93.81 ± 3.97$^{a, A}$	88.38 ± 3.20$^{a, A}$	95.62 ± 2.94$^{a, A}$	**92.60 ± 4.28**	94.08 ± 0.26$^{a, A}$	92.45 ± 1.28$^{a, A}$	**93.26 ± 1.21**	93.62 ± 3.71$^{a, A}$	97.88 ± 2.05$^{a, A}$	91.09 ± 0.64$^{a, A}$	**94.20 ± 3.62**
LIAA	Met + Cys	Trp	Trp		Met + Cys	Met + Cys		Trp	Met + Cys	Met + Cys	
In vitro DIAAS (%)	30.35 ± 0.51^{a,B}	24.78 ± 10.07^{a,B}	32.24 ± 0.28^{a,B}		28.28 ± 0.71$^{b, B}$	37.94 ± 1.44$^{a, B}$		41.97 ± 14.04$^{a, AB}$	66.33 ± 1.71$^{a, A}$	37.04 ± 1.93$^{a, B}$	

CM, commercial mixture; EAA, essential amino acids; EAAI, essential amino acids index; IVPD, *In vitro* protein digestibility; LIAA, limiting indispensable amino acid; Met, methionine; Cys, cysteine; Trp, tryptophan; DIAAS, digestible indispensable amino acid score. $^{a-c}$ Denotes statistical differences within species ($p < 0.05$). A-E Denotes statistical differences between species ($p < 0.05$).

studies [6,39,77,78]. In another work, *L. mutabilis* also showed minimal variation across the studied cultivars [23]. Consistent with previous studies [6,23,39], *L. luteus* cultivars stood out for their higher cysteine content.

Significant variation was also noted in the total essential amino acid content (ΣEAA) among cultivars of the same species. *L. angustifolius* Giribita exhibited the highest ΣEAA (32.68 · 16 g^{-1} N) while *L. angustifolius* Carabor had the lowest (26.39 · 16 g^{-1} N). Based on FAO guidelines for adult amino acid requirements [39], lupin seeds are particularly rich in leucine (5.55–7.28 g · 16 g^{-1} N), lysine (3.88–4.93 g · 16 g^{-1} N) and isoleucine (3.39–4.23 g · 16 g^{-1} N). However, lupin seeds contained relatively low levels of sulphur-containing amino acids (methionine and cysteine), as well as tryptophan, which is consistent with previous studies [6,39,65,77,78].

Protein quality was assessed based on amino acid composition and digestibility. While *in vivo* experiments are the standard for determining protein digestibility, their cost and time constraints make the *in vitro* assays a practical alternative. This study employed an *in vitro* digestibility assay, the 3-enzyme method, previously shown to correlate well with *in vivo* data [40]. Across all analysed lupin species, protein digestibility exceeded 88 %, surpassing the values reported by Tinus et al. [37] for ground cowpea and those reported by Stone et al. [79] for various legume flours. This high digestibility enhances amino acids bioavailability, thereby improving the nutritional value of lupins as a protein source for human consumption. Two further parameters were considered to evaluate protein quality: EAAI and *in vitro* DIAAS. The EAAI values of the studied cultivars ranged from 81.8 % to 103 %, with *Lupinus angustifolius* Giribita and *Lupinus luteus* exhibiting the highest values (103 % and 99.8 %, respectively). According to FAO/WHO/UNU [80] standards, these cultivars contain high-quality protein. Domínguez et al. [77] observed slight variations in the EAAI profile of lupin PI obtained via wet extraction compared to lupin flour. Thus, *L. angustifolius* Giribita and L. *luteus* CM flours, represent a promising source of high-quality protein. Regarding the *in vitro* DIAAS, *L. luteus* Cardiga had the highest value among the studied cultivars. The *in vitro* DIAAS values ranged between 25 and 66 %, in agreement with previously published data on the *in vitro* protein digestibility-corrected amino acid score (PDCAAS) of legumes [79,81].

