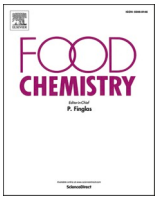
Food Chemistry 442 (2024) 138411

Contents lists available at ScienceDirect

Food Chemistry

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



Flavonoid diversity in bitter and debittered seeds of Andean lupin (*Lupinus mutabilis* Sweet)

Ye Tian [a,1], Paola Cortés-Avendaño [b,1], Baoru Yang [a], Patricia Glorio-Paulet [c], Ritva Repo-Carrasco-Valencia [d], Jukka-Pekka Suomela [a,*]

[a] Food Sciences, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland
[b] Facultad de Industrias Alimentarias, Innovative Technology, Food and Health Research Group, Universidad Nacional Agraria La Molina-UNALM, Av. La Molina s/n, Lima, Peru
[c] Food Chemistry and Biochemistry Associated with Food Safety and Stability Group, Instituto de Investigación de Bioquímica y Biología Molecular (UNALM-IIBBM), Av. La Molina s/n, Lima, Peru
[d] Centro de Investigación e Innovación en Productos Derivados de Cultivos Andinos (CIINCA), Universidad Nacional Agraria La Molina-UNALM, Av. La Molina s/n, Lima, Peru

ARTICLE INFO

Keywords:
Lupinus mutabilis Sweet
Seed
Ecotype
Aqueous debittering treatment
Isoflavone
Flavone

ABSTRACT

Seeds of ten Andean lupin (*Lupinus mutabilis* Sweet) ecotypes were collected from different regions of Peru and treated with an aqueous debittering method. Both untreated and treated seeds were analyzed by using LC-MS to investigate flavonoid profiles of different ecotypes and impact of debittering process on these compounds. Thirteen isoflavones (mainly as glycosides of genistein and methoxy-genistein) and eight flavones (glycosylated apigenins and methyl-luteolins) were characterized as the main flavonoids in the seed samples. The untreated lupin seeds contained 187–252 mg/100 g (dry weight) of flavonoids. The main difference among lupin ecotypes was observed in the levels of genistein-malonylhexoside, methoxy-genistein-malonylhexoside, and methyl-luteolin-malonylhexoside. After the debittering treatment, the total flavonoid content in the seeds was decreased to 125–203 mg/100 g dry weight, the aglycones of genistein, methoxy-genistein, and methyl-luteolin being the key distinguishing compounds of ecotypes. The aqueous treatment was effective in degrading flavonoid glycosides and releasing the corresponding aglycones.

1. Introduction

As a native legume in Europe and South America, lupin (*Lupinus* spp.) is a promising source of plant-based proteins. The seed of lupin has been considered as an important alternative to soybean, since it has a high protein content (33–55 % of dry seeds, mostly as globulins) and its proteins have similar properties as soybean proteins (size, appearance, and protein composition) (Lo et al., 2021). Lupin is rich in lysine but lacks cysteine and methionine. This amino acid profile is an ideal complement to cereals (high in cysteine and methionine, low in lysine), which improves the protein quality in vegetarian diet (Shrestha et al., 2021). Besides these macronutrients, bioactive compounds, such as flavonoids, phytosterols, tocopherols, and triterpenes, are also abundant in lupins, which may provide therapeutic health benefits due to their anti-oxidative, anti-inflammatory, anti-bacterial, and anti-carcinogenic effects (Arnoldi et al., 2015; Khan et al., 2015).

L. albus (white lupin), *L. luteus* (yellow lupin), *L. angustifolius* (narrow-leafed or blue lupin) and *L. mutabilis* (Andean lupin) are four species commonly cultivated nowadays (Lucas et al., 2015). In comparison with the other three species, *L. mutabilis* Sweet (also called “tarwi” or “chocho”) is cultivated mostly in the Andean region of South America. Its seeds are rich in proteins, but the alkaloid content in *L. mutabilis* is also higher than that detected in other species (Carvajal-Larenas et al., 2016; Gulisano et al., 2019). Alkaloids in lupins (mostly as quinolizidine alkaloids) provide a bitter taste and more importantly, exert acute toxicity. As observed in many cases, the patients who accidentally ate unripe/un-debittered lupin seeds suffered from neurological (such as weakness, dizziness, mydriasis, anxiety, confusion, malaise, loss of coordination, visual disturbances, or dry mouth), cardiovascular (dysrhythmias) and gastrointestinal (nausea or vomiting) symptoms (Koleva

* Corresponding author.
E-mail address: jusuom@utu.fi (J.-P. Suomela).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2024.138411
Received 5 October 2023; Received in revised form 5 January 2024; Accepted 8 January 2024

et al., 2012). Therefore, in the Andean region, a traditional water debittering process is commonly used before consumption of *L. mutabilis* to reduce toxic alkaloid levels in seeds. The treatment using cold or warm water for several hours can largely remove alkaloids from the seeds (Carvajal-Larenas et al., 2013). Further processing of debittered seeds as drying, milling, or extrusion is applied to develop more suitable ingredients of high nutritional value for modern food industry (Córdova-Ramos et al., 2020).

Aside from proteins and alkaloids, flavonoids are also key components affecting the application of *L. mutabilis* Sweet in the food industry. These compounds have positive effects on cardiovascular health (Ciumărnean et al., 2020), but they may also possess certain disadvantages in food products. For example, apigenin *C*-glycosides could interact with lupin globulin proteins and form flavonoid–protein complex, which might impair digestibility of lupin proteins (Czubinski et al., 2012). Genistein conferred the bitter taste to the foods by activating human bitter taste receptors (Roland et al., 2011). Monitoring the total quantity of flavonoids, as well as the changes in their composition, will assist food industry to choose optimal *L. mutabilis* seeds for developing novel foods. Nevertheless, up to date, only a few studies have suggested that aqueous debittering process can alter flavonoid composition in *L. mutabilis* seeds, and the variation in flavonoid profile may also be associated with the geographic locations where lupins are cultivated (Brandolini et al., 2022). In the current research of this field, a thorough determination of flavonoid profile in *L. mutabilis* seeds is often missing. As lacking accurate characterization of these compounds, the impacts of geographic areas and debittering process remain unclear.

Therefore, in the present study, we collected the seeds of ten ecotypes of *L. mutabilis* Sweet that were cultivated in Southern, Central, and Northern Peru. The aim of our study was to investigate the diversity of flavonoids among the seeds of different ecotypes, and the impact of debittering treatment (soaking, boiling, and washing with water) on the flavonoid composition. The raw and the debittered seeds were both analyzed with liquid chromatography tandem mass spectrometry (LC-MS) to determine flavonoid profile (mainly as isoflavones and flavones) at the molecular level. As the novelty, we provided accurate characterization of flavonoids in *L. mutabilis* seeds. Through systematic comparison using statistical models, the study filled the knowledge gap in flavonoid diversity among *L. mutabilis* ecotypes. We also revealed the pattern of changes in flavonoid composition triggered by aqueous debittering process. This novel knowledge will offer important reference for developing new products from Andean lupins.

2. Materials and methods

2.1. Plant materials

Ten ecotypes of Andean lupin (*L. mutabilis* Sweet) were collected in different regions of Peru, as shown in Supplemental Table 1. The ecotypes were chosen based on their geographic distribution, production area, and commercial interest. The seeds of Andean lupin were harvested in year 2017. The harvesting time was defined by local producers according to the color of optimally ripe pods. After harvesting, all seeds were stored in a cold room (around 10 °C) until moved to the laboratory.

2.2. Chemicals

Reference standards of apigenin, chrysoeriol, genistein, and tectorigenin were purchased from Extrasynthese (Genay, France). Acetic acid was purchased from Merck Group (Darmstadt, Germany). The solvents of LC and MS grade (acetonitrile, methanol, and formic acid) were purchased from VWR International Oy (Espoo, Finland).

2.3. Aqueous debittering of lupin seeds

The method of aqueous debittering treatment was described in our previous study (Cortés-Avendaño et al., 2020). Briefly, 1 kg of seeds of each ecotype were soaked with water at a solid/water ratio of 1:6 (*w/v*) for 12 h (at room temperature). The soaked seeds were further boiled for 1 h (seeds/water, 1:3, *w/v*) while the water was changed once after 30 min. After boiling, the seeds were washed with running water for 5 days. Both treated and untreated seeds were dried at 50 °C for 18 h and ground into fine powder (particle sides between 100 and 500 μm). All powder samples were stored at −20 °C until analysis.

2.4. Extraction of free flavonoids

The extraction of free-formed flavonoids from lupin seeds was conducted based on previous method with modification (Liu et al., 2014; Tian et al., 2017). The lupin seed powders were defatted with *n*-hexane for 4 h. All defatted powders were dried at room temperature to remove hexane (Adeloye et al., 2020). Subsequently, 0.4 g of untreated powder samples or 1 g of treated samples was mixed with 3 × 5 mL of acidified methanol (methanol: acetic acid, 99:1, *v/v*) and homogenized using T25 digital Ultra-Turrax high-performance disperser (IKAWerke GmbH & Co.KG, Staufen, Germany) at a speed of 10,600 rpm (for 3 min in the first-time extraction and for 1 min in the second and third extraction). The mixture was centrifuged at 18,000 × *g* for 30 min (Sorvall Tc-6 Centrifuge H400, Du Pont, Newtown, CT, United States) and the supernatant was collected. The supernatants from three-time extraction were combined and evaporated to completely dry by using a rotary evaporator (at 35 °C, vacuumed). The residues were re-dissolved in 4 mL of methanol and filtered through 0.22 μm filters before the following analyses.

2.5. Identification of flavonoids by HPLC-DAD-ESI-MS

The flavonoid characterization in lupin samples was performed by using a Waters Acquity ultra performance liquid chromatography (UPLC) system equipped with 2996 DAD detector, an electrospray ionization interface (ESI) and a Waters Quattro Premier mass spectrometer (Waters Corp., Milford, MA, United States). The liquid chromatographic separation was carried out with a binary elution method at 25 °C (Tian et al., 2019). A Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 μm, Torrance, CA, United States) was applied. The extracts of 10 μL was injected into LC system at a flow rate of 1 mL/min. Water (A) and acetonitrile (B) were used as mobile phase, both containing 0.1 % (*v/v*) of formic acid. The LC gradient was set as: 0–15 min with 8–10 % solvent B, 15–20 min with 10–13 % B, 20–25 min with 13—16 % B, 25–30 min with 16–18 % B, 30–35 min. with 18–20 % B, 35–40 min. with 20–22 % B, 40–45 min with 22–25 % B, 45–50 min with 25–60 % B, 50–55 min with 60–8 % B, 55–57 min with 8 % B. For MS and MS^2 analyses, both positive and negative ion scans were applied. The analytical parameters were described in our previous research (Tian et al., 2017).

2.6. Quantification of flavonoids by HPLC-DAD

The quantification of identified compounds was carried out by using a Shimadzu liquid chromatography system (Shimadzu Corp., Kyoto, Japan), consisting of a SIL-10A autosampler, a CTO-10 column oven, two LC-10ATvp pumps, and an SPD-M10AVP diode array detector (DAD). The chromatographic condition was the same as that applied in the LC-MS identification. Chromatograms were recorded at 260 nm (for isoflavones) and 340 nm (flavones). The quantification was conducted in triplicates. The content of each identified compound was calculated with calibration curves of external reference standards. Approximately 1 mg of reference compounds were dissolved in 10 mL methanol and diluted to four different concentrations. The calibration curves were constructed by plotting the peak areas at the recorded wavelengths of HPLC chromatogram as a function of the concentrations. Detailed information of calibration curves is given in Supplementary Table 2.

