Food Research International 230 (2026) 118563

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Multivariate Chemometric analysis of chemical diversity in French winter and spring *Lupinus albus* L. varieties

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ARTICLE INFO

*Keywords:*
Nutritional profiling
Functional ingredients
Chemometric analysis
Dietary applications

ABSTRACT

White lupin (*Lupinus albus* L.) is a nutrient-rich legume well-adapted to Mediterranean climates and tolerant of abiotic stress. Therefore, lupins represent a sustainable crop option with high potential for use in areas where climate change impacts are intensifying.

This study analysed eight cultivars, four winter and four spring ecotypes, to assess differences in nutritional and bioactive compounds. Key compounds studied included proteins, fats, carbohydrates, tocopherols, and phenolics. Spring ecotypes like 'Sulimo' and 'Energy' showed higher protein and tocopherol content, which may be ideal for protein-rich, antioxidant-rich diets. Winter ecotypes such as 'Angus' had more carbohydrates, especially fructose and raffinose, which could benefit energy and gut health. Both ecotypes showed high fibre levels, supporting digestive health. The findings suggest that ecotype influences chemical composition significantly, and each offers distinct benefits for targeted nutritional and functional food applications.

## 1. Introduction

Legumes are a rich source of essential nutrients for humans and animals, including protein, carbohydrates, and unsaturated fatty acids. They are commonly used in crop fields as rotation crops or intercrops to reduce disease, pest, or weed problems, thus improving productivity. In sustainable farming systems, they improve soil fertility by fixing atmospheric nitrogen (Valente et al., 2023).

Climate change is expected to worsen the impact of abiotic stresses, affecting crop productivity. In the case of legumes, the impact will be more significant because the soil microbial community will be modified, which will affect the symbiotic relationship of these organisms with their roots (Araújo et al., 2015). According to these latest predictions, the agricultural industry is moving towards climate-smart agriculture. This shift will enhance crop productivity and resilience, fulfilling the regional food security and development goals (Chandra et al., 2018; Dutta et al., 2022). Therefore, identifying the most suitable legume species and cultivars is crucial for maximizing grain productivity and protein production, especially in the Mediterranean region, where climate change has worsened due to stress events.

The *Lupinus* genus belongs to the Fabaceae family and is distributed worldwide, presenting different cultivated species, such as *L. albus, L. angustifolius, L. luteus* and *L. mutabilis*. Among them, white lupin (*Lupinus albus* L.) is a minor crop, considered one of the most important domesticated lupin species due to its high-protein grain content and utilization as a functional ingredient (Berger et al., 2017). Moreover, it is well-adapted to Mediterranean climatic conditions and soils and is more tolerant of abiotic stresses than other legume species, such as soybeans. Wild lupins accumulate high amounts of secondary metabolites, such as the quinolizidine alkaloid (QA) family, which are bitter and toxic to humans and farm animals (Osorio & Till, 2022). However, these levels are below the safe consumption limit of 200 mg/kg in the different commercial cultivars artificially selected during plant breeding (Mavromatis et al., 2023).

Minor crops are of great historical and cultural importance at the regional level, even with low marketability. They are grown on a small

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https://doi.org/10.1016/j.foodres.2026.118563
Received 8 November 2025; Received in revised form 5 January 2026; Accepted 1 February 2026
Available online 2 February 2026

scale, often positively promoting sustainable development, and preserving local agriculture biodiversity (Mattas et al., 2024). Crop diversification is one of the objectives to be reinforced by common European agricultural policies to achieve greater sustainability, production, profitability, and mitigation of climate effects (Peltonen-Sainio et al., 2016). Although lupins are considered a minor winter crop (sown in autumn and producing seeds in the early spring months), there are also spring ecotypes (they are not sensitive to vernalisation and their production is harvested in summer) (Annicchiarico et al., 2010). Thus, these species cultivars are an ideal candidate to obtain a high yield and be used as a rotation crop all year round. However, identifying traits economically beneficial for farmers and the food industry is essential to guide agronomic decision-making and the development of value-added crops.

Since winter and spring ecotypes are cultivated at distinct times of the year, they are exposed to different seasonal growth conditions, such as variable temperatures, light incidence and precipitation, which may result in genetic diversity over time and reflect in differences in their nutritional and bioactive profiles (Ferchichi et al., 2021). Therefore, this study aimed to perform a comprehensive chemical characterisation of eight L. *albus* ecotypes, including four winter and four spring ecotypes. Comparing the chemical composition and identifying compositional traits that may be associated with their ecotype classification will provide valuable data for future breeding programs focused on improving the most promising cultivars.

## 2. Material and methods

### 2.1. Plant material

*Lupinus albus* L. kernels of eight different local cultivars were provided by the French agronomic company Cérience. The sweet lupins were grown in 2021 at the research station of Cérience at Saint Sauvant, France (46°23′32.7"N 0°05′35.6″E).

Four of the eight cultivars studied, 'Angus', 'Magnus', 'Ulysse', and 'Orus', were winter ecotypes sown on the 24th of September 2020 and harvested on the 18th of July 2021. The remaining four—'Feodora', 'Sulimo', 'Figaro', and 'Energy'—were spring ecotypes sown on the 25th of February 2021 and harvested on the 25th of August 2021.

### 2.2. Chemical characterisation

#### 2.2.1. Nutritional characterisation

The nutritional profile of the samples was analysed following the official AOAC methodology (Latimer, 2023).

The moisture content (MC) was analysed following AOAC method 925.10. Protein content (PC) was calculated by the Macro-Kjedahl method, following the AOAC 920.87 method, using a conversion factor of 6.25. A Soxhlet apparatus was used to extract and quantify the crude fats (CF), using petroleum ether as an extracting solvent (AOAC 920.85). Total mineral content (TMC) was calculated following the AOAC 923.03. The dietary fibre (DF) was calculated using the AOAC procedure 993.19 through the enzymatic–gravimetric method. Total carbohydrate content (CC) was calculated by difference, according to the following equation:

$$CC = 100 - (MC + PC + CF + TMC + DF)$$

and therefore, includes digestible carbohydrates (such as starch and soluble sugars). Protein content, CF, TMC, DF, and CC were expressed as g/100 g of fresh weight (FW). Finally, the total energy (TE) was calculated using the European Parliament and Council Regulation No. 1169/2011 Formula (1):

$$\text{`Energy`}\ (\text{kcal}/100\ \text{g FW}) = 4 \times (PC + CC) + 2 \times (DF) + 9 \times (CF)$$