With regard to fatty acid composition, lupin cultivars showed significant differences (Table 5). The lipid fraction of lupin seeds generally contains a low proportion of saturated fatty acids (SFA). Palmitic acid (C16:0) was the most prevalent SFA across all varieties, accounting from 5.60 % to 12.10 % of the total fat content. The highest concentration of palmitic acid was found in *L. angustifolius* Giribita, while the lowest was in *L. luteus* Cardiga. The second most common SFA was stearic acid (C18:0) in *L. angustifolius* (5.14 %–5.58 %), and behenic acid (C22:0) in *L. albus* (3.50 %–4.19 %) and *L. luteus* (4.59 %–5.57 %) species.

Oleic acid (C18:1 n-9) was the predominant monounsaturated fatty acid (MUFA) in all cases. However, *L. albus* showed higher oleic acid contents than other lupin species which agrees with Chiofalo et al. [82] *L. albus* Estoril and Frieda had the highest levels of oleic acid (48.42 % and 48.49 %), while *L. luteus* Acos and Cardiga had the lowest content (23.14 % and 22.77 %). The second most abundant MUFA was gadoleic acid (C20:1 n-9), ranging from 4.21 % in *L. albus* Celina to 0.25 % in

Table 5
Concentration of main fatty acids (mean ± SD) in the studied lupin cultivars (g/100 g of total fatty acids).