2.7. Statistical analysis

The contents of identified compounds were expressed as mean ± standard deviation. Statistical difference among phenolic contents was performed with one way-ANOVA and Tukey's post hoc test ($p < 0.05$) by using IBM SPSS Statistics 26 for Windows (SPSS Inc., New York, Unite States). Principal component analysis (PCA) and hierarchical clustering heatmap were applied to investigate variation in flavonoid contents among samples. The PCA models with full cross validation were created by Unscrambler 11 (Camo Process AS, Oslo, Norway). The heatmap was performed using open-source platform of MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). The data used in the heatmap was normalized with auto-scaling in average values.

3. Results and discussion

3.1. Characterization of flavonoids in lupin seed samples

Flavonoids in lupin seed extracts were characterized by comparing maximum UV absorption, LC retention time, typical MS ions, and MS fragmentation pattern with external reference standards and data from previous literatures (Aisyah et al., 2016; Kachlicki et al., 2016; Muth et al., 2008; Siger et al., 2012; Stobiecki et al., 2010). As a result, 21 compounds were identified from the samples. Both isoflavones (13 compounds) and flavones (8 compounds) formed the major flavonoids in the lupin seeds. The LC chromatograms of the extracts are given in Supplemental Fig. 1 and the MS data of identified phenolics is shown in Table 1.

The isoflavones were identified in the lupin extracts primarily as genistein (4′,5,7-trihydroxyisoflavone) and its glycosides with mono-, di-, and tri-saccharides as sugar moieties (Table 1). Certain saccharides (such as hexose) were acylated with malonic acid or acetic acid, showing the ion of $[M+H-162-86]^+$ or $[M+H-162-42]^+$ in the MS spectra. For identified genistein glycosides, it was likely that the sugar substitution might have occurred only on hydroxyl groups of genistein, since the ion of $[M+H-120]^+$ was not found in the MS^2 spectra of any genistein derivatives identified. This assumption is based on the study of Waridel et al. (2001) where *O*-glycosides of flavonoids did not produce $[M+H-120]^+$ fragment ions in comparison with their *C*-glycosylated counterparts. For flavones, glycosylated apigenins were the major flavones in the seeds of *Lupinus* species, as reported previously (Dueñas et al., 2009; Siger et al., 2012; Wojakowska et al., 2013). Yet, in this study, the seed

Table 1
Identification of flavonoids in lupine samples by HPLC-DAD-ESI-MS.

Peak No.	Identification	UV (nm)**	$[M+H]^+$/$[M-H]^-$ in MS (*m/z*)	$[A+H]^+$/$[A-H]^-$ and other ions in MS (*m/z*)	Product ions of $[M+H]^+$ or other ions in MS^2 (*m/z*)	Identification by *
1	genistein 4′,7-*O*-diglucoside	**260**,330	595/593	271/431,269	595 → 271	UV, MS, literature[1,2,3]
2	methoxy-genistein-pentoside-pentoside-hexoside	**260**,327	727/725	595,463,301/593,461,299	**301** → **286**,**269**,**241**,229,213,199,153	UV, MS
3	apigenin-pentoside-6,8-di-*C*-glucoside	271,**337**	727/725	595,577/605,593,473,455,383,353,335,269		UV, MS
4	apigenin 7-*O*-apiofuranosyl-6,8-di-*C*-glucoside	270,**337**	727/725	595,577/605,593,473,455,383,353,335,269		UV, MS, literature[4]
5	genistein 7-*O*-glucoside	**260**,330	433/431	271/269	433 → 271,253,243,241,215,153	UV, MS, literature[1,2,3,5]
6	methoxy-genistein-hexoside 1	**261**,330	463/461	301/299	463 → **301**,**286**,**269**,**241**,229,213,199,153	
7	genistein 4′-*O*-glucoside	**260**,330	433/431	271/269	433 → 271,253,243,241,215,153	UV, MS, literature[1,2]
8	genistein 7-*O*-xylosylglucoside	**265**,330	565/563	433,271/269	433 → 271,153	UV, MS, literature[3]
9	methoxy-genistein-hexoside 2	**260**,330	463/461	301/299	463 → **301**,**286**,**269**,**241**,229,213,199,153	UV, MS
10	methyl-luteolin-hexoside 1	267,**345**	463/461	301/299	463 → **301**,**286**,**258**	UV, MS
11	methyl-luteolin-hexoside-pentoside	270,**345**	595/593	463,301/461,299	595 → **301**,**286**,**258**	UV, MS
12	methyl-luteolin-hexoside 2	268,**347**	463/461	301/299	463 → **301**,**286**,**258**	UV, MS
13	genistein-malonylhexoside	**265**,335	475/473	519,271/269		UV, MS
14	methoxy-genistein-malonylhexoside	**261**,330	549/547	505,301/503,299	549 → **301**,**286**,**269**,**241**,153	UV, MS
15	methyl-luteolin-malonylhexoside	267,**345**	549/547	505,301/503,299	549 → **301**,**286**,**258**	UV, MS
16	genistein-acetylhexoside	**261**,330	475/473	271/269		UV, MS
17	methoxy-genistein-acetylhexoside	**262**,330	505/503	301/299	505 → **301**,**286**,**269**,**241**,153	UV, MS
18	methyl-luteolin-acetylhexoside	268,**343**	505/503	301/299	505 → **301**,**286**,**258**	UV, MS
19	genistein aglycone	**261**,330	271/269		271 → 253,197,169,153	UV, MS
20	methoxy-genistein aglycone	**262**,330	301/299	-/284,269	**301** → **286**,**269**,**241**,229,213,201,187,153	UV, MS
21	methyl-luteolin aglycone	268,**346**	301/299	-/284,269	**301** → **286**,**258**,229,203,187,153	UV, MS

* Reference literatures are:
[1] Aisyah et al. (2016).
[2] Stobiecki et al. (2010).
[3] Muth et al. (2008).
[4] Siger et al. (2012).
[5] Kachlicki et al. (2016).
** λ_{max} is marked in bold font

extracts of *L. mutabilis* contained only two apigenin derivatives, identified as apigenin 7-*O*-apiofuranosyl-6,8-di-*C*-glucoside and apigenin-pentoside-6,8-di-*C*-glucoside (Table 1).

Six unknown compounds (compounds 2, 6, 9, 14, 17, and 20) were found in the treated and untreated lupin seeds, each of which showed a typical UV maximum absorption of isoflavone at 260 nm (Table 1 and Supplemental Fig. 1). Compound 20, as shown in Supplemental Fig. 2, produced $[M-H]^-$ ion at *m/z* 299 and $[M+H]^+$ ion at *m/z* 301, which suggested it might be a derivative of genistein substituted with a methoxy group (—OCH_3) at benzene ring. The compound was further confirmed not to be tectorigenin (4′,5,7-trihydroxy-6-methoxyisoflavone) due to different retention time shown in LC chromatogram. Compound 20 might have been 3′-*O*-methylorobol (4′,5,7-trihydroxy-3′-methoxyisoflavone), which was previously isolated from the leaf extracts of white lupin (*L. albus*, cv. Kievskij Mutant) (Tahara et al., 1984). It is also possible that compound 20 may have been a methylated derivative of 2′-hydroxygenistein (2′,4′,5,7-tetrahydroxyisoflavone), since 2′-hydroxygenistein (and its varying glycosides) has been identified from the roots, seeds, and leaves of lupin species of Mexican and European origins (Aisyah et al., 2016; Kachlicki et al., 2005; Stobiecki et al., 2010; Wojakowska et al., 2013). Other unknown isoflavones (such as compounds 2, 6, 9, 14 and 17) detected from the lupin samples might have been *O*-glycosides of compound 20. By showing a similar MS fragmentation pattern, all these compounds generated the ions at *m/z* 301, 286, 269, and 241, which were also observed in the MS^2 spectrum of compound 20 (Table 1).

Unknown compounds 10, 11, 12, 15, 18, and 21 were tentatively identified as flavones for exhibiting a maximum UV absorption at approximately 340 nm (Table 1 and Supplemental Fig. 1). As shown in Supplemental Fig. 3, compound 21 produced $[M-H]^-$ ion at *m/z* 299 and $[M+H]^+$ ion at *m/z* 301, which suggested it might be chrysoeriol (luteolin 3′-methyl ether) as suggested in previous research(Muth et al., 2008; Stobiecki et al., 2010; Wojakowska et al., 2013). Nevertheless, this was later proved as incorrect by a LC co-injection (containing both seed extract and reference standard of chrysoeriol), where chrysoeriol was eluted later than compound 21 in the chromatographic separation. Dueñas et al. (2009) suggested that the unknown compound was diosmetin (luteolin 4′-*O*-methyl ether), which was isolated in the seeds of *L. angustifolius*. Yet, our study was not able to confirm this due to lack of reference standard. Compounds 10, 11, 12, 15 and 18 all produced the ions at *m/z* 301, 286, and 258 in their MS^2 spectra, indicating they could be glycosides of compound 21 (Table 1). The sugar moieties of these unknown compounds might be linked to the different hydroxy groups of compound 21.

3.2. Diversity of flavonoid profile in lupin seeds before debittering process

Table 2 shows the contents of flavonoids identified from the seed extracts of ten lupin ecotypes. In the untreated seeds, the total content of identified flavonoids varied from 187 (‘Cajamarca’, E1) to 252 mg/100 g of dry seeds (‘Compuesto blanco semi precoz INIA’, E6). The value was calculated as the sum of the contents of all isoflavones and flavones obtained from HPLC quantification. Isoflavones were the main flavonoids presented in the lupin seeds, representing 77–84 % of total flavonoids. The highest content of isoflavones were detected from the seeds of ‘Compuesto blanco semi precoz INIA’ (E6, 212 mg/100 g dry weight,

Table 2
Concentration (mg/100 g dry weight) of identified flavonoids in the extracts of untreated lupin seeds.