For detailed compositional purposes, the individual composition of soluble sugars (SS) were determined using a High-Performance Liquid Chromatography (HPLC) system coupled to a refraction index (RI detector Knauer Smartline 2300, Berlin, Germany) detector. Chromatographic separation was achieved with a Eurospher100–5 NH2 column (5 μm, 4.6, 250 mm, Knauer) at 35 °C. Isocratic elution contained acetonitrile/deionized water 70:30 (*v*/v) as the mobile phase, with a flow rate of 1 mL/min and injection volume of 10 μL of sample. The samples (1 g) were extracted in a hydroethanolic (80:20 v/v) solution during 30 min at 80 °C. Melezitose was added as an internal standard (IS). The extracts were centrifuged (15,000 *g*, 10 min) and the supernatants were collected and concentrated under reduced pressure in a rotatory evaporator. Finally, the samples were defatted in ethyl ether (three times) and filtered using 0.2 μm Whatman's nylon filters for HPLC analysis. The quantification was based on the RI signal response of each standard, depending on the IS method, and using calibration curves from commercial standards of each compound. The results were expressed in g/100 g of fresh weight (FW).

The lipidic fraction obtained previously in the crude fat analysis were subjected to a transesterification procedure that consisted of adding 5 mL of methanol:sulfuric acid:toluene solution (2:1:1, *v*/v/v) and letting incubate for 12 h at 50 °C and 160 rpm. After incubation, 3 mL of distilled water and 3 mL of diethyl ether were added to each sample and vigorously stirred using a vortex. The upper phase containing the fatty acid methyl esters (FAME) was removed, dehydrated with anhydrous sodium sulfate and filtered with 0.2 μm Whatman's nylon filters prior to their determination. Fatty acid (FA) analysis was performed via gas chromatography coupled to a flame ionisation detector (GC-FID), using a YOUNG IN Chromass 6500 GC System instrument equipped with a *split/splitless* injector at 250 °C and split injection at 1:80, FID at 260 °C and Zebron-Fame column (20 m × 0.18 mm × 0.15 μm df). The oven temperature program obeyed the following configuration: the initial column temperature was 80 °C, for 1.5 min; then, the temperature was increased at 40 °C/min to 160 °C, 5 °C/min to 185 °C, 30 °C/min to 260 °C for 4 min. The hydrogen (carrier gas) had a flow rate of 0.6 mL/min (0.61 bar), measured at 250 °C. For each analysis, 1 μL of the sample was injected. Identification was performed by comparing the relative retention times of FAME peaks from samples with commercial standards. The quantification was processed using the Clarity 4.0.1.7 software and expressed as a relative percentage of each fatty acid. The crude fat value for each sample was used to calculate the fatty acid concentration in g/100 g FW.

#### 2.2.2. Bioactive compounds characterisation

Tocopherol content was determined following a procedure previously described (Fernandes et al., 2011). Briefly, BHT and tocol solution (internal standard) were added to a lyophilized powder sample. The sample was homogenized twice with methanol and n-hexane, followed by an addition of saturated NaCl aqueous solution. After centrifugation, the clear supernatant was transferred into a vial, and the sample was re-extracted with n-hexane twice. The extracts were dried under a nitrogen stream, redissolved in n-hexane, filtered (0.2 μm nylon filters), and analysed using HPLC.

The High-Performance Liquid Chromatography (HPLC) (Knauer, Smartline system 1000, Berlin, Germany) was coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed at 290 nm for excitation and at 330 nm for emission. Hexane:ethyl acetate (70:30, *v*/v, hexane and ethyl acetate composed the mobile phase mixture, and the chromatographic separation was achieved in a Polyamide II column (250 × 4.6 mm, 5 μm; YMC, Kyoto, Japan). The quantification was performed by the internal standard (tocol) method. The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and express in mg/100 g FW.

Regarding phenolic compound determination, lyophilized powder samples were subjected to solid-liquid extraction in 80% aqueous ethanol by maceration. After filtering, liquid extracts were concentrated under reduced pressure using a rotatory evaporator, and their remaining aqueous phase was removed by lyophilization. The resulting dry extracts