	L. albus				*L. angustifolius*			*L. luteus*			
	Celina	Estoril	Frieda	Mean	Carabor	Giribita	Mean	Acos	Cardiga	CM	Mean
SFA											
C16:0	9.67 ± 0.00$^{a, C}$	9.70 ± 0.12$^{a, C}$	7.93 ± 0.03$^{b, D}$	**9.10 ± 0.91**	11.28 ± 0.05$^{b, B}$	12.10 ± 0.01$^{a, A}$	**11.63 ± 0.47**	6.20 ± 0.02$^{b, E}$	5.60 ± 0.05$^{c, F}$	7.82 ± 0.13$^{a, D}$	**6.54 ± 1.03**
C18:0	1.99 ± 0.01$^{a, EF}$	1.73 ± 0.03$^{b, G}$	2.13 ± 0.07$^{a, E}$	**1.95 ± 0.19**	5.58 ± 0.14$^{a, A}$	5.14 ± 0.00$^{b, B}$	**5.36 ± 0.27**	1.86 ± 0.00$^{c, FG}$	2.98 ± 0.00$^{b, D}$	3.58 ± 0.05$^{a, C}$	**2.81 ± 0.78**
C20:0	1.14 ± 0.00$^{a, D}$	0.88 ± 0.01$^{b, E}$	1.16 ± 0.02$^{a, D}$	**1.06 ± 0.14**	0.73 ± 0.01$^{a, F}$	0.71 ± 0.00$^{a, F}$	**0.72 ± 0.01**	1.63 ± 0.02$^{c, C}$	2.40 ± 0.00$^{b, B}$	2.85 ± 0.03$^{a, A}$	**2.29 ± 0.55**
C22:0	4.19 ± 0.01$^{a, D}$	3.50 ± 0.04$^{c, E}$	3.66 ± 0.04$^{b, E}$	**3.78 ± 0.32**	1.87 ± 0.00$^{a, F}$	1.81 ± 0.02$^{b, F}$	**1.84 ± 0.04**	4.85 ± 0.06$^{b, B}$	4.59 ± 0.04$^{b, C}$	5.57 ± 0.10$^{a, A}$	**5.00 ± 0.46**
C24:0	0.97 ± 0.00$^{a, A}$	1.06 ± 0.04$^{a, A}$	0.79 ± 0.01$^{b, B}$	**0.94 ± 0.13**	0.42 ± 0.01$^{b, D}$	0.51 ± 0.01$^{a, D}$	**0.47 ± 0.05**	0.79 ± 0.04$^{a, B}$	0.67 ± 0.02$^{b, C}$	0.69 ± 0.01$^{ab, C}$	**0.72 ± 0.06**
MUFA											
C16:1 n-7	0.50 ± 0.01$^{a, A}$	0.52 ± 0.02$^{a, A}$	0.36 ± 0.01$^{b, B}$	**0.46 ± 0.08**	0.067 ± 0.002$^{b, D}$	0.090 ± 0.003$^{a, CD}$	**0.078 ± 0.013**	0.10 ± 0.01$^{a, C}$	0.071 ± 0.005$^{a, CD}$	0.078 ± 0.010$^{a, CD}$	**0.083 ± 0.015**
C18:1 n-9	46.93 ± 0.22$^{b, B}$	48.42 ± 0.52$^{a, A}$	48.49 ± 0.23$^{a, A}$	**47.94 ± 0.83**	34.46 ± 0.05$^{a, C}$	29.63 ± 0.26$^{b, D}$	**32.05 ± 2.80**	23.14 ± 0.07$^{b, E}$	22.77 ± 0.14 b$^{, E}$	35.60 ± 0.59$^{a, C}$	**27.17 ± 6.54**
C20:1 n-9	4.21 ± 0.01$^{a, A}$	3.99 ± 0.09$^{a, B}$	3.99 ± 0.09$^{a, B}$	**4.06 ± 0.13**	0.25 ± 0.00$^{a, E}$	0.25 ± 0.00$^{a, E}$	**0.25 ± 0.00**	1.95 ± 0.00$^{a, C}$	1.65 ± 0.01$^{c, D}$	1.84 ± 0.02$^{b, C}$	**1.81 ± 0.14**
C22:1 n-9	1.95 ± 0.02$^{a, A}$	2.05 ± 0.04$^{a, A}$	1.41 ± 0.06$^{b, B}$	**1.81 ± 0.31**	0.025 ± 0.002$^{a, E}$	0.026 ± 0.002$^{a, E}$	**0.026 ± 0.002**	0.98 ± 0.03$^{a, C}$	0.69 ± 0.01$^{c, D}$	5.57 ± 0.10$^{b, D}$	**0.82 ± 0.14**
PUFA											
C18:2 n-6	16.73 ± 0.18$^{b, G}$	18.94 ± 0.20$^{a, F}$	18.19 ± 0.39$^{a, F}$	**17.95 ± 1.03**	38.95 ± 0.10$^{b, D}$	43.95 ± 0.08$^{a, C}$	**41.45 ± 2.89**	49.86 ± 0.26$^{a, A}$	48.74 ± 0.22$^{b, B}$	33.73 ± 0.15$^{c, E}$	**44.11 ± 8.06**
C20:2 n-6	0.29 ± 0.00$^{ab, AB}$	0.29 ± 0.01$^{a, A}$	0.25 ± 0.01$^{b, CD}$	**0.28 ± 0.02**	0.037 ± 0.001$^{a, F}$	0.042 ± 0.002$^{a, F}$	**0.039 ± 0.003**	0.26 ± 0.00$^{a, BC}$	0.24 ± 0.01$^{b, D}$	0.18 ± 0.01$^{c, E}$	**0.23 ± 0.04**
C18:3 n-3	10.54 ± 0.03$^{b, B}$	8.00 ± 0.15$^{c, D}$	10.95 ± 0.05$^{a, A}$	**9.83 ± 1.43**	5.50 ± 0.09$^{a, G}$	5.11 ± 0.05$^{b, H}$	**5.31 ± 0.23**	6.76 ± 0.00$^{b, E}$	8.61 ± 0.00$^{a, C}$	6.10 ± 0.03$^{c, F}$	**7.16 ± 1.16**
Nutritional values											
U/S	4.50 ± 0.01$^{c, D}$	4.86 ± 0.09$^{b, C}$	5.32 ± 0.03$^{a, A}$	**4.89 ± 0.37**	3.99 ± 0.02$^{a, E}$	3.90 ± 0.02$^{a, EF}$	**3.94 ± 0.05**	5.40 ± 0.05$^{a, A}$	5.08 ± 0.05$^{b, B}$	3.81 ± 0.05$^{c, F}$	**4.77 ± 0.76**
n-6/n-3	1.59 ± 0.01$^{b, F}$	2.37 ± 0.02$^{a, E}$	1.66 ± 0.04$^{b, F}$	**1.87 ± 0.39**	7.08 ± 0.13$^{b, C}$	8.59 ± 0.08$^{a, A}$	**7.88 ± 0.88**	7.37 ± 0.55$^{a, B}$	5.66 ± 0.44$^{b, D}$	5.53 ± 0.08$^{c, D}$	**6.19 ± 0.92**

CM, commercial mixture; MUFA, monosaturated fatty acids; n-6/n-3, omega 6/omega 3 ratio; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; U/S, unsaturated/saturated.
[a-c] Statistical differences within species ($p < 0.05$).
A-H Statistical differences between species ($p < 0.05$).