	Ecotypes									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Total flavonoids	186.6 ± 1.1^{a}	241.0 ± 6.3^{efg}	242.1 ± 3.8^{fg}	224.5 ± 3.8^{def}	206.6 ± 8.9^{bc}	252.4 ± 0.6^{g}	246.6 ± 6.9^{g}	223.5 ± 3.2^{cde}	198.4 ± 4.9^{ab}	220.2 ± 4.9^{cd}
Total isoflavones	152.8 ± 0.9^{a}	201.2 ± 6.1^{cd}	201.8 ± 3.2^{d}	184.1 ± 3.5^{b}	159.5 ± 7.8^{a}	212.2 ± 0.4^{d}	208.2 ± 6.0^{d}	185.8 ± 3.0^{bc}	163.7 ± 4.3^{a}	183.4 ± 4.3^{b}
Ge-diGlu	7.2 ± 0.0^{a}	18.5 ± 0.2^{e}	17.8 ± 0.5^{de}	14.0 ± 0.3^{c}	19.2 ± 1.3^{e}	15.6 ± 0.2^{c}	17.8 ± 0.8^{de}	16.0 ± 0.6^{cd}	15.7 ± 0.6^{c}	11.5 ± 0.4^{b}
methoxyGe-Pent-Pent-Hex	6.1 ± 0.2^{a}	18.0 ± 0.6^{e}	14.7 ± 0.3^{d}	12.2 ± 0.2^{c}	10.6 ± 0.6^{b}	21.1 ± 0.0^{f}	17.8 ± 0.1^{e}	24.7 ± 0.3^{g}	17.1 ± 0.3^{e}	14.6 ± 0.2^{d}
methoxyGe-Hex 1	22.6 ± 0.1^{a}	62.3 ± 2.1^{d}	55.2 ± 0.6^{c}	45.9 ± 0.6^{b}	46.0 ± 1.8^{b}	70.3 ± 0.2^{e}	69.3 ± 2.0^{e}	63.8 ± 2.2^{d}	56.7 ± 2.4^{c}	55.1 ± 1.2^{c}
Ge-Glu 2	4.5 ± 0.2^{cd}	5.9 ± 0.2^{f}	4.7 ± 0.1^{de}	4.1 ± 0.1^{bc}	5.7 ± 0.2^{f}	4.9 ± 0.1^{de}	4.0 ± 0.0^{bc}	3.7 ± 0.2^{b}	2.8 ± 0.1^{a}	5.1 ± 0.1^{e}
methoxyGe-Hex 2	2.9 ± 0.1^{b}	7.0 ± 0.2^{f}	2.5 ± 0.0^{a}	3.1 ± 0.1^{b}	3.7 ± 0.2^{c}	7.7 ± 0.0^{g}	7.2 ± 0.2^{f}	5.3 ± 0.1^{e}	2.4 ± 0.0^{a}	4.8 ± 0.1^{d}
Ge-malonylHex	34.5 ± 0.6^{e}	15.7 ± 0.9^{b}	30.9 ± 0.8^{d}	30.1 ± 0.7^{d}	19.1 ± 1.4^{c}	12.5 ± 0.1^{a}	20.7 ± 0.7^{c}	11.2 ± 0.1^{a}	10.7 ± 0.6^{a}	15.8 ± 0.6^{b}
methoxyGe-malonylHex	65.9 ± 0.4^{e}	46.0 ± 1.4^{c}	62.6 ± 1.1^{e}	63.7 ± 1.5^{e}	30.5 ± 1.5^{a}	46.1 ± 0.4^{c}	52.2 ± 2.1^{d}	41.9 ± 0.5^{bc}	38.0 ± 0.8^{b}	51.6 ± 2.1^{d}
Ge aglycone	3.9 ± 0.1^{a}	10.3 ± 0.1^{e}	5.6 ± 0.1^{bc}	4.6 ± 0.2^{ab}	12.8 ± 0.6^{f}	10.7 ± 0.1^{e}	7.7 ± 1.0^{d}	6.2 ± 0.2^{c}	6.5 ± 0.1^{cd}	7.6 ± 0.0^{d}
methoxyGe aglycone	5.2 ± 0.1^{a}	17.5 ± 0.6f	7.8 ± 0.2^{b}	6.4 ± 0.1^{a}	12.0 ± 0.6^{cd}	23.4 ± 0.1^{g}	11.5 ± 0.7^{c}	13.0 ± 0.2^{de}	13.8 ± 0.3^{e}	17.2 ± 0.1^{f}
Total flavones	33.8 ± 0.3^{a}	39.8 ± 0.5^{de}	40.3 ± 0.6^{e}	40.4 ± 0.3^{e}	47.1 ± 1.3^{f}	40.2 ± 0.2^{e}	38.4 ± 0.9^{cde}	37.8 ± 0.2^{cd}	34.7 ± 0.7^{ab}	36.8 ± 0.6^{bc}
Ap-Pent-diGlu	7.0 ± 0.1^{c}	8.5 ± 0.3^{d}	6.3 ± 0.1^{b}	9.9 ± 0.2^{e}	10.1 ± 0.3^{e}	8.2 ± 0.0^{d}	6.4 ± 0.1^{bc}	5.1 ± 0.0^{a}	6.2 ± 0.1^{b}	6.6 ± 0.2^{bc}
Ap-Apio-diGlu	13.1 ± 0.2^{b}	17.7 ± 0.1^{d}	11.1 ± 0.2^{a}	17.6 ± 0.3^{d}	18.0 ± 0.5^{d}	16.4 ± 0.0^{c}	12.6 ± 0.2^{b}	10.8 ± 0.1^{a}	11.3 ± 0.3^{a}	12.7 ± 0.3^{b}
methylLu-Hex-Pent	1.1 ± 0.0^{a}	1.3 ± 0.0^{b}	1.7 ± 0.0^{g}	1.3 ± 0.0^{b}	1.5 ± 0.0^{cd}	1.5 ± 0.0^{cde}	1.6 ± 0.0^{de}	1.7 ± 0.0^{fg}	1.6 ± 0.0^{ef}	1.5 ± 0.0^{c}
methylLu-Hex 2	3.5 ± 0.0^{a}	4.9 ± 0.1^{b}	7.7 ± 0.0^{ef}	4.0 ± 0.1^{a}	8.1 ± 0.4^{f}	6.1 ± 0.1^{c}	7.9 ± 0.2^{f}	9.8 ± 0.2^{g}	7.2 ± 0.3^{de}	7.0 ± 0.1^{d}
methylLu-malonylHex	8.0 ± 0.1^{cd}	5.9 ± 0.1^{a}	12.2 ± 0.3^{e}	6.4 ± 0.1^{ab}	8.1 ± 0.4^{cd}	6.6 ± 0.1^{ab}	8.5 ± 0.4^{d}	8.7 ± 0.2^{d}	6.9 ± 0.0^{b}	7.7 ± 0.1^{c}
methylLu-acetylHex	1.0 ± 0.0^{a}	1.4 ± 0.0^{ef}	1.3 ± 0.0^{c}	1.2 ± 0.0^{b}	1.3 ± 0.0^{c}	1.4 ± 0.0^{d}	1.4 ± 0.0^{def}	1.6 ± 0.0^{g}	1.5 ± 0.0^{f}	1.4 ± 0.0^{de}

Statistical differences are based on one way-ANOVA and Tukey’s post hoc test ($p < 0.05$); significant differences are shown with different superscript letters a–g. The *L. mutabilis* ecotypes (E) are Cajamarca (E1), Altagracia (E2), Paton grande (E3), Cholo fuerte (E4), Huanuco I (E5), Compuesto blanco semi precoz INIA (E6), H6 INIA (E7), Moteado beige (E8), Andenes INIA (E9), and Yunguyo (E10).

DW), followed by 'H6 INIA' (E7, 208 mg/100 g DW) and 'Paton grande' (E3, 202 mg/100 g DW). In contrast, 'Cajamarca' (E1), 'Huanuco I' (E5), and 'Andenes INIA' (E9) were low in isoflavones (153–164 mg/100 g DW). Despite the variation in total content, the isoflavones in the lupin seeds were presented mostly as methoxy-genistein-malonylhexoside (methoxyGe-malonylHex) and a derivative of methoxy-genistein-hexoside (methoxyGe-Hex 1). The contents of these two compounds accounted for 48–60 % of the total content of isoflavones. Flavones were found in the lupin seeds at lower levels in comparison with isoflavones. The total content of identified flavones was in a range of 34–47 mg/100 g of dry seeds, apigenin 7-*O*-apiofuranosyl-6,8-di-*C*-glucoside (Ap-Apio-diGlu) being the dominant.

Previous study on flavonoid composition of *L. mutabilis* Sweet seed is scarce. Czubinski et al. (2021) extracted flavonoids from *L. mutabilis* seeds of certain sweet cultivar with 80 % aqueous methanol and analyzed the extracts by using a LC-ESI-MS^n method. The MS results suggested that no isoflavones were presented in the seed extract, which was not in accordance with our study. The major compounds detected in the extract were apigenin-6.8-di-*C*-*β*-glucopyranoside (36 mg/100 g dry matter, DM) and apigenin 7-*O*-*β*-apiofuranosyl-6.8-di-*C*-

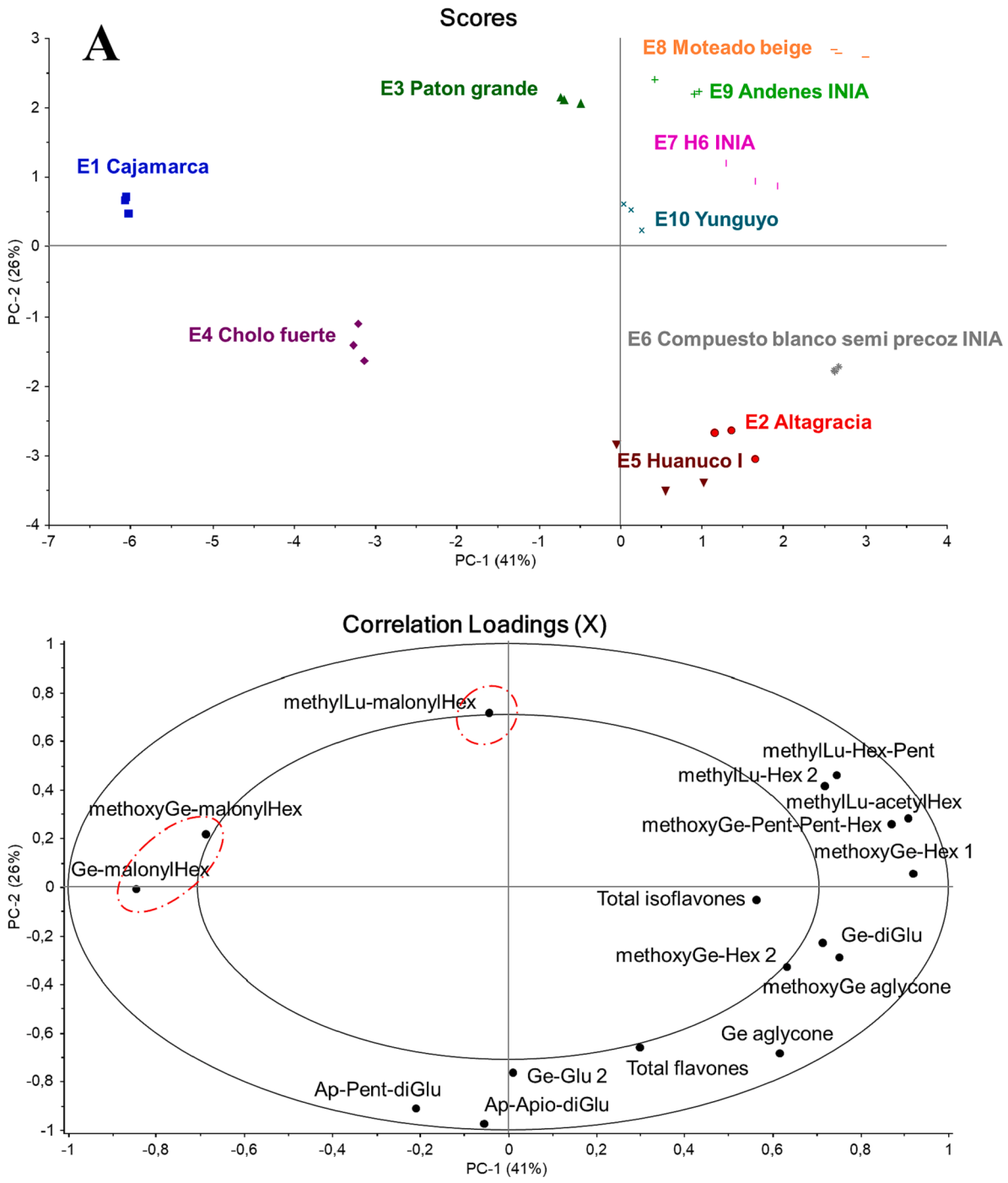


Fig. 1. PCA models for comparison of flavonoid profile among lupin seed extracts: untreated seeds (**A**, all ecotypes; **B**, the ecotypes without E1, E3, and E4) and treated seeds (**C**, all ecotypes; **D**, the ecotypes without E1). The abbreviation of compounds used in the figure are: **Ge**, genistein; **methoxyGe**, methoxy genistein; **Ap**, apigenin; **Lu**, luteolin; **methylLu**, methyl luteolin; **diGlu**, 4′,7-*O*-diglucoside; **Glu 1**, 7-*O*-glucoside; **Glu 2**, 4′-*O*-glucoside; **Apio**, Apiofuranoside; **Xyl**, xyloside; **Hex**, hexoside; **malonylHex**, malonylhexoside; **acetylHex**, acetylhexoside; and **Pent**, pentoside.