**Table 1**
Nutritional profile, soluble sugars, and fatty acids of the different *Lupinus albus* cultivars.

| Cultivar | 'Angus' | 'Magnus' | 'Ulysse' | 'Orus' | 'Feodora' | 'Sulimo' | 'Figaro' | 'Energy' |
|---|---|---|---|---|---|---|---|---|
| **Ecotype** | **W** | **W** | **W** | **W** | **S** | **S** | **S** | **S** |
| **Moisture** | 4.48 ± 0.35 **cd** | 4.53 ± 0.27 **cd** | 4.3 ± 0.06 **d** | 5.06 ± 0.00 **b** | 5.39 ± 0.24 **b** | 5.16 ± 0.25 **b** | 4.93 ± 0.00 **bc** | 6.35 ± 0.19 **a** |
| **Crude Fats** | 3.74 ± 0.49 **bc** | 4.52 ± 0.06 **abc** | 4.24 ± 0.25 **abc** | 3.51 ± 0.12 **c** | 4.7 ± 0.27 **abc** | 5.01 ± 0.88 **ab** | 5.47 ± 0.68 **a** | 4.43 ± 0.49 **abc** |
| **Protein** | 25.51 ± 1.68 **b** | 24.97 ± 0.16 **bc** | 23.04 ± 0.02 **bc** | 22.52 ± 0.07 **c** | 24.6 ± 0.74 **bc** | 29.67 ± 1.61 **a** | 29.73 ± 1.64 **a** | 28.28 ± 0.45 **a** |
| **Ash** | 3.04 ± 0.04 **c** | 3.10 ± 0.09 **bc** | 3.33 ± 0.24 **ab** | 3.50 ± 0.02 **a** | 3.24 ± 0.04 **bc** | 3.18 ± 0.04 **bc** | 3.56 ± 0.06 **a** | 3.18 ± 0.02 **bc** |
| **Total carbohydrates** | 63.24 ± 2.48 **a** | 62.89 ± 0.28 **a** | 65.08 ± 0.54 **a** | 65.41 ± 0.02 **a** | 62.07 ± 0.28 **a** | 56.98 ± 1.03 **b** | 56.32 ± 2.38 **b** | 57.76 ± 0.77 **b** |
| ***Dietary fibre*** | 19.2 ± 0.5 **a** | 16.0 ± 0.8 **d** | 16.7 ± 0.9 **bcd** | 18.8 ± 0.3 **a** | 17.9 ± 0.3 **ab** | 16.1 ± 0.5 **cd** | 17.4 ± 0.6 **bc** | 17.4 ± 0.3 **bcd** |
| ***Total sugars*** | 3.71 ± 0.09 **de** | 3.63 ± 0.02 **e** | 4.29 ± 0.06 **a** | 4.21 ± 0.02 **ab** | 3.8 ± 0.05 **d** | 3.75 ± 0.04 **de** | 4.09 ± 0.05 **bc** | 3.99 ± 0.04 **c** |
| Fructose | 0.35 ± 0.02 **a** | 0.33 ± 0.01 **abc** | 0.35 ± 0.01 **a** | 0.32 ± 0.01 **abc** | 0.31 ± 0.01 **c** | 0.34 ± 0.01 **ab** | 0.32 ± 0.01 **abc** | 0.31 ± 0.01 **bc** |
| Glucose | 0.4 ± 0.02 **bc** | 0.41 ± 0.01 **bc** | 0.47 ± 0.01 **a** | 0.41 ± 0.01 **bc** | 0.43 ± 0.00 **b** | 0.39 ± 0.02 **c** | 0.4 ± 0.02 **bc** | 0.42 ± 0.01 **bc** |
| Sucrose | 2.6 ± 0.04 **e** | 2.56 ± 0.05 **e** | 3.17 ± 0.04 **ab** | 3.18 ± 0.01 **a** | 2.77 ± 0.04 **d** | 2.73 ± 0.05 **d** | 3.07 ± 0.06 **bc** | 2.99 ± 0.04 **c** |
| Raffinose | 0.35 ± 0.01 **a** | 0.33 ± 0.01 **b** | 0.30 ± 0.01 **c** | 0.30 ± 0.00 **cd** | 0.29 ± 0.01 **cd** | 0.29 ± 0.01 **cd** | 0.29 ± 0.01 **cd** | 0.28 ± 0.01 **d** |
| **Energy** | 350.21 ± 2.09 **bc** | 360.23 ± 2.60 **a** | 357.33 ± 1.78 **a** | 345.66 ± 0.19 **c** | 353.15 ± 0.10 **ab** | 359.42 ± 4.51 **a** | 358.56 ± 4.43 **a** | 349.34 ± 2.66 **bc** |
| ***Fatty acids*** | | | | | | | | |
| C14:0 [1] | 0.004 ± 0.001 **b** | 0.007 ± 0.001 **b** | 0.007 ± 0.002 **b** | 0.004 ± 0.000 **b** | 0.011 ± 0.003 **a** | 0.006 ± 0.000 **b** | 0.006 ± 0.001 **b** | 0.004 ± 0.001 **b** |
| C16:0 [2] | 0.35 ± 0.05 **de** | 0.53 ± 0.03 **abc** | 0.47 ± 0.06 **bcd** | 0.33 ± 0.01 **e** | 0.58 ± 0.02 **ab** | 0.56 ± 0.05 **ab** | 0.62 ± 0.09 **a** | 0.43 ± 0.05 **cde** |
| C16:1 [3] | 0.004 ± 0.001 | 0.007 ± 0.002 **ab** | 0.007 ± 0.001 **ab** | 0.004 ± 0.000 | 0.011 ± 0.004 **a** | 0.009 ± 0.001 **a** | 0.009 ± 0.001 **a** | 0.007 ± 0.001 **ab** |
| C18:1n9c [4] | 2.49 ± 0.32 **ab** | 2.95 ± 0.04 **ab** | 2.82 ± 0.15 **ab** | 2.22 ± 0.07 **b** | 2.63 ± 0.05 **ab** | 3.18 ± 0.54 **a** | 3.20 ± 0.40 **a** | 2.90 ± 0.33 **ab** |
| C18:2n6c [5] | 0.32 ± 0.04 **d** | 0.43 ± 0.01 **bc** | 0.32 ± 0.02 **d** | 0.35 ± 0.01 **cd** | 0.46 ± 0.01 **ab** | 0.40 ± 0.06 **bcd** | 0.54 ± 0.07 **a** | 0.35 ± 0.04 **cd** |
| C18:3n3 [6] | 0.14 ± 0.02 **b** | 0.19 ± 0.00 **ab** | 0.16 ± 0.01 **b** | 0.15 ± 0.00 **b** | 0.23 ± 0.06 **a** | 0.06 ± 0.01 **c** | 0.23 ± 0.03 **a** | 0.06 ± 0.01 **c** |
| C20:0 [7] | 0.06 ± 0.01 **b** | 0.06 ± 0.00 **b** | 0.06 ± 0.00 **b** | 0.06 ± 0.00 **b** | 0.12 ± 0.02 **a** | 0.10 ± 0.03 **ab** | 0.13 ± 0.01 **a** | 0.10 ± 0.01 **a** |
| C20:1 [8] | 0.10 ± 0.01 **d** | 0.11 ± 0.00 **cd** | 0.11 ± 0.00 **cd** | 0.11 ± 0.00 **cd** | 0.21 ± 0.07 **ab** | 0.19 ± 0.05 **abc** | 0.24 ± 0.03 **a** | 0.15 ± 0.02 **bcd** |
| C22:0 [9] | 0.18 ± 0.02 **d** | 0.16 ± 0.01 **d** | 0.15 ± 0.00 **d** | 0.20 ± 0.01 **cd** | 0.21 ± 0.02 **cd** | 0.27 ± 0.07 **bc** | 0.39 ± 0.04 **a** | 0.30 ± 0.03 **b** |
| C24:0 [10] | 0.025 ± 0.002 **nd** | 0.021 ± 0.002 **nd** | 0.020 ± 0.002 **nd** | 0.028 ± 0.003 **nd** | 0.026 ± 0.007 **nd** | 0.025 ± 0.010 **nd** | 0.030 ± 0.002 **nd** | 0.030 ± 0.003 **nd** |
| ***MUFA*** | 2.60 ± 0.34 **ab** | 3.07 ± 0.04 **ab** | 2.93 ± 0.15 **ab** | 2.33 ± 0.07 **b** | 2.85 ± 0.02 **ab** | 3.38 ± 0.59 **a** | 3.45 ± 0.43 **a** | 3.06 ± 0.34 **ab** |
| ***PUFA*** | 0.47 ± 0.06 **d** | 0.63 ± 0.01 **bc** | 0.48 ± 0.03 **cd** | 0.50 ± 0.02 **cd** | 0.69 ± 0.07 **ab** | 0.47 ± 0.08 **cd** | 0.78 ± 0.10 **a** | 0.42 ± 0.05 **d** |
| ***SFA*** | 0.67 ± 0.09 **b** | 0.83 ± 0.01 **b** | 0.83 ± 0.07 **b** | 0.68 ± 0.03 **b** | 1.15 ± 0.17 **a** | 1.15 ± 0.21 **a** | 1.22 ± 0.15 **a** | 0.96 ± 0.1 **ab** |