L. angustifolius cultivars.

In agreement with Chiofalo et al. [82], linoleic acid (C18:2 n-6) was the most prevalent polyunsaturated fatty acid (PUFA). The highest concentration was found in *L. luteus* Acos, while the lowest was in *L. albus* Celina. Alpha-linolenic acid (C18:3 n-3) was the second most common PUFA, with concentrations ranging from 10.95 % in *L. albus* Frieda to 5.11 % in *L. angustifolius* Giribita. These fatty acid contents are consistent with those reported by other authors [49,65,82]. In another work, *L. mutabilis* exhibited a fatty acid profile comparable to the studied cultivars of the present work, with palmitic acid as predominant SFA (9–11 %), oleic acid as the most abundant MUFA (42–54 %), and linoleic acid as the principal PUFA (23–34 %) [83]. In Western diets, the dietary omega-6 to omega-3 (n-6/n-3) ratio can be as high as 15:1, whereas the recommended ratio for preventing cardio-vascular diseases is 2:1 [49, 84]. The unsaturated to saturated (U/S) fatty acid ratio in lupin oil is higher than in many vegetable oils [84]. For instance, *L. albus* Frieda exhibited both a high U/S ratio and a n-6/n-3 ratio of 4.89 and 1.66, respectively, indicating a healthier fatty acid profile. Conversely, *L. angustifolius* Giribita has a lower U/S ratio and a higher n-6/n-3 ratio, suggesting a less favourable fatty acid profile.

3.3. Lupin protein isolates (LPI)

All cultivars were subjected to a standard alkaline extraction stage followed by isoelectric point precipitation to assess the protein extraction potential and characteristics of the obtained LPI. Table 6 shows the protein content and protein extraction yield of the LPI and the resulting insoluble fibre fraction (FF), derived from the protein extraction process. As expected, the obtained LPI had a high protein content ranging between 78.3 and 86.8 %. *L. luteus* Acos and CM, as well as *L. albus* Celina and Estoril, produced richer PI under the studied extraction conditions (86.8, 83.2, 84.8 and 84.4 %, respectively). The difference in protein purity may be linked to the presence of ions, carbohydrates, phytates or other small-molecular compounds that interact with proteins during the acid precipitation stage [85]. Larger differences are observed between the protein extraction yield values of the different lupin cultivars studied, ranging between 31.7 and 53.9 %. *L. angustifolius* Giribita and L. *albus* Estoril showed the highest protein extraction yields (53.9 and 47.0 %, respectively). Higher globulins/albumin ratio favours higher protein extraction yields after isoelectric precipitation [3]. Therefore, the differences in protein extraction yields can be explained by the different proportions of globulin/albumin in the studied cultivars as well as differences in proteins isoelectric points. In general, PI extracted from all cultivars had high purities and yields which agrees with similar studies [86]. It is worth noting that part of the seeds' protein content remains in the insoluble FF but apparently a bigger fraction is not recovered upon isoelectric precipitation. Therefore, a selective precipitation or the use of membranes may be advised for a higher recovery of soluble proteins.

The use of PI can be limited by its solubility and other techno-functional properties. Among them, colour can be crucial as it can determine many foods purchasing decisions. As for LPI colour properties, higher L* values denote lighter colours (Table 6). Among the studied cultivars, *L. albus* cultivars exhibited LPI with a higher lightness, particularly Estoril (66.4), which is significantly lighter than the other cultivars within this species. In contrast, *L. luteus* evaluated cultivars produced darker LPI, with Cardiga (47.1) and CM (47.3) being the darkest. *L. angustifolius* cultivars showed intermediate lightness, with

Table 6
Protein content and extraction yield of the lupin protein isolates (LPI) and fibre fractions (FF), and colour (CIE L*a*b*) of the protein isolates from the different cultivars (mean ± SD).