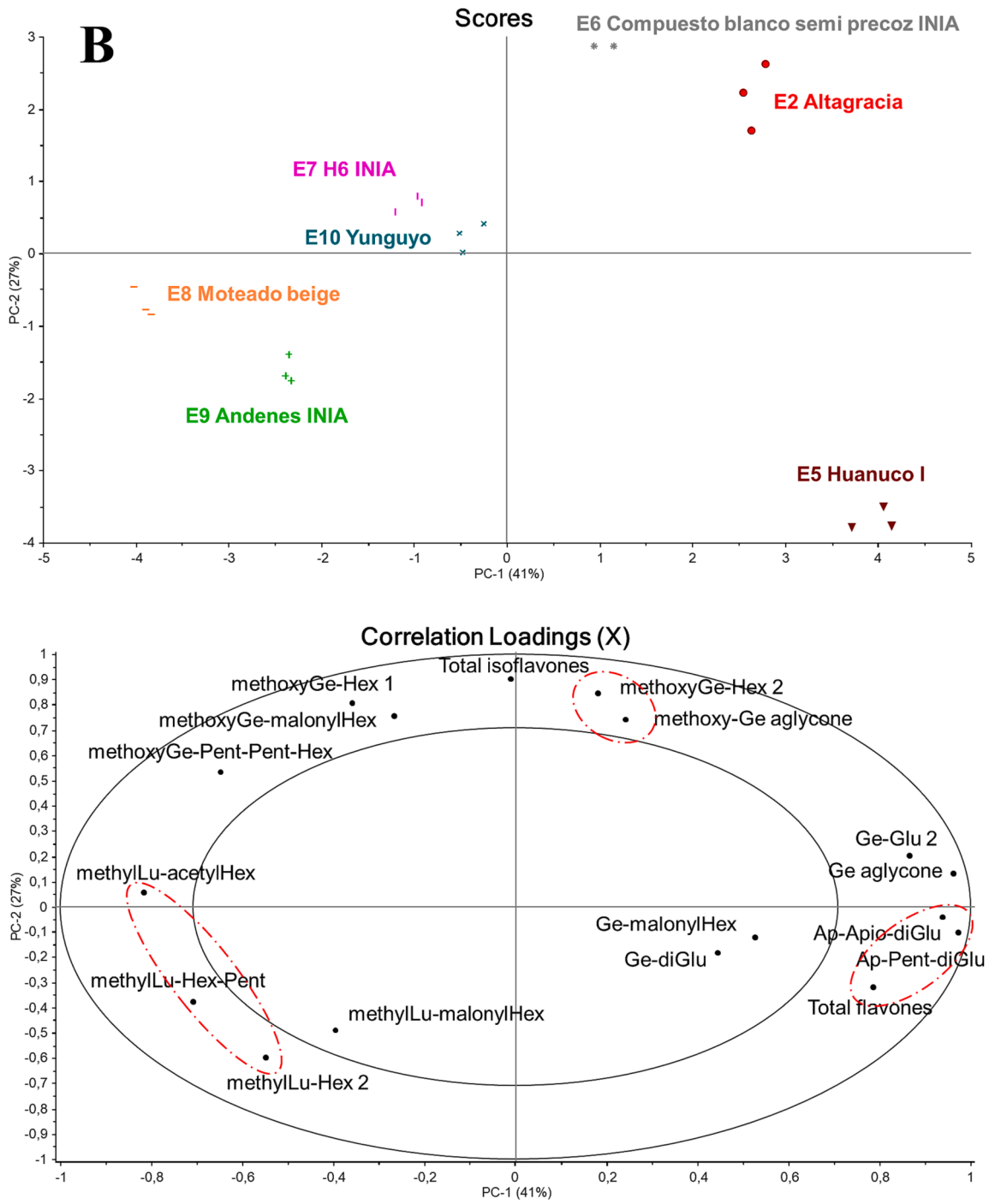


Fig. 1. (*continued*).

β-glucopyranoside (81 mg/100 g DM). Two unknown flavone derivatives were also observed at a low amount, the identification of which was made based on typical UV absorption at both 271–273 nm and 323–348 nm (Czubinski et al., 2021). Brandolini et al. (2022) investigated three genotypes of *L. mutabilis* Sweet ('Altagracia', 'Andenes', and 'Yunguyo') that were cultivated in different regions of Peru. The obtained seed extracts (using 80 % aqueous methanol) contained approximately 300 mg/100 g DM of free flavonoids, presenting mostly as derivatives of genistein (accounting for 44 % of total flavonoids), catechin (32 %), diosmin (diosmetin 7-*O*-rutinoside, 8 %), apigenin (6 %), and naringenin (4 %). Yet, it was noticed that the flavonoid compounds were identified only by comparing UV spectrum and retention time with reference standards. For each derivative of genistein, catechin, diosmin, apigenin, or naringenin, no compositional information was provided. Therefore, the results may not be comparable to those obtained in our study.

The diversity of isoflavones and flavones among the seeds of lupin ecotypes was revealed by statistical models. In the PCA model of Fig. 1**A** (containing 67 % of chemical varieties in PC-1 and PC-2), the studied ecotypes were classified into different groups (as shown in the score plot). It is noticed that the grouping of the studied ecotypes is not always in accordance with their cultivation sites (Supplemental Table 1). The ecotypes from higher latitudes ('H6 INIA', 'Moteado beige', 'Andenes INIA', and 'Yunguyo') are close to each other. Yet, 'Altagracia' was separated from 'Cajamarca' and 'Paton grande' although they were all grown at lower latitudes. This indicates that the geographic location

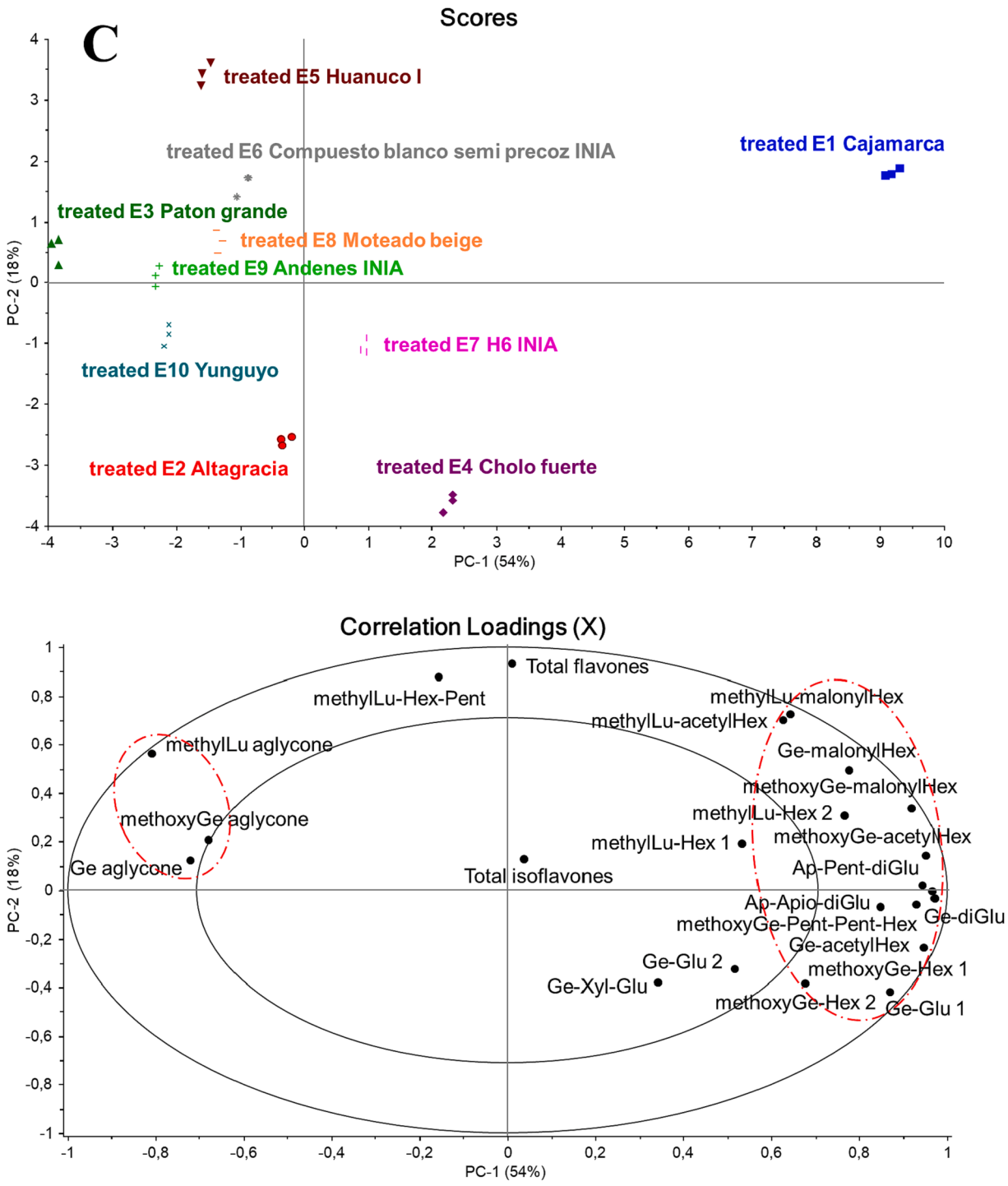


Fig. 1. (*continued*).

may not be the only factor influencing chemical profile of lupin seeds. For the identified flavonoids, the seeds of 'Cajamarca', 'Cholo fuerte', and 'Paton grande' had considerably high levels of methoxy-genistein-malonylhexoside and genistein-malonylhexoside (Ge-malonylHex), which explained positive correlation with these two compounds in the loading plot. Another positive correlation was observed between 'Paton grande' and methyl-luteolin-malonylhexoside (methylLu-malonylHex) as a higher content of which detected from the seed samples. The rest of the ecotypes were compared in the model of Fig. 1B. The samples of 'Altagracia' and 'Compuesto blanco semi precoz INIA' strongly associated with methoxy-genistein (methoxy-Ge aglycone) and a methoxy-genistein hexoside isomer (methoxyGe-Hex 2) along PC-1 (with 41 % of variables included, Fig. 1B). Higher level of flavones detected in the 'Huanuco I' seeds, which was ascribed to the abundance of apigenin 7-*O*-apiofuranosyl-6,8-di-*C*-glucoside and its isomer (Ap-Pent-diGlu). The derivatives of methyl-luteolin were concentrated mainly in the sample of 'Moteado beige', such as methyl-luteolin-acetylhexoside (methylLu-acetylHex), methyl-luteolin-hexoside-pentoside (methylLu-Hex-Pen), and methyl-luteolin-hexoside (methylLu-Hex 2). Only a few studies have reported the potential influence of lupin ecotype on phenolic profile in seeds. The studies in this field have focused mostly on other *Lupinus* species rather than *L. mutabilis* Sweet. Ferchichi et al. (2021) compared