Results are expressed as mean ± standard deviation represented in g/100 g FW, except for energy which was represented in kcal/100 g FW. Statistical differences were assessed by one-way ANOVA, followed by Tukey's HSD post hoc test ($\alpha = 0.05$): lower-case letters indicate significant differences in each row. [1] myristic acid; [2] palmitic acid; [3] palmitoleic acid; [4] oleic acid; [5] linoleic acid; [6] α-linolenic acid; [7] arachidic acid; [8] docosanoic acid; [9] behenic acid; [10] lignoceric acid; MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

were re-dissolved in water and purified by a C-18 (EC) Telos® SPE cartridge (500 mg / 3 mL) previously activated with 5 mL of methanol followed by water. Sugars and other polar substances were removed by water washing (5 mL), while phenolic compounds were selectively eluted in 5 mL of methanol solution (Bastos et al., 2015). The methanolic fractions containing purified phenolic compounds were subsequently analysed using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA), equipped with a quaternary pump, an auto-sampler (kept at 5 °C), a degasser and an automated thermos-stated column compartment. Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column at 35 °C. The mobile phase was composed of (A) 0.1% formic acid in water, and (B) acetonitrile. The gradient elution for legume samples was: 0%–15% B (5 min), 15% B to 20% B (5 min), 20–25% B (10 min), 25–35% B (10 min), 35–50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 mL/min. The detection was carried out with a diode array detector (DAD) using 280 and 370 nm as the preferred wavelengths and connected in line with a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source and working in negative mode. For MS detection, nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325 °C, and a capillary voltage of −20 V. The tube lens offset was kept at a voltage of −66 V. The full scan covered the mass range from $m/z$ 100–1800. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with the Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The identification of phenolic compounds was carried out by comparing their retention times and UV–Vis spectra with those of available commercial standards. In cases where standards were not available, tentative identification was performed based on spectral characteristics and supporting information from the literature. Quantification was conducted using calibration curves constructed from the UV–Vis responses of the analogue's available phenolic standards HPLC grade from Sigma (> 95%): ferulic acid ($y = 633{,}126\times - 185{,}462$; $R^2 = 0.999$; Limit of detection, LOD =1.85 μg/mL; Limit of quantification, LOQ = 5.61 μg/mL), apigenin-7-O-glucoside ($y = 10{,}683\times - 45{,}794$; $R^2 = 0.996$; LOD = 13.70 μg/mL; LOQ = 41.50 μg/mL), and apigenin 6-C-glucoside ($y = 107{,}025\times + 61{,}531$; $R^2 = 0.9989$; LOD = 4.45 $\mu g/mL^{-1}$; LOQ = 13.49 μg/mL). The results were expressed in mg/100 g FW.

### 2.3. Statistical analysis

For each cultivar, lupins were collected in three independent paper bags, each harvested from different plants. Each bag was considered an independent biological replicate, and all analyses were performed using these biological replicates. All data are available in an open-access repository: https://doi.org/10.34620/dadosipb/YHMGVN. One-way ANOVA was used to determine significant differences between cultivars, followed by Tukey's HSD post hoc test. Before this, the homogeneity of variances was verified using Levene's test. These analyses were performed using SPSS statistics software (IBM SPSS Statistics v. 25., IBM

**Table 2**
Identification of phenolic acids (PCPA) and flavonoids (PCF) by HPLC-DAD-ESI/$MS^n$.

| Peak | RT (min) | $\lambda_{max}$ (nm) | [M-H]$^-$ | $MS^2$ | $MS^3$ | Tentative identification |
|---|---|---|---|---|---|---|
| PCPA1 | 12.22 | 223, 276 | 355 | 193 | 178, 149, 134 | Ferulic acid hexoside |
| PCF2 | 13.37 | 337 | 725 | 605, 455, 635, 689, 335, 575, 485, 353 | 473, 455, 545, 335, 409 | Apigenin-7-*O*-apiofuranosyl-6,8-di-*C*– glucoside |
| PCF3 | 14.00 | 337 | 593 | 473, 353, 383, 575, 503 | | Apigenin-6,8-di-*C*-glucoside |
| PCF4 | 14.63 | 336 | 739 | 619, 649, 455, 485, 383, 335 | 455, 335, 353, 383, 499 | Apigenin-6,8-di-*C*-glucoside-rhamnoside |
| PCF5 | 14.75 | 337 | 753 | 725, 633, 605, 575, 533 | 575, 605, 353, 473, 455,335 | Apigenin-pentoside-di-*C*-hexoside derivative |
| PCF6 | 16.30 | 335 | 753 | 707, 603, 635, 679, 587, 455, 383, 353 | 409, 455, 575 | Apigenin-pentoside-di-*C*-hexoside derivative |
| PCF7 | 18.01 | 336 | 753 | 605, 335, 473, 383, 587, 531, 707 | 455, 485, 383, 353, 335 | Apigenin-pentoside-di-*C*-hexoside derivative |
| PCF8 | 19.97 | 324 | 1063 | 605, 901, 751, 707, 725, 353, 635, 383, 725 | 455 | Hexoside of apigenin-feruloyl-6,8-di-*C*-hexoside-pentoside |
| PCF9 | 21.07 | 324 | 1063 | 901, 635, 605, 707, 725, 751, 383 | 707, 781, 631, 335 | Hexoside of apigenin-feruloyl-6,8-di-*C*-hexoside-pentoside isomer |

Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), mass spectral data ([M-H]$^-$), and fragment ions ($MS^2$ and $MS^3$). PCPA: Phenolic acids; PCF: Flavonoids.

Corp, Armonk, NY, USA). Chemometric multivariate analysis was conducted after converting the obtained data to g/100 g fresh weight. Data were standardised by subtracting the mean and dividing by the standard deviation of each variable. To better understand the generated models, dependent variables calculated using other dependent variables were eliminated (carbohydrates, total sugars, energy, MUFA, PUFA, and SFA). Differences between ecotypes ($n = 12$) were evaluated through partial least squares discriminant analysis (PLS-DA) of the chemical composition data using MetaboAnalyst 6.0, and fold changes between groups were visualized via a heatmap generated in GraphPad Prism 10.2.3.

## 3. Results and discussion

### 3.1. Chemical characterisation

Table 1 summarises the nutritional profile of the eight lupin varieties. All cultivars presented a high protein content, which aligns with previous research identifying lupin seeds as a valuable plant-based protein source (Shrestha et al., 2021). Notably, the spring ecotypes 'Sulimo', 'Figaro', and 'Energy' showed the highest protein concentrations.)