	LPI protein content (%; w/w)	LPI Protein extraction yield (%; w/w)	FF protein content (%; w/w)	FF Protein extraction yield (%; w/w)	LPI colour			
					L*	a*	b*	
Lupinus albus								
cv. Celina	84.8 ± 0.3$^{a, AB}$	36.6 ± 0.2$^{b, DE}$	12.0 ± 0.1$^{a, A}$	12.5 ± 0.4$^{a, A}$	55.6 ± 1.0$^{b, B}$	12.1 ± 0.5$^{a, A}$	33.2 ± 0.2$^{a, A}$	
cv. Estoril	84.4 ± 1.2$^{a, AB}$	47.0 ± 4.0$^{a, AB}$	10.9 ± 0.8$^{a, A}$	12.8 ± 1.4$^{a, A}$	66.4 ± 0.4$^{a, A}$	8.4 ± 0.2$^{c, D}$	31.4 ± 0.4$^{b, B}$	
cv. Frieda	78.3 ± 0.9$^{b, C}$	31.7 ± 1.4$^{b, E}$	11.0 ± 1.0$^{a, A}$	13.3 ± 1.7$^{a, A}$	57.1 ± 1.3$^{b, B}$	11.0 ± 0.5$^{b, B}$	33.5 ± 0.4$^{a, A}$	
Mean *L. albus*	**82.5 ± 3.3**	**38.5 ± 7.3**	**11.3 ± 0.8**	**12.9 ± 1.1**	**59.7 ± 5.0**	**10.5 ± 1.7**	**32.7 ± 1.0**	
Lupinus angustifolius								
cv. Carabor	81.0 ± 1.3$^{a, BC}$	42.8 ± 2.0$^{b, BCD}$	6.1 ± 0.0$^{a, B}$	7.9 ± 0.1$^{a, C}$	49.1 ± 1.3$^{b, CD}$	9.4 ± 0.3$^{b, C}$	28.6 ± 1.0$^{b, C}$	
cv. Giribita	81.5 ± 0.1$^{a, BC}$	53.9 ± 1.7$^{a, A}$	6.4 ± 0.3$^{a, B}$	8.8 ± 0.7$^{a, BC}$	57.1 ± 0.7$^{a, B}$	12.5 ± 0.4$^{a, A}$	33.3 ± 0.8$^{a, A}$	
Mean *L. angustifolius*	**81.3 ± 0.8**	**48.3 ± 6.6**	**6.2 ± 0.25**	**8.3 ± 0.7**	**53.1 ± 4.4**	**10.9 ± 1.7**	**31.0 ± 2.6**	
Lupinus luteus								
cv. Acos	86.8 ± 1.0$^{a, A}$	39.8 ± 1.5$^{b, BCD}$	11.4 ± 0.3$^{a, A}$	12.8 ± 0.6$^{a, A}$	50.8 ± 1.6$^{a, C}$	9.5 ± 0.3$^{b, C}$	25.5 ± 1.0$^{b, D}$	
cv. Cardiga	78.1 ± 0.5$^{b, C}$	44.3 ± 0.1$^{a, BC}$	7.8 ± 0.2$^{b, B}$	8.2 ± 0.5$^{b, C}$	47.1 ± 0.8$^{b, D}$	7.1 ± 0.5$^{c, E}$	21.6 ± 0.5$^{c, E}$	
cv. CM	83.2 ± 1.5$^{a, AB}$	39.3 ± 0.9$^{b, CD}$	11.1 ± 0.2$^{a, A}$	12.1 ± 0.1$^{a, AB}$	47.3 ± 0.6$^{b, D}$	12.1 ± 0.3$^{a, A}$	30.3 ± 0.9$^{a, BC}$	
Mean *L. luteus*	**82.7 ± 4.0**	**41.1 ± 2.6**	**10.1 ± 1.8**	**11.0 ± 2.2**	**48.4 ± 2.1**	**9.6 ± 2.2**	**25.8 ± 3.8**	

CM, commercial mixture; FF, fibre fraction; LPI, lupin protein isolate.
[a-c] Statistical differences within species ($p < 0.05$).
A-E Statistical differences between species ($p < 0.05$).