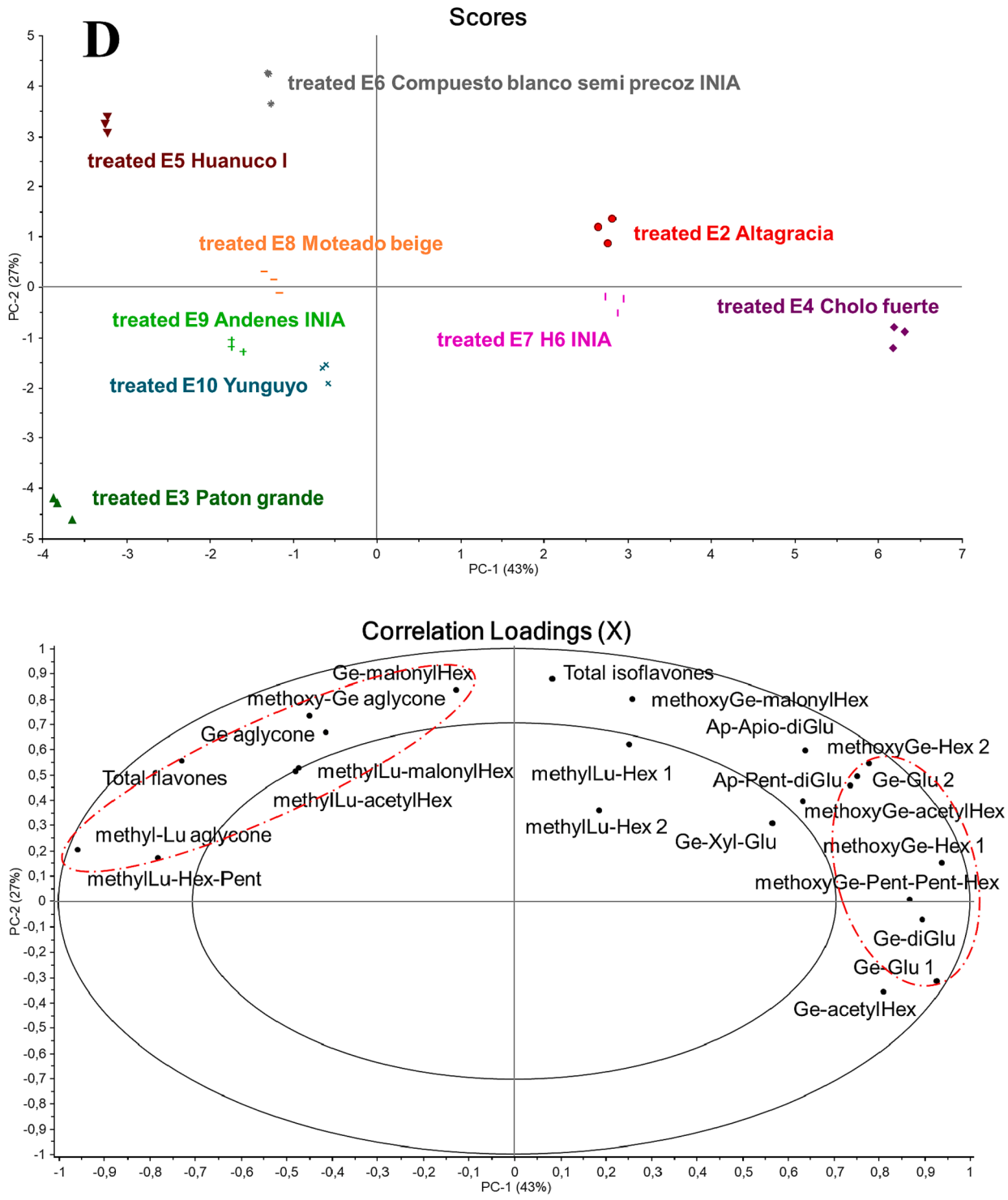


Fig. 1. (*continued*).

phenolic composition in the methanol extracts (using 80 % of methanol) of *L. albus* (including two ecotypes), *L. luteus* (three ecotypes), and *L. angustifolius* (two ecotypes) seeds, to determine the effect of Tunisian ecotypes on these compounds. The results from LC-MS analysis suggested that different geographical origins led to a variation in the contents of benzoic acids, hydroxycinnamic acids, flavones, flavanones, and flavonols (21 compounds in total). The main differentiation in flavones was observed in morin dihydrate, luteolin 7-*O*-glucoside, luteolin, hesperetin 7-*O*-rutinoside (hesperidin), apigenin 7-*O*-glucoside, and apigenin 7-*O*-apioglucoside (apiin). The chemical diversity among lupin seeds of different ecotypes might be associated with the environment of lupin cultivation. Ferchichi et al. (2021) also reported the origins of lupin samples (geographical location) in their study, as well as the climate and the soil characteristics in each origin. Yet, the authors did not investigate the relationship between environmental factors and phenolic profile in lupin seeds, despite of a clear variation observed in geographical origins, such as annual rainfall, soil pH, and soil texture (Ferchichi et al., 2021).

3.3. Diversity of flavonoid profile in lupin seeds after debittering process

The contents of all identified flavonoids in the extracts of treated lupin seeds are given in Table 3. Approximately 125–203 mg/100 g DW of flavonoids were quantified from the extracts of debittered seeds. Like in untreated seeds, isoflavones still formed the major group of flavonoids in the treated samples, accounting for 88–92 % of total content of flavonoids. After aqueous debittering process, the seeds of 'Compuesto blanco semi precoz INIA' (E6) contained the highest level of isoflavones (185 mg/100 g DW), whereas the sample of 'Paton grande' (E3) had the lowest (110 mg/100 g DW). As the major isoflavone in the debittered seeds, the content of methoxy-genistein (aglycone) represented 23–59 % of total content of isoflavones. Genistein aglycone was also rich in most of treated samples (12–31 % of total isoflavones). A considerably low level of flavones (12–22 mg/100 g DW) remained in the lupin seeds after the debittering treatment. The aglycone of methylated luteolin became the main compound of flavones (3–11 mg/100 g DW).

The diversity of flavonoid composition also appears in the seeds after aqueous debittering treatment. As shown in the plots of Fig. 1C (including 72 % of chemical variables in PC-1 and PC-2), the treated sample of 'Cajamarca' correlated strongly with varying glycosides of genistein, methoxy-genistein, and apigenin. Strong correlations of 'Cajamarca' were also found with methyl-luteolin-malonylhexoside, methyl-luteolin-acetylhexoside, and methyl-luteolin-hexoside isomer 2. On the contrary, the seeds of other ecotypes after the debittering process were highly associated with the aglycones of genistein, methoxy-genistein, and methyl-luteolin. Aside from 'Cajamarca', the rest of ecotypes were further compared with the model in Fig. 1D, where 70 % of variables was contained in the first two PCs. The debittered seed of 'Paton grande' contained low contents of isoflavones and flavones, and thus showed negative correlations with the most identified compounds along PC-1 and PC-2. The samples of 'Altagracia', 'H6 INIA', and 'Cholo fuerte' were close to each other in the score plot of Fig. 1D, which was due to high levels of genistein 7-*O*-glucoside (Ge-Glu 1), genistein 4′-*O*-glucoside (Ge-Glu 2), methoxy-genistein-hexoside (methoxyGe-Hex 1&2), and methoxy-genistein-pentoside-pentoside-hexoside (methoxyGe-Pent-Pent-Hex). The seeds of 'Huanuco I' and 'Compuesto blanco semi precoz INIA' had similar flavonoid profile after the treatment, both of which were rich in genistein, methoxy-genistein, and methyl-luteolin aglycones. Strong correlations of 'Huanuco I' and 'Compuesto blanco semi precoz INIA' were also found with genistein-malonylhexoside, methyl-luteolin-malonylhexoside, and methyl-luteolin-acetylhexoside. In addition, the PCA models of treated seed samples suggested that no clear relationship was found between cultivation sites and flavonoid composition. The compositional diversity among debittered seeds may not be attributed to the variation in geographic areas.

Like that of untreated seeds, the flavonoid profile of debittered seeds of *L. mutabilis* ecotypes has also seldom been reported. Estivi et al. (2022) assessed 33 ecotypes of *L. mutabilis* with HPLC to reveal the impact of geographic location on the chemical composition of the seeds. Although phenolic identification in this study was performed based only on the comparison of LC retention time and UV spectrum with limited numbers of reference standards, it was confirmed that *L. mutabilis* seeds contained high levels of flavonoids (mostly as derivatives of genistein

Table 3
Concentration (mg/100 g dry weight) of identified flavonoids in the extracts of treated lupin seeds.