The fat content across all cultivars was found to be below 6%. Additionally, this fat composition was rich in beneficial unsaturated fatty acids. This low-fat concentration with a high content of unsaturated fatty acids is described as usual for other legumes, except soybeans (Grela & Günter, 1995; Iqbal et al., 2006). Furthermore, as previously described for lupins, the oleic acid was the major fatty acid among those identified (Uzun et al., 2007). Their higher protein content is ideal for dietary formulations to enhance protein intake, particularly in vegetarian or vegan diets. Their rich content of unsaturated fatty acids further contributes to heart-healthy diets, though their overall low-fat content limits their role in daily fat intake (Kapoor et al., 2021).

Regarding fibre content, the winter ecotypes 'Angus' and 'Orus' presented higher concentrations than other cultivars. Lupins are rich in fibre compared with other legumes, which enhances the functional value of these cultivars, especially in promoting gut health and improving digestion (Gullón et al., 2015). Finally, the sugar content was most notable in the winter cultivars 'Ulysse' and 'Orus'. Sugars are generally present in low concentrations in lupin and other legume seeds (Kaczmarska et al., 2017).

Table 2 presented the tentative identification of phenolic compounds in the different cultivars. A total of ten phenolic compounds were assigned, one phenolic acid and nine flavonoids (see Fig. 1.). Among phenolic acids, peak PCPA1 was assigned as ferulic acid hexoside, according to (Hellal et al., 2021). This compound was reported previously by Dueñas et al. (2009) and Zhong et al. (2019) in *L. angustifolius* and Hellal et al. (2021) in *L. albus* fractions.

The phenolic profile of lupin species previously described in the literature includes predominantly flavones, and isoflavones (Dueñas et al., 2009; Farag et al., 2019; Hellal et al., 2021; Tian et al., 2024; Zhong et al., 2019). Among these, flavones, specifically apigenin derivatives, have been described as prevalent substances in various lupin species. This agrees with our findings since peaks 2–9 were tentatively assigned as apigenin derivatives.

Compounds 2–9 consistently showed diagnostic fragment ions of apigenin-di-*C*-derivatives at *m/z* 383 (apigenin aglycone +113) and m/z 353 (apigenin aglycone +83) (Siger et al., 2012; Vukics & Guttman, 2010). Additionally, their fragmentation patterns showed neutral losses of 90 and 120 Da, indicative of the presence of apigenin-*C*-glycosides (Ferreres et al., 2007). Further neutral losses of 162 Da, 146 Da, and 150 Da were also observed, revealing the occurrence of *O*-hexoses, *O*-deoxyhexoses and pentose units (Vukics & Guttman, 2010).

Based on these data, peak PCF2 was assigned as apigenin-7-*O*-apiofuranosyl-6,8-di-*C*–glucoside, peak PCF3 was identified as apigenin-6,8-di-*C*-glucoside, and PCF4 as apigenin-6,8-di-*C*-glucoside-rhamnoside. Peaks PCF8 and PCF9 (isomers) were annotated as hexoside of apigenin-feruloyl-6,8-di-*C*-hexoside-pentoside due to minor fragments indicative of ferulic acid in the structure (neutral loss of 176 Da and 194 Da) (Kamel, 2003). Compounds PCF5, PCF6, and PCF7 also exhibited diagnostic fragment associated with apigenin-di-*C*-hexoside derivatives. However, assigning a structure to these molecules was impossible.

All the described compounds have been reported in previous research on lupin whole seeds (*L. hartwegii, L. termis, L. albus,* and *L. luteus*) and their presence was confirmed using nuclear magnetic

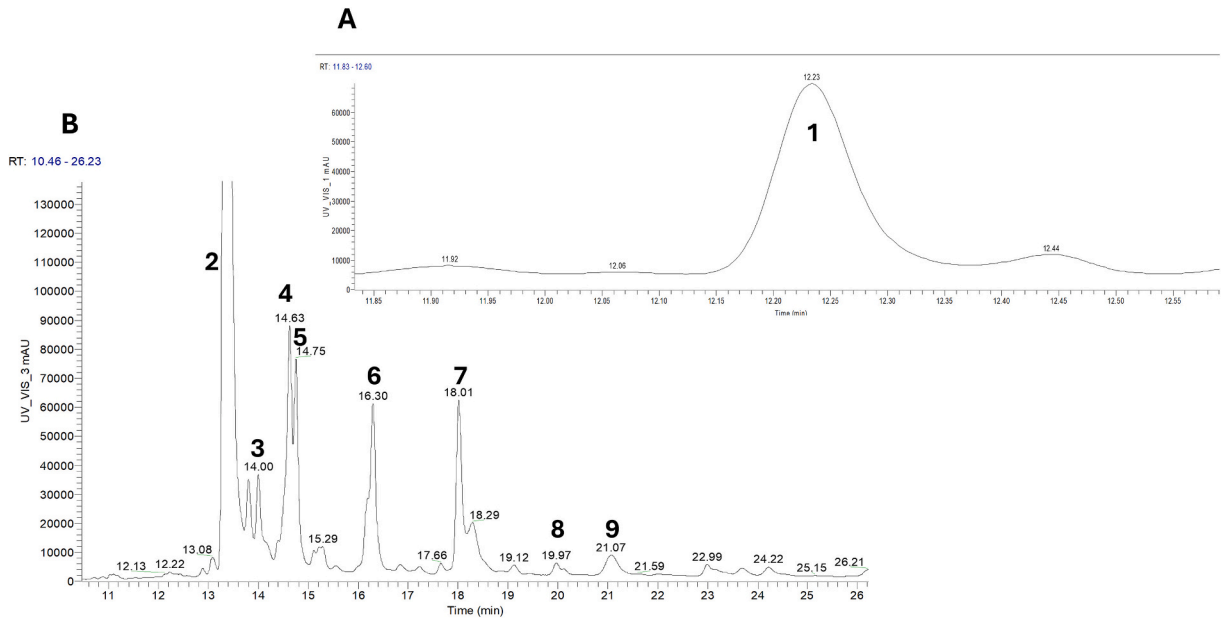


**Fig. 1.** HPLC profile of the ten tentatively identified phenolic compounds. A) UV: 280 nm; B) UV: 370 nm. Peak identification details in Table 2.

resonance (NMR) spectra in some studies (Dueñas et al., 2009; Elbandy & Rho, 2014; Farag et al., 2019; Kamel, 2003; Siger et al., 2012; Zhong et al., 2019). In contrast to our results, other phenolic acids than ferulic acid hexoside have been reported in lupin species, including *p*-coumaric, caffeic, protocatechuic acids and their glycoside counterparts, as well as other flavonoids, including quercetin and luteolin derivatives (Dueñas et al., 2009; Siger et al., 2012; Zhong et al., 2019). Additionally, previous research have registered the presence of isoflavones in lupin species (Wojakowska et al., 2013), but they could not be detected in the present study.