Giribita (57.1) being the lightest. During the alkaline extraction process, oxidation of phenolic compounds occurs, leading to a darker PI colour [87]. Furthermore, L* value of the different LPI obtained were correlated with the amount of TPC presented in the seeds. Notably, *L. luteus* Cardiga and CM had the highest TPC content and correspondingly displayed the lowest L* values. Redness intensity (a* values) revealed interesting differences across the species. *L. albus* cultivars, particularly Celina (12.1) and Frieda (11.0), exhibited the highest redness intensity. In *L. angustifolius* species, Giribita showed higher redness intensity (12.5) than Carabor (9.4). Regarding *L. luteus*, Cardiga (7.1) showed the lowest reddish values among all cultivars studied. However, the CM had high a* values across species and cultivars, thus underscoring the diversity of LPI pigmentation. These results are probably due to differences in carotenoids, but also in anthocyanin and other phenolics. Yellowness (b* values) indicated that both *L. albus* and *L. angustifolius* evaluated cultivars exhibited a tendency toward higher values. For instance, Celina (33.2) and. Giribita (33.3) had the highest yellow intensities. In contrast, *L. luteus* cultivars, particularly Cardiga (21.6), showed significantly lower yellow tones. LPI colour traits can be influenced by anthocyanin and carotenoid contents in seeds, which in turn are affected by genetic, environmental and agronomic factors [72]. Nevertheless, among the evaluated cultivars, those of *L. albus* can be regarded as suitable for most food applications in which the colour of the final product can be critical.

4. Conclusions

The chemical composition of eight European lupin cultivars highlights the existing variability in protein, total dietary fibre (TDF), fat, ash, and phytochemicals. Lupin seeds exhibited high protein content, comparable to soybean, and are notably higher than most legumes, suggesting their potential as a sustainable alternative in Europe for PI in food applications.

Among the studied cultivars, *L. albus* Celina stands out for its high protein content, making it a promising candidate for PI production. Additionally, the low-fat content of lupins facilitates protein extraction, providing a cost-effective and sustainable alternative to other legume-based proteins by eliminating the need for defatting processes. The amino acid profile further supports lupins as a valuable protein source, especially for plant-based diets. Moreover, the high dietary fibre content, particularly in studied *L. angustifolius* and *L. luteus* cultivars, position them as an ideal ingredient for fibre-enriched food products.

The study also identified the presence of phytochemicals, such as phenolic compounds, phytic acid, saponins, and QAs, which may pose both a challenge and an opportunity. Cultivars like *L. luteus* Cardiga (high in phenolics), *L. albus* Celina (rich in saponins), and *L. luteus Acos* (elevated phytic acid levels), may be valuable for functional food and nutraceutical applications. Furthermore, significant differences in QA content and profile were found among the studied cultivars. *L. angustifolius* Giribita exhibited the lowest QA levels, while *L. luteus* cultivars had the highest. Although QAs content can be influenced by environmental and agronomic factors, the distinct alkaloid profiles observed among the studied cultivars highlight the importance of cultivar selection for optimal valorisation in the protein isolation process.

Additionally, this study demonstrated that evaluated lupin cultivars PI can achieve high purity and protein extraction yield, with notable cultivar-specific differences in colour. *L. luteus* Acos showed the highest protein content in LPI, while *L. angustifolius* Giribita had the highest extraction yield, positioning them as optimal for PI production among the other studied cultivars.

However, given the diverse environmental conditions under which these crops were grown, these findings should be carefully interpreted. The observed variations among species and cultivars may, in part, reflect environmental influences on the studied parameters. Future research should further explore these interactions to optimize cultivar selection and enhance lupin's potential in food applications.

CRediT authorship contribution statement

Pau Taberner-Pibernat: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Albert Ribas-Agustí:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Gisela Quinteros:** Supervision, Methodology, Investigation. **Gerard Sabeña:** Supervision, Methodology, Investigation. **Miguel López-Gómez:** Writing – review & editing, Investigation, Conceptualization. **Rubén Domínguez-Valencia:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Ricard Bou:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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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.

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