	Ecotypes									
	treated E1	treated E2	treated E3	treated E4	treated E5	treated E6	treated E7	treated E8	treated E9	treated E10
Total flavonoids	161.4 ± 0.5^{de}	191.5 ± 1.8^{f}	125.2 ± 3.0^{a}	148.5 ± 1.6^{b}	185.0 ± 1.4^{f}	203.2 ± 2.8^{g}	156.1 ± 1.6^{cd}	152.4 ± 2.6^{bc}	161.6 ± 0.5^{de}	163.6 ± 1.5^{e}
Total isoflavones	142.8 ± 0.6^{cd}	176.4 ± 1.7^{f}	109.5 ± 2.7^{a}	136.1 ± 1.5^{b}	163.5 ± 1.4^{e}	185.2 ± 2.7^{g}	139.9 ± 1.6^{bc}	134.2 ± 2.3^{b}	144.8 ± 0.4^{cd}	147.1 ± 1.2^{d}
Ge-diGlu	3.8 ± 0.0 ^{h}	0.6 ± 0.0^{e}	0.0 ± 0.0^{a}	1.3 ± 0.0^{f}	0.1 ± 0.0^{b}	0.2 ± 0.0^{c}	1.5 ± 0.0^{g}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{b}	0.5 ± 0.0^{d}
methoxyGe-Pent-Pent-Hex	3.4 ± 0.1^{g}	0.7 ± 0.1^{d}	0.0 ± 0.0^{a}	1.0 ± 0.0^{e}	0.2 ± 0.0^{b}	0.4 ± 0.0^{c}	1.4 ± 0.0^{f}	0.4 ± 0.0^{c}	0.3 ± 0.0^{b}	0.7 ± 0.0^{d}
Ge-Glu 1	14.7 ± 0.2 ^{h}	5.8 ± 0.0^{e}	2.4 ± 0.0^{b}	12.4 ± 0.2^{g}	0.3 ± 0.0^{a}	0.5 ± 0.0^{a}	7.0 ± 0.1^{f}	2.5 ± 0.0^{b}	3.3 ± 0.0^{c}	3.6 ± 0.0^{d}
methoxyGe-Hex 1	32.8 ± 0.2^{g}	16.2 ± 0.0^{e}	4.8 ± 0.2^{a}	22.9 ± 0.3^{f}	8.8 ± 0.2^{b}	12.9 ± 0.3^{d}	22.2 ± 0.3^{f}	8.5 ± 0.0^{b}	10.3 ± 0.0^{c}	10.9 ± 0.1^{c}
Ge-Glu 2	1.9 ± 0.0^{e}	2.3 ± 0.0^{f}	0.4 ± 0.0^{a}	2.8 ± 0.0^{g}	2.2 ± 0.0^{f}	1.5 ± 0.0^{d}	2.2 ± 0.1^{f}	1.4 ± 0.0^{d}	1.1 ± 0.0^{c}	0.9 ± 0.0^{b}
Ge-Xyl-Glu	0.7 ± 0.0^{cd}	0.9 ± 0.0^{e}	0.2 ± 0.0^{a}	0.6 ± 0.0^{b}	0.3 ± 0.0^{a}	0.8 ± 0.0^{e}	1.1 ± 0.0^{f}	0.7 ± 0.0^{d}	0.7 ± 0.0^{bc}	0.6 ± 0.0^{b}
methoxyGe-Hex 2	3.1 ± 0.0^{ef}	3.6 ± 0.1^{g}	0.5 ± 0.0^{a}	3.2 ± 0.0^{f}	2.0 ± 0.0^{b}	2.8 ± 0.0^{d}	3.0 ± 0.1^{e}	2.5 ± 0.0^{c}	1.8 ± 0.0^{b}	2.0 ± 0.1^{b}
Ge-malonylHex	5.5 ± 0.3^{f}	2.2 ± 0.1^{bcd}	1.5 ± 0.0^{a}	2.6 ± 0.1^{d}	3.3 ± 0.0^{e}	3.4 ± 0.1^{e}	1.7 ± 0.1^{ab}	2.7 ± 0.2^{d}	2.4 ± 0.2^{cd}	2.0 ± 0.1^{abc}
methoxyGe-malonylHex	11.3 ± 0.2^{f}	2.9 ± 0.1^{c}	1.8 ± 0.1^{b}	3.5 ± 0.0^{d}	3.6 ± 0.1^{d}	4.2 ± 0.1^{e}	2.7 ± 0.0^{c}	3.0 ± 0.0^{c}	1.8 ± 0.1^{b}	1.3 ± 0.2^{a}
Ge-acetylHex	5.7 ± 0.3^{f}	1.5 ± 0.0^{d}	1.3 ± 0.1^{cd}	3.0 ± 0.1^{e}	0.7 ± 0.1^{a}	0.6 ± 0.0^{a}	1.4 ± 0.0^{cd}	1.1 ± 0.0^{bc}	0.9 ± 0.0^{ab}	1.0 ± 0.0^{ab}
methoxyGe-acetylHex	10.1 ± 0.2^{f}	2.8 ± 0.0^{b}	2.1 ± 0.0^{a}	5.5 ± 0.1^{e}	3.3 ± 0.1^{c}	4.2 ± 0.0^{d}	3.0 ± 0.0^{bc}	2.2 ± 0.0^{a}	2.0 ± 0.0^{a}	2.2 ± 0.1^{a}
Ge aglycone	17.3 ± 0.1^{a}	43.9 ± 0.6^{f}	33.7 ± 0.7^{d}	26.7 ± 0.4^{b}	43.4 ± 0.6^{f}	44.7 ± 0.4^{f}	30.7 ± 0.5^{c}	31.1 ± 0.7^{c}	35.4 ± 0.2^{de}	36.0 ± 1.2^{e}
methoxyGe aglycone	32.5 ± 0.1^{a}	92.8 ± 1.0^{f}	60.7 ± 1.9^{c}	50.5 ± 0.7^{b}	95.4 ± 0.9^{f}	108.9 ± 2.5^{g}	62.1 ± 0.6^{c}	78.0 ± 1.7^{d}	84.8 ± 0.4^{e}	85.6 ± 0.4^{e}
Total flavones	18.6 ± 0.1^{e}	15.2 ± 0.1^{b}	15.7 ± 0.3^{bc}	12.4 ± 0.1^{a}	21.6 ± 0.3^{f}	18.0 ± 0.1^{e}	16.2 ± 0.1^{cd}	18.2 ± 0.3^{e}	16.8 ± 0.2^{d}	16.5 ± 0.3^{d}
Ap-Pent-diGlu	1.5 ± 0.0 ^{h}	0.8 ± 0.0^{f}	0.5 ± 0.0^{a}	1.0 ± 0.0^{g}	0.7 ± 0.0^{d}	0.8 ± 0.0^{e}	0.6 ± 0.0^{b}	0.6 ± 0.0^{c}	0.6 ± 0.0^{b}	0.6 ± 0.0^{bc}
Ap-Apio-diGlu	2.9 ± 0.0 ^{h}	2.3 ± 0.1^{g}	0.6 ± 0.0^{a}	2.0 ± 0.0^{f}	1.5 ± 0.0^{d}	1.7 ± 0.0^{e}	1.2 ± 0.0^{b}	1.4 ± 0.0^{c}	1.2 ± 0.0^{b}	1.3 ± 0.0^{c}
methylLu-Hex 1	0.6 ± 0.0^{fg}	0.6 ± 0.0^{de}	0.5 ± 0.0^{a}	0.5 ± 0.0^{b}	0.6 ± 0.0^{e}	0.6 ± 0.0^{e}	0.6 ± 0.0^{g}	0.6 ± 0.0^{f}	0.5 ± 0.0^{cd}	0.5 ± 0.0^{c}
methylLu-Hex-Pent	0.8 ± 0.0^{c}	0.7 ± 0.0^{a}	0.8 ± 0.0^{c}	0.7 ± 0.0^{a}	0.8 ± 0.0^{d}	0.8 ± 0.0^{c}	0.8 ± 0.0^{c}	0.8 ± 0.0^{c}	0.8 ± 0.0^{c}	0.7 ± 0.0^{b}
methylLu-Hex 2	6.3 ± 0.1 ^{h}	3.4 ± 0.1^{bc}	2.4 ± 0.1^{a}	3.4 ± 0.0^{bc}	4.5 ± 0.1^{f}	3.3 ± 0.1^{b}	5.3 ± 0.1^{g}	4.0 ± 0.0^{e}	3.9 ± 0.1^{de}	3.6 ± 0.2^{cd}
methylLu-malonylHex	1.9 ± 0.0^{f}	0.5 ± 0.0^{a}	0.9 ± 0.0^{cd}	0.8 ± 0.0^{b}	1.4 ± 0.1^{e}	1.3 ± 0.1^{e}	1.1 ± 0.0^{d}	1.0 ± 0.0^{d}	0.8 ± 0.0^{bc}	0.8 ± 0.0^{b}
methylLu-acetylHex	1.6 ± 0.0^{f}	1.0 ± 0.0^{a}	1.2 ± 0.0^{d}	1.1 ± 0.0^{bc}	1.4 ± 0.0^{e}	1.3 ± 0.0^{e}	1.1 ± 0.0^{c}	1.1 ± 0.0^{c}	1.0 ± 0.0^{ab}	1.0 ± 0.0^{a}
methyl-Lu aglycone	2.9 ± 0.0^{a}	5.9 ± 0.0^{b}	8.9 ± 0.3^{e}	3.1 ± 0.0^{a}	10.8 ± 0.2^{f}	8.2 ± 0.1^{cd}	5.6 ± 0.1^{b}	8.7 ± 0.2^{de}	8.0 ± 0.1^{c}	7.9 ± 0.1^{c}

Statistical differences are based on one way-ANOVA and Tukey's post hoc test ($p < 0.05$); significant differences are shown with different superscript letters a–h. The *L. mutabilis* ecotypes (E) are Cajamarca (E1), Altagracia (E2), Paton grande (E3), Cholo fuerte (E4), Huanuco I (E5), Compuesto blanco semi precoz INIA (E6), H6 INIA (E7), Moteado beige (E8), Andenes INIA (E9), and Yunguyo (E10).

and diosmetin) after debittering process. The results suggested a diversity of these compounds among the debittered seeds. Higher contents of genisteins and diosmetins were observed in certain ecotypes cultivated in the central regions of Peru (Junin and Huanuco), such as 'H6 INIA BP' (130 mg/100 g DM), 'Huanuco II' (120 mg/100 g DM), and 'Moteado beige' (116 mg/100 g DM). The debittered seeds of 'Altagracia' and 'Paton grande' (harvested in La Libertad, Northern Peru) contained lower levels of these compounds (69 and 58 mg/100 g DM, respectively). In agreement with our results, no significant correlation was found between geographical areas and phenolic compounds in debittered seeds (Estivi et al., 2022). This may have been due to the fact that the seed samples were collected in only one harvesting year, which is not sufficient to discover the effect of environmental factors on phenolic profile. The genotype of *L. mutabilis* may also play a role in regulating flavonoid composition of lupin seeds. Estivi et al. (2022) reported that the debittered seeds collected even from the same region showed different profile of flavonoids. Similar findings were also observed in our study when comparing the samples from regions of La Libertad ('Altagracia' and 'Paton grande') or Junin ('Compuesto blanco semi precoz INIA', 'H6 INIA', and 'Moteado beige'). Unfortunately, our study could not provide any information on gene expression of the studied ecotypes. The effect of *L. mutabilis* genotype remained unknown. Moreover, the quality of soil used in lupin cultivation is also responsible for changes in flavonoid profile in debittered *L. mutabilis* seeds. Urrego-Pava and Coy-Barrera (2023) evaluated the impacts of both silty loam and sandy clay loam on nutrients and bioactive compounds in *L. mutabilis* ecotypes (from Cajica and Pasto, Colombia). Compared to sandy clay loam, the silty loam soil was superior in pH, humidity saturation, organic matter, and total nitrogen, which enhanced the accumulation of isoflavones (characterized with LC-MS) in the debittered seeds of *L. mutabilis* Sweet; however, the use of silty loam soil was not effective in increasing total content of phenolic compounds in the samples (Urrego-Pava & Coy-Barrera, 2023).

3.4. Impact of aqueous debittering treatment on flavonoid profile of lupin seeds

The applied debittering treatment was effective in reducing flavonoid levels in lupin seeds. Fig. 2 suggests that the impacts of the treatment varied among lupin ecotype and the type of flavonoids. In most seed samples, a clear decrease in total content of isoflavones was observed after the treatment (Fig. 2A). 'Paton grande' (E3) showed the largest reduction. The total content of isoflavones in the treated 'Paton grande' seeds was 46 % lower than that detected in the untreated ones. High decrease (26–33 % of reduction rate) was also found in the samples of 'H6 INIA'(E7), 'Moteado' (E8), and 'Cholo fuerte' (E4). 'Cajamarca' (E1) showed a decrease of 7 % in total isoflavones. Interestingly, similar levels of isoflavones were detected in both treated and untreated seeds of 'Huanuco I' (E5; 163.5 ± 1.4 *vs*.159.5 ± 7.8 mg/100 g DW, Table 2 & Table 3). As another major group of flavonoids, flavones in lupin seeds were mostly removed during debittering process. After the treatment, the level of total flavones in the seeds decreased by 56 % on average (Fig. 2B). The seeds of 'Cholo fuerte' (E4) had the highest decrease rate (69 %). The lowest decrease was still found in 'Cajamarca' (E1) seeds where approximately 45 % of flavones were reduced during the treatment.

A hierarchical clustering heatmap was applied to visualize compositional diversity among treated and untreated lupin seeds. As shown in Fig. 3, the seed samples obtained before and after debittering were classified into two clusters based on the contents of twenty-one identified flavonoids. The red zones on the map represented high amounts of compounds. Isoflavones and flavones present in the untreated seeds were primarily in the form of glycosylated derivatives; however, in the treated samples, the aglycones of genistein, methoxy-genistein, and methyl-luteolin became the major flavonoids. This suggests that the debittering process may cause the cleavage of *C*- or *O*-glycosylated linkage to release the corresponding flavonoid aglycones. The variation was also observed among glycosylated isoflavones and flavones. The untreated seeds were rich in tri- and di-glycosides of genistein, methoxy-genistein, apigenin, and methyl-luteolin with pentoside-pentoside-hexoside, pentoside-hexoside-hexoside, hexoside-hexoside, or hexoside-pentoside as sugar moieties. After the debittering treatment, these tri- and di-glycosylated derivatives were mostly removed, whereas high content of genistein 7-*O*-xylosylglucoside (Ge-Xyl-Glu) appeared in the most treated seeds. The applied treatment led to a significant decrease in the content of genistein 4′-*O*-glucoside (Ge-Glu 2). As its isomers, genistein 7-*O*-glucoside (Ge-Glu 1) was detected only in the treated seed samples. Moreover, the debittering process might also have an impact on the acylation of isoflavone glycosides. Genistein and methoxy-genistein were presented in the untreated lupin seeds mostly as malonylhexoside. Yet, in the samples after the treatment, acetylhexoside became the main sugar moiety of isoflavones. The debittering-induced variation in flavonoids of Andean lupin seeds has also been observed previously. As reported by Brandolini et al. (2022), after applying the