The differences observed between our results and previous works could be attributed to several factors. Indeed, a study performed by (Ranilla et al., 2009) found no isoflavones in L. *albus* seed coats, cotyledons, and hypocotyls. Furthermore, literature data indicate that in lupin plants the content of isoflavones is affected by the plant organ and development stage, being negligible in seeds and high in leaves and roots(D'Agostina et al., 2008). Different extraction techniques and extraction solvents utilised can also affect the extraction of phenolic compounds. For example, some studies have applied acidified solvent mixtures, using hydrochloric acid or acetic acid, for phenolics recovery from lupin species (Dueñas et al., 2009; Tian et al., 2024). Further factors contributing to modifying the phytochemical composition may include different lupin species, cultivars and plants' growth conditions.

PCF2 (apigenin-7-*O*-apiofuranosyl-6,8-di-*C*–glucoside) was the compound found in the highest concentration in all cultivars. These results were in concordance with other investigations on *Lupinus* species, where the major compounds found were apigenin derivatives, with PCF2 also being identified in the highest concentration (Siger et al., 2012; Tian et al., 2024; Zhong et al., 2019). In addition, in these works, phenolic acids also appear as minor compounds compared with flavonoids. The 'Feodora' spring ecotype showed the highest concentration of phenolic compounds, also exhibiting the highest concentration of the

**Table 3**
Quantification of tocopherols and phenolic compounds of the different *Lupinus albus* cultivars.

| Cultivar | 'Angus' | 'Magnus' | 'Ulysse' | 'Orus' | 'Feodora' | 'Sulimo' | 'Figaro' | 'Energy' |
|---|---|---|---|---|---|---|---|---|
| **Ecotype** | **W** | **W** | **W** | **W** | **S** | **S** | **S** | **S** |
| ***Tocopherols*** | | | | | | | | |
| α-tocopherol | 0.032 ± 0.005 **c** | 0.057 ± 0.002 **ab** | 0.051 ± 0.003 **b** | 0.063 ± 0.005 **ab** | 0.067 ± 0.010 **a** | 0.063 ± 0.001 **ab** | 0.065 ± 0.001 **a** | 0.070 ± 0.006 **a** |
| γ -tocopherol | 3.39 ± 0.51 **e** | 6.18 ± 0.26 **bc** | 4.67 ± 0.10 **d** | 5.01 ± 0.42 **cd** | 6.51 ± 1.17 **ab** | 5.56 ± 0.06 **bcd** | 7.49 ± 0.21 **a** | 5.98 ± 0.24 **bc** |
| δ -tocopherol | 0.054 ± 0.015 **c** | 0.112 ± 0.014 **a** | 0.066 ± 0.005 **bc** | 0.106 ± 0.019 **a** | 0.041 ± 0.005 **c** | 0.050 ± 0.003 **c** | 0.089 ± 0.004 **ab** | 0.060 ± 0.013 **c** |
| ***Phenolic compounds*** | | | | | | | | |
| PCPA1 | 1.10 ± 0.01 **d** | 1.64 ± 0.05 **c** | 1.59 ± 0.07 **c** | 2.12 ± 0.08 **a** | 1.55 ± 0.06 **c** | 1.94 ± 0.07 **b** | 1.16 ± 0.02 **d** | 1.58 ± 0.03 **c** |
| PCF2 | 101.6 ± 0.4 **e** | 115.4 ± 0.9 **d** | 157.7 ± 0.1 **c** | 116.8 ± 0.5 **d** | 305.1 ± 2.0 **a** | 230.5 ± 2.7 **b** | 90.6 ± 3.3 **f** | 154.5 ± 0.7 **c** |
| PCF3 | 0.72 ± 0.10 **e** | 0.91 ± 0.07 **e** | 1.79 ± 0.34 **d** | 0.78 ± 0.07 **e** | 4.17 ± 0.37 **a** | 3.16 ± 0.29 **b** | 2.40 ± 0.07 **c** | 2.13 ± 0.08 **cd** |
| PCF4 | 4.14 ± 0.04 **e** | 4.49 ± 0.04 **e** | 7.75 ± 0.11 **cd** | 6.68 ± 2.33 **d** | 21.28 ± 0.18 **a** | 14.88 ± 0.40 **b** | 7.16 ± 0.33 **cd** | 9.18 ± 0.19 **c** |
| PCF5 | 4.03 ± 0.02 **e** | 4.37 ± 0.05 **de** | 7.75 ± 0.11 **c** | 5.91 ± 1.79 **d** | 12.56 ± 0.06 **b** | 6.07 ± 0.50 **d** | 15.81 ± 0.61 **a** | 12.11 ± 0.12 **b** |
| PCF6 | 2.93 ± 0.02 **d** | 3.41 ± 0.02 **cd** | 0.59 ± 0.11 **e** | 5.01 ± 1.62 **b** | 8.84 ± 0.17 **a** | 4.57 ± 0.21 **bc** | 8.56 ± 0.29 **a** | 8.22 ± 0.25 **a** |
| PCF7 | 4.19 ± 0.03 **d** | 4.40 ± 0.12 **d** | 0.37 ± 0.05 **e** | 6.24 ± 2.12 **c** | 13.71 ± 0.08 **b** | 6.60 ± 0.24 **c** | 15.91 ± 0.32 **a** | 12.96 ± 0.13 **b** |
| PCF8 | 1.33 ± 0.02 **a** | 1.51 ± 0.16 **a** | 0.71 ± 0.03 **b** | 1.59 ± 0.28 **a** | 0.80 ± 0.28 **b** | 0.46 ± 0.01 **bc** | 0.59 ± 0.15 **bc** | 0.25 ± 0.05 **c** |
| PCF9 | 2.76 ± 0.01 **c** | 3.13 ± 0.08 **b** | 3.39 ± 0.22 **b** | 3.21 ± 0.03 **b** | 4.35 ± 0.19 **a** | 1.74 ± 0.04 **e** | 0.93 ± 0.17 **f** | 2.25 ± 0.10 **d** |
| Total phenolic acids | 1.1 ± 0.01 **d** | 1.55 ± 0.06 **c** | 1.94 ± 0.07 **b** | 1.58 ± 0.03 **c** | 1.64 ± 0.05 **c** | 1.59 ± 0.07 **c** | 1.16 ± 0.02 **d** | 2.12 ± 0.08 **a** |
| Total flavonoids | 121.7 ± 0.4 **f** | 137.6 ± 1.4 **e** | 180.1 ± 0.3 **d** | 146.2 ± 7.6 **e** | 370.8 ± 1.7 **a** | 268.0 ± 4.3 **b** | 142.0 ± 3.9 **e** | 201.6 ± 0.7 **c** |

Results are expressed as mean ± standard deviation represented in mg/100 g FW. Statistical differences were assessed by one-way ANOVA, followed by Tukey's HSD post hoc test (α = 0.05): lower-case letters indicate significant differences in each row. PCPA: Phenolic acids; PCF: Flavonoids. Phenolic compound abbreviations used in this table are explained in Table 2.

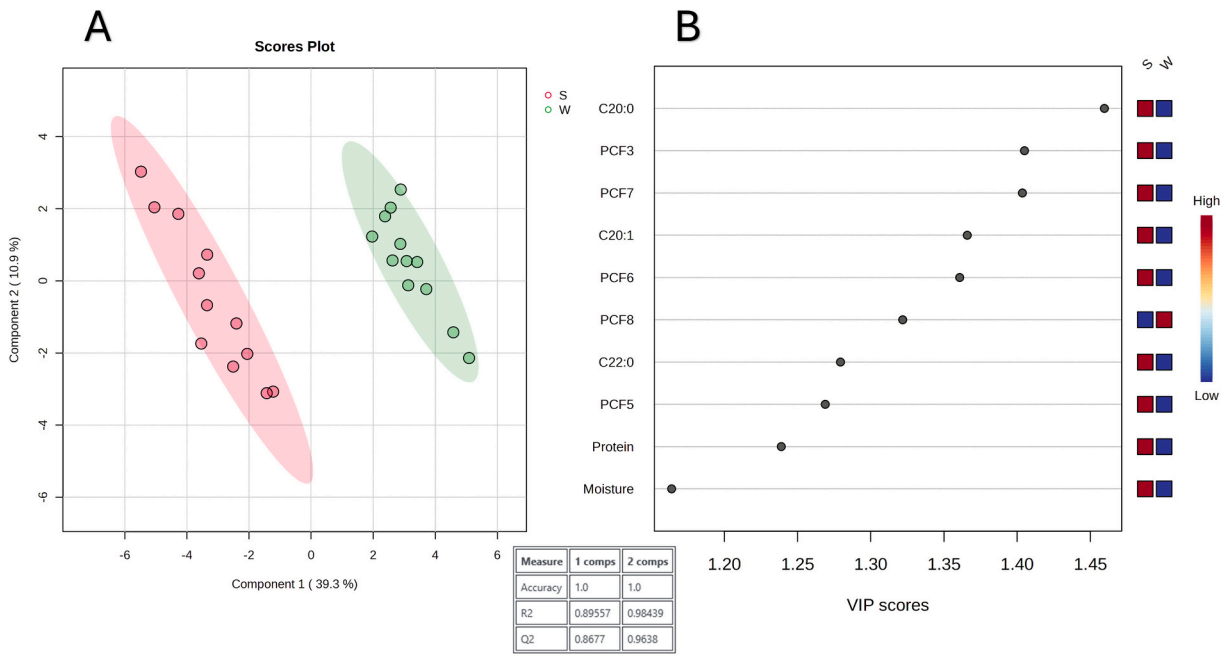


**Fig. 2.** (A) PLS-DA score plot illustrating the discrimination between Spring (red) and Winter (green) ecotypes based on their chemical composition, including the model $R^2$ and $Q^2$ values. (B) PLS-DA VIP-scores indicate the most discriminating nutritional and bioactive compounds between ecotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compound PCF2, three times higher than the 'Figaro' cultivar, which showed the lowest concentration.

The elevated levels of flavonoids, such as apigenin derivatives, showed their potential for providing significant bioactive properties. The cardioprotective role of apigenin has been studied in several experimental studies to assess the impact of its supplementation. In both clinical and preclinical trials, it has shown efficacy in alleviating hypertension, diabetes, dyslipidaemia, obesity, and metabolic syndrome, which highly predispose to cardiovascular diseases (Tomou et al., 2023).

Table 3 showed the quantification results of the phenolic compounds and vitamin E isomers (tocopherols). Three tocopherol isomers—alpha-, gamma-, and delta-tocopherol—were identified, with gamma-tocopherol being the most abundant across all cultivars, especially in 'Figaro'. This finding aligns with previous research that has noted the dominance of gamma-tocopherol in lupin seeds, contributing to their antioxidant properties (Berru et al., 2021; Lampart-Szczapa et al., 2003).

### 3.2. Multivariate analysis

A PLS-DA was performed to reduce the number of study variables and better identify the most differentiating compounds. The generated model explained 50.2% of the total variability and showed two well-separated clusters between the two ecotypes (Fig. 2A). Additionally, the model showed strong explanatory and predictive performance, with $R^2$ and $Q^2$ values of 0.98 and 0.96, respectively, indicating a robust discrimination between ecotypes. Based on the VIP score value (Fig. 2B), the most discriminant compounds were found to be arachidic and docosanoic fatty acids, and the phenolic compounds PCF3, PCF6 and PCF7, which were present in higher concentrations in the spring ecotypes. PCF9 presented the highest VIP score and was most concentrated in the winter ecotypes.

A heatmap was performed to obtain a better visualization of the fold changes between the analysed groups. In the generated fold-change heatmap, some of the trends observed in the cultivars were confirmed, as shown in Fig. 3. The winter ecotype cultivars presented a higher concentration of carbohydrates, such as fructose and raffinose. Regarding bioactive compounds, delta-tocopherol and PCF8 were significantly associated with winter ecotypes. Spring ecotypes showed higher concentration of proteins, crude fats, and several saturated and unsaturated fatty acids. Additionally, alpha and gamma tocopherol, and apigenin derivatives (from PCF2 to PCF7) were higher in this ecotype.

Since the studied ecotypes were grown at the same experimental station, the effect of geographic origin on nutritional and bioactive composition can be considered negligible. The observed differences could be attributed to genetic and/or environmental factors which occur during their growing season due to the distinct sowing dates. Ferchichi et al. (2021) found that the nutritional and bioactive composition of Tunisian ecotypes of L. *luteus*, L. *angustifolius* and *L. albus* varied significantly when grown in the same country under the same conditions, suggesting that variations could be fixed genetically. On the other hand, differences in environmental conditions may generate diversity between ecotypes chemical composition, as suggested in previous studies (Ferchichi et al., 2021; Piergiovanni et al., 2011).