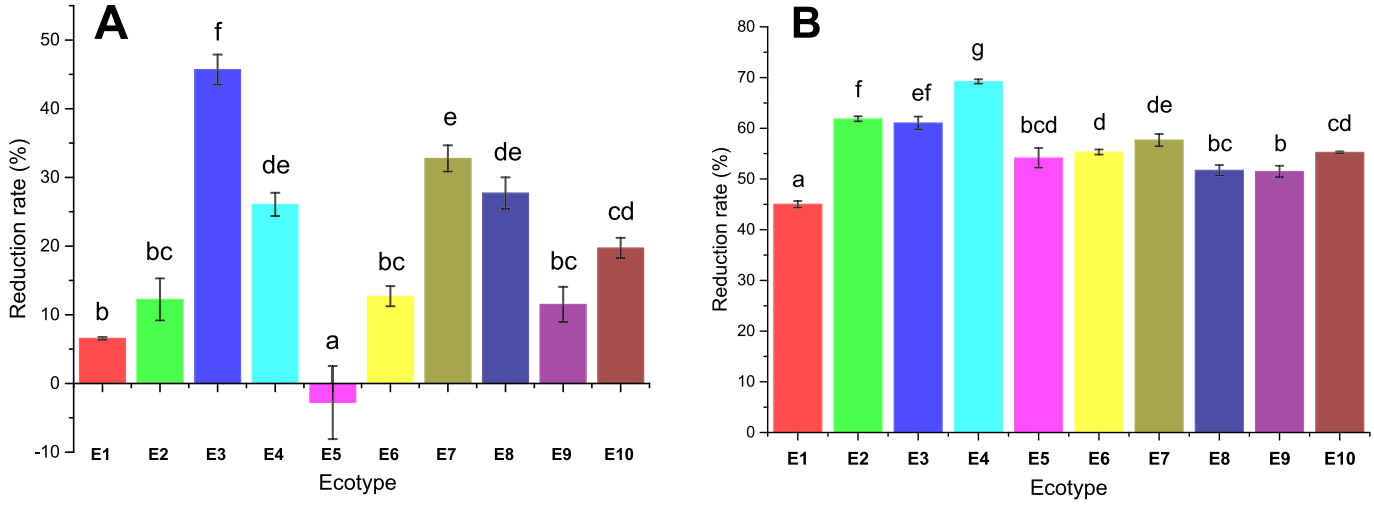


Fig. 2. The reduction rate of identified isoflavones (**A**) and flavones (**B**) after debittering treatment. Statistical differences are based on one way-ANOVA and Tukey's post hoc test ($p < 0.05$); significant differences are shown with different superscript letters a-g. The *L. mutabilis* ecotypes (E) are Cajamarca (**E1**), Altagracia (**E2**), Paton grande (**E3**), Cholo fuerte (**E4**), Huanuco I (**E5**), Compuesto blanco semi precoz INIA (**E6**), H6 INIA (**E7**), Moteado beige (**E8**), Andenes INIA (**E9**), and Yunguyo (**E10**).

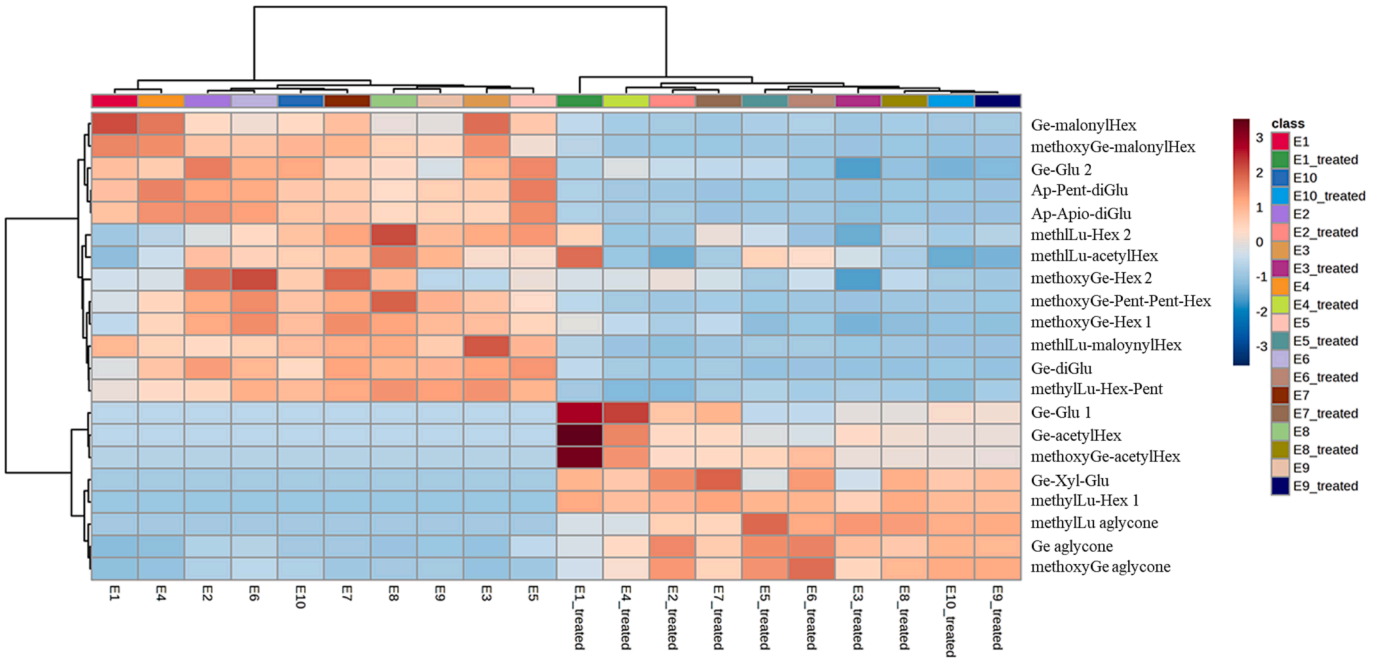


Fig. 3. Hierarchical clustering analysis of isoflavones and flavones in the extracts of untreated and treated lupin seeds. The abbreviation of compounds used in the figure includes **Ge**, genistein; **methoxyGe**, methoxy genistein; **Ap**, apigenin; **Lu**, luteolin; **methylLu**, methyl luteolin; **diGlu**, 4′,7-*O*-diglucoside; **Glu 1**, 7-*O*-glucoside; **Glu 2**, 4′-*O*-glucoside; **Apio**, Apiofuranoside; **Xyl**, xyloside; **Hex**, hexoside; **malonylHex**, malonylhexoside; **acetylHex**, acetylhexoside; and **Pent**, pentoside. The *L. mutabilis* ecotypes (E) are Cajamarca (**E1**), Altagracia (**E2**), Paton grande (**E3**), Cholo fuerte (**E4**), Huanuco I (**E5**), Compuesto blanco semi precoz INIA (**E6**), H6 INIA (**E7**), Moteado beige (**E8**), Andenes INIA (**E9**), and Yunguyo (**E10**).

same debittering treatment as in our study, over 70 % of free flavonoids were removed from the seeds of three *L. mutabilis* genotypes. Clear decrease was found in the contents of naringenin derivatives (reduced by 94 %), apigenin derivatives (90 %), diosmin derivatives (71 %), and genistein derivatives (around 50 %); whereas the level of genistein aglycone in the debittered samples was 8-fold more than that detected in the raw seeds. However, in the study of Brandolini et al. (2022), most of the compounds were identified as the derivatives of naringenin, apigenin, diosmin, or genistein by using a reversed phase HPLC-DAD system. The characterization was conducted based on the similitude in their UV absorption spectrum with reference standards. No accurate information is available on their molecular structures.

The aqueous debittering process is originally designed to reduce alkaloid level in lupin seeds. After 17–144 h of soaking, boiling, and washing in water, the alkaloid content in lupin seeds was decreased by 93–99 % (Carvajal-Larenas et al., 2016). Varying alkaloid compounds may response differently to aqueous treatment. Our previous study evaluated the profile and residual levels of quinolizidine alkaloids in the *L. mutabilis* seeds of the same ecotypes as studied in the present research after aqueous debittering process. The results obtained from GC–MS analysis showed that, among eight alkaloid compounds present originally in the seed samples, only lupanine and sparteine remained in the samples (at an amount of 1.2–1.4 mg/100 g DM) after the debittering process (Cortés-Avendaño et al., 2020). Recent studies have shown that the aqueous debittering process can also alter the profiles of chemical components in lupin seeds other than alkaloids. Villacrés et al. (2020) proposed that applying aqueous debittering treatment resulted in a remarkable decrease in some anti-nutritional compounds of *L. mutabilis* Sweet, such as nitrates, tannins, phytic acid, and trypsin inhibitors. It should be highlighted that the process can affect the content of essential nutrients and health-promoting compounds in lupin seeds. Carvajal-Larenas et al. (2016) reported that the *L. mutabilis* seeds obtained after debittering treatment had higher content of crude proteins than the untreated ones; however, the levels of crude lipids, fibres, and carbohydrates were decreased after aqueous processing. The aqueous debittering resulted in a slight increase in fatty acid content in *L. mutabilis* seeds, but it did not significantly alter the composition of fatty acids. This treatment could enhance the accumulation of calcium, iron, and zinc in the *L. mutabilis* seeds, whereas a considerable amount of potassium and magnesium were lost during the process (Carvajal-Larenas et al., 2016). Moreover, after debittering, higher content of tocopherol (mostly as γ-tocopherol) and lower level of ascorbic acid were both detected in the seeds, but carotenoid content (mainly as lutein and zeaxanthin) seemed not to be affected by the treatment (Brandolini et al., 2022; Villacrés et al., 2020).

Since aqueous debittering improved the nutritional value of seeds through removal of anti-nutritional agents and enrichment of essential nutrients and health-related bioactive components (Carvajal-Larenas et al., 2016), the debittered seeds of *L. mutabilis* could be used either as ready-to-eat foods or as functional ingredients in food products. Flavonoids in the debittered seeds may possess their significant effects of reducing the risk of cardiovascular and neurodegenerative diseases, and providing potent bioactivities against oxidation, cancers, and multiple parasites (Rees et al., 2018; Yang et al., 2018). In our study, we found the seeds of *L. mutabilis* ecotypes after debittering process still varied significantly in both composition and concentration of flavonoids. Although debittering process causes a large reduction of flavonoids in lupin seeds, it may be rational to select the debittered seeds containing the lower flavonoid content as potential ingredients used in foods. This is due to the concern that high levels of flavonoids may reduce the bioavailability of proteins and minerals in lupin seeds. Previous research has shown the interaction of dietary flavonoid with proteins and minerals (Kamiloglu et al., 2021). Both proteins and metal ions form stable complexes with flavonoids and may diminish the digestion and absorption of each other in human gastrointestinal tract (Halake et al., 2016; Li et al., 2021). In addition, flavonoids are often perceived as having undesirable bitter taste, since some of these compounds activate human bitter receptors (such as hTAS2R14 and hTAS2R39) (Roland, Van Buren, & Gruppen et al., 2013). Using the ecotype rich in flavonoids might have a negative effect on consumers' acceptance of lupin-derived

products. Therefore, before the optimal concentration and composition of flavonoids are determined with thorough investigation on protein bioavailability and sensory property of lupin debittered seeds, the ecotype of 'Paton grande' (E3) containing low flavonoid content may be the best option for further investigation of applying *L. mutabilis* Sweet in novel food development.

4. Conclusion

The flavonoid profile in the seeds of *L. mutabilis* Sweet was investigated on the molecule level by using a LC-MS method. Isoflavones and flavones formed the major groups of flavonoids in *L. mutabilis* seeds; however, the selected 10 ecotypes exhibited a large variation in contents and composition of both groups. The compositional diversity of lupin ecotypes was found mainly in the levels of dominant compounds of isoflavones and flavones. This might have been ascribed to both cultivating condition and genotype of lupin samples. An aqueous debittering process was applied, initially aimed at reducing the alkaloid level in lupin seeds by soaking, boiling, and washing with water. Our study showed that the debittering treatment was an effective approach of altering isoflavone and flavone composition, as well as decreasing the total flavonoid contents in the seeds. The seeds of different ecotypes showed varying behavior in response to the treatment by showing significant deviation in contents of individual compounds. Due to the presence of isoflavones and flavones, the debittered lupin seeds could be potentially used in food products for possessing multiple health-beneficial functions. Future studies should include the evaluation on sensory property and bioaccessibility of debittered seeds to assist the development of lupin-derived food products.

To our best knowledge, this is the first study to systematically investigate flavonoid profile in seeds of *L. mutabilis* ecotypes and reveal the impact aqueous debittering process on flavonoid composition in the lupin seeds. This knowledge is essential for food industry selecting optimal seeds and for farmers breeding new cultivars of Andean lupins. Besides yields, micronutrient contents, and removal of anti-nutritional components, the quality of health-promoting phytochemicals in *L. mutabilis* seeds and related products should be emphasized more in the future.

Funding

This research was funded by European Union's Horizon 2020 ("PROTEIN2FOOD: Development of high quality food protein through sustainable production and processing" project, project decision number 635727), and Business Finland (Formerly, Tekes-the Finnish Funding Agency for Innovation; "Sustainable utilization of Andean and Finnish crops/perucrop" project, project decision number 1084/31/2016). Financial supports were also given by the China Scholarship Council (as a researcher grant to Dr. Ye Tian, Decision no. 201309150008) and Cienciactiva of CONCYTEC (a researcher grant to Dr. Paola Cortés-Avendaño, Decision no. 179-2015-FONDECYT).

CRediT authorship contribution statement

Ye Tian: Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Paola Cortés-Avendaño:** Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Baoru Yang:** Supervision, Writing – review & editing. **Patricia Glorio-Paulet:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Ritva Repo-Carrasco-Valencia:** Conceptualization, Supervision, Writing – review & editing. **Jukka-Pekka Suomela:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ritva Repo-Carrasco-Valencia reports financial support was provided by European Commission. Jukka-Pekka Suomela reports financial support was provided by Business Finland. Ye Tian reports financial support was provided by China Scholarship Council. Paola Cortes-Avendano reports financial support was provided by Cienciactiva of CONCYTEC.

Data availability

Data will be made available on request.

Acknowledgement

We thank the PNIA project for Tarwi from the Program of Legumes of National Agraria La Molina University (Lima, Peru) for providing the lupin seeds.

Appendix A. Abbreviation used

The Abbreviation used in the study are **Ge**, genistein; **methoxyGe**, methoxy genistein; **Ap**, apigenin; **Lu**, luteolin; **methylLu**, methyl luteolin; **diGlu**, 4′,7-*O*-diglucoside; **Glu 1**, 7-*O*-glucoside; **Glu 2**, 4′-*O*-glucoside; **Apio**, Apiofuranoside; **Xyl**, xyloside; **Hex**, hexoside; **malonylHex**, malonylhexoside; **acetylHex**, acetylhexoside; and **Pent**, pentoside.

Appendix B. Supplementary material

The supplemental materials include: 1) Information of the Andean lupin ecotypes (Supplemental Table 1); 2) Information of external standards applied in flavonoid quantification (Supplemental Table 2); 3) LC chromatogram of flavonoids in the extracts of treated and untreated lupin seeds (Supplemental Fig. 1); 4) Information of compound 20 (methoxy-genistein aglycone, Supplemental Fig. 2); and 5) Information of the compound 21 (methyl-luteolin aglycone, Supplemental Fig. 3). Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138411.

References

Adeloye, J. B., Osho, H., & Idris, L. O. (2020). Defatted coconut flour improved the bioactive components, dietary fibre, antioxidant and sensory properties of nixtamalized maize flour. *Journal of Agriculture and Food Research, 2*, Article 100042. https://doi.org/10.1016/j.jafr.2020.100042

Aisyah, S., Vincken, J. P., Andini, S., Mardiah, Z., & Gruppen, H. (2016). Compositional changes in (iso)flavonoids and estrogenic activity of three edible Lupinus species by germination and Rhizopus-elicitation. *Phytochemistry, 122*, 65–75. https://doi.org/10.1016/j.phytochem.2015.12.015

Arnoldi, A., Boschin, G., Zanoni, C., & Lammi, C. (2015). The health benefits of sweet lupin seed flours and isolated proteins. *Journal of Functional Foods, 18*, 550–563. https://doi.org/10.1016/j.jff.2015.08.012

Brandolini, A., Glorio-Paulet, P., Estivi, L., Locatelli, N., Cordova-Ramos, J. S., & Hidalgo, A. (2022). Tocopherols, carotenoids and phenolics changes during Andean lupin (*Lupinus mutabilis* Sweet) seeds processing. *Journal of Food Composition and Analysis, 106*, Article 104335. https://doi.org/10.1016/j.jfca.2021.104335

Carvajal-Larenas, F. E., Linnemann, A. R., Nout, M. J. R., Koziol, M., & van Boekel, M. A. J. S. (2016). *Lupinus mutabilis*: Composition, uses, toxicology, and debittering. *Critical Reviews in Food Science and Nutrition, 56*(9), 1454–1487. https://doi.org/10.1080/10408398.2013.772089

Carvajal-Larenas, F. E., Nout, M. J. R., van Boekel, M. A. J. S., Koziol, M., & Linnemann, A. R. (2013). Modelling of the aqueous debittering process of *Lupinus mutabilis* Sweet. *LWT, 53*(2), 507–516. https://doi.org/10.1016/j.lwt.2013.03.017

Ciumărnean, L., Vasile Milaciu, M., Runcan, O., Vesa, C., Răchis, A. L., Negrean, V., Perné, M.-G., Donca, V. I., Alexescu, T.-G., Para, I., & Dogaru, G. (2020). The effects of flavonoids in cardiovascular diseases. *Molecules, 25*(18), Article 4320. https://doi.org/10.3390/molecules25184320

Córdova-Ramos, J. S., Glorio-Paulet, P., Camarena, F., Brandolini, A., & Hidalgo, A. (2020). Andean lupin (*Lupinus mutabilis* Sweet): Processing effects on chemical composition, heat damage, and in vitro protein digestibility. *Cereal Chemistry, 97*(4), 827–835. https://doi.org/10.1002/cche.10303

Cortés-Avendaño, P., Tarvainen, M., Suomela, J.-P., Glorio-Paulet, P., Yang, B., & Repo-Carrasco-Valencia, R. (2020). Profile and content of residual alkaloids in ten ecotypes of *Lupinus mutabilis* Sweet after aqueous debittering process. *Plant Foods for Human Nutrition, 75*(2), 184–191. https://doi.org/10.1007/s11130-020-00799-y

Czubinski, J., Dwiecki, K., Siger, A., Kachlicki, P., Neunert, G., Lampart-Szczapa, E., & Nogala-Kalucka, M. (2012). Release of flavonoids from lupin globulin proteins during digestion in a model system. *Journal of Agricultural and Food Chemistry, 60*(7), 1830–1836. https://doi.org/10.1021/jf2042592

Czubinski, J., Grygier, A., & Siger, A. (2021). *Lupinus mutabilis* seed composition and its comparison with other lupin species. *Journal of Food Composition and Analysis, 99*, Article 103875. https://doi.org/10.1016/j.jfca.2021.103875

Dueñas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chemistry, 117*(4), 599–607. doi:10.1016/J.FOODCHEM.2009.04.051.

Estivi, L., Grassi, S., Briceño-Berrú, L., Glorio-Paulet, P., Camarena, F., Hidalgo, A., Brandolini, A., & Molina, L. (2022). Free phenolic compounds, antioxidant capacity and FT-NIR survey of debittered *Lupinus mutabilis* seeds. *Processes, 10*(8), Article 1637. https://doi.org/10.3390/pr10081637

Ferchichi, N., Toukabri, W., Vrhovsek, U., Nouairi, I., Angeli, A., Masuero, D., Mhamdi, R., & Trabelsi, D. (2021). Proximate composition, lipid and phenolic profiles, and antioxidant activity of different ecotypes of *Lupinus albus*, *Lupinus luteus* and *Lupinus angustifolius*. *Journal of Food Measurement and Characterization, 15*, 1241–1257. https://doi.org/10.1007/s11694-020-00722-8

Gulisano, A., Alves, S., Martins, J. N., & Trindade, L. M. (2019). Genetics and breeding of *Lupinus mutabilis*: An emerging protein crop. *Frontiers in Plant Science, 10*, 1385. https://doi.org/10.3389/fpls.2019.01385

Halake, K., Birajdar, M., & Lee, J. (2016). Structural implications of polyphenolic antioxidants. *Journal of Industrial and Engineering Chemistry, 35*, 1–7. https://doi.org/10.1016/j.jiec.2016.01.003

Kachlicki, P., Marczak, Ł., Kerhoas, L., Einhorn, J., & Stobiecki, M. (2005). Profiling isoflavone conjugates in root extracts of lupine species with LC/ESI/MS^n systems. *Journal of Mass Spectrometry, 40*, 1088–1103. https://doi.org/10.1002/jms.884

Kachlicki, P., Piasecka, A., Stobiecki, M., Marczak, Ł., Feliciano, A. S., & Santos-Buelga, C. (2016). Structural characterization of flavonoid glycoconjugates and their derivatives with mass spectrometric techniques. *Molecules, 21*(11), Article 1494. https://doi.org/10.3390/molecules21111494

Kamiloglu, S., Tomas, M., Ozdal, T., & Capanoglu, E. (2021). Effect of food matrix on the content and bioavailability of flavonoids. *Trends in Food Science & Technology, 117*, 15–33. https://doi.org/10.1016/j.tifs.2020.10.030

Khan, M. K., Karnpanit, W., Nasar-Abbas, S. M., Huma, Z., & Jayasena, V. (2015). Phytochemical composition and bioactivities of lupin: A review. *International Journal of Food Science & Technology, 50*(9), 2004–2012. https://doi.org/10.1111/ijfs.12796

Koleva, I. I., van Beek, T. A., Soffers, A. E. M. F., Dusemund, B., & Rietjens, I. M. C. M. (2012). Alkaloids in the human food chain – Natural occurrence and possible adverse effects. *Molecular Nutrition & Food Research, 56*, 30–52. https://doi.org/10.1002/mnfr.201100165

Li, J., Tian, R., Liang, G., Shi, R., Hu, J., & Jiang, Z. (2021). Interaction mechanism of flavonoids with whey protein isolate: A spectrofluorometric and theoretical investigation. *Food Chemistry, 355*, Article 129617. https://doi.org/10.1016/j.foodchem.2021.129617

Liu, P., Lindstedt, A., Markkinen, N., Sinkkonen, J., Suomela, J.-P., & Yang, B. (2014). Characterization of metabolite profiles of leaves of bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.). *Journal of Agricultural and Food Chemistry, 62*(49), 12015–12026. https://doi.org/10.1021/jf503521m

Lo, B., Kasapis, S., & Farahnaky, A. (2021). Lupin protein: Isolation and techno-functional properties, a review. *Food Hydrocolloids, 112*, Article 106318. https://doi.org/10.1016/j.foodhyd.2020.106318

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frías, J., Martínez-Villaluenga, C., Sussmann, D., Duranti, M., Seger, A., Zander, P. M., & Pueyo, J. J. (2015). The future of lupin as a protein crop in Europe. *Frontiers in Plant Science, 6*, Article 160197. https://doi.org/10.3389/fpls.2015.00705

Muth, D., Marsden-Edwards, E., Kachlicki, P., & Stobiecki, M. (2008). Differentiation of isomeric malonylated flavonoid glyconjugates in plant extracts with UPLC-ESI/