Tocopherols play a crucial role in the low-temperature adaptation of some species; nevertheless, the warm temperatures could increase the alpha isomers during grain development in legumes (Britz & Kremer, 2002; Maeda et al., 2006). The presence of high concentration of carbohydrates, including fructose and raffinose in winter ecotypes, could suggest that the colder growing conditions may induce their accumulation as a protective mechanism (Bourion et al., 2003). Indeed, the accumulation of soluble sugars in seeds subjected to cold stress has been reported in other legume species, as well as a higher accumulation of these compounds in cold-tolerant chickpea genotypes (Kumar et al., 2010; Nayyar et al., 2005). These changes have been associated with increased expression of C-repeat binding factor (CBF), cold-regulated (COR), and late embryogenesis abundant (LEA) genes, which are involved in the accumulation of sugars acting as osmolytes in plant tissues(Thapa et al., 2025). In addition, the upregulation of CBF, COR, and LEA genes has been linked to modifications in cell wall composition, which may also help explain the higher fibre content observed in the winter ecotypes 'Angus' and 'Orus' compared to the other cultivars. Additionally, winter ecotypes generally showed lower flavonoid concentrations, which may reflect a trade-off in carbon allocation between primary and secondary metabolism. Under cold or seasonal stress conditions, plants often prioritise the accumulation of soluble carbohydrates, which function as osmolytes and cryoprotectants, as well as

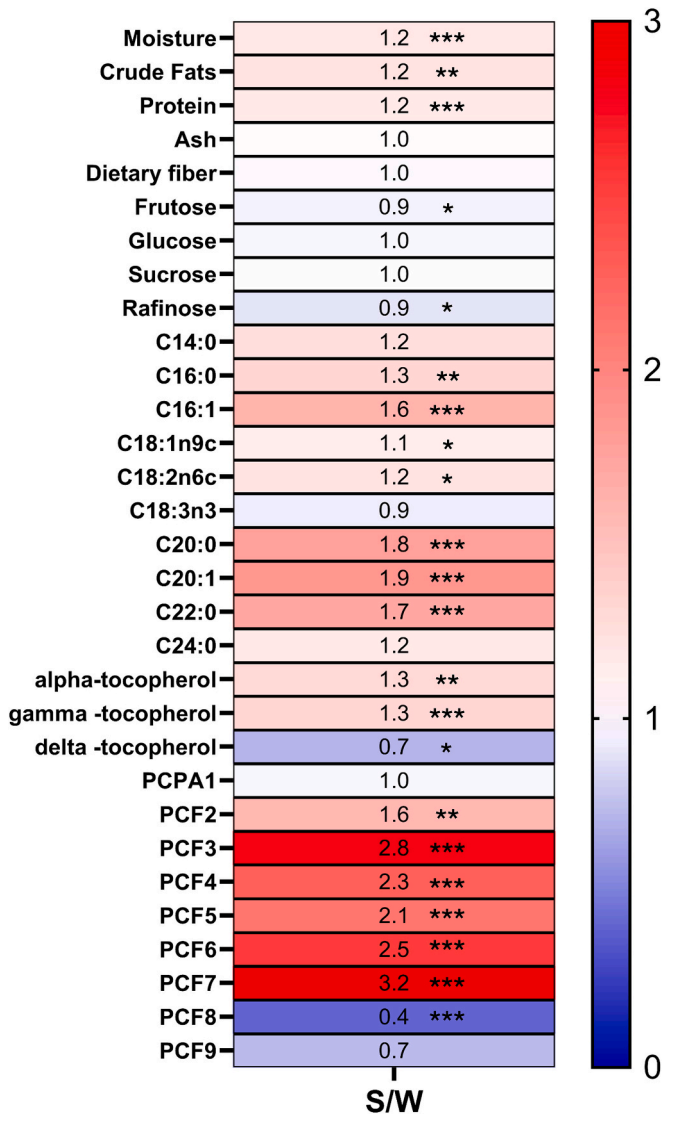


**Fig. 3.** Heat-map of foldchange for the nutritional and bioactive compounds of different ecotype studied (W: Winter; S: Spring). Asterisks mean significant differences between ecotypes by *t*-test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

structural polysaccharides that reinforce cell walls(Morin et al., 2022). This shift may occur at the expense of secondary metabolite biosynthesis. In fact, recent research found that flavonoid metabolism plays distinct roles in the early and late stages of plant cold acclimation, and its deficiency affects pyruvate, citrate and glutamate metabolism, indicating a role in stabilising C/N metabolism (Kitashova et al., 2024).

## 4. Conclusions

This study highlights compositional differences between winter and spring ecotypes of *Lupinus albus*, reflecting distinct nutritional and bioactive profiles. Winter cultivars showed higher carbohydrate levels, particularly slower-digesting sugars and fibres. Spring cultivars were characterized by higher protein and fat contents, as well as increased levels of unsaturated fatty acids, tocopherols, and apigenin derivatives, indicating their potential relevance for protein-rich and nutritionally balanced food applications. Overall, these findings demonstrate that winter and spring lupin ecotypes differ in their chemical composition, which may inform cultivar selection for targeted food applications and crop diversification strategies.

## CRediT authorship contribution statement

**Manuel Ayuso:** Writing – original draft, Investigation, Formal analysis, Data curation. **Tayse F.F. da Silveira:** Writing – original draft, Methodology, Investigation, Formal analysis. **Daiana Almeida:** Writing – original draft, Formal analysis. **Natalie Harzic:** Formal analysis, Conceptualization. **Valérie Gagnaire:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Ingrid Aguiló-Aguayo:** Writing – review & editing, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lillian Barros:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation, 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.

## Acknowledgments

The authors are thankful to the LOCALNUTLEG project (PRIMA programme, Call 2020, Section 1 2021 Agrofood Value Chain topic 1.3.1.). Additionally, this work was also supported by national funds through FCT/MCTES (PIDDAC): CIMO, UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); and national funding by FCT, P.I., through the institutional scientific employment program contract for L. Barros (CEEC-INST, DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002). T. Siveira thanks to the Agenda VIIAFOOD - Platform for Valorization, Industrialization and Commercial Innovation for Agrifood (no. C644929456-00000040), a project supported under the PRR (www.recuperarportugal.gov.pt) and financed by the European Union /Next Generation EU, for her contract. Manuel Ayuso acknowledges the contract as part of grant JDC2022-049135-I, funded by MCIU/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

## Data availability

Data will be made available on request.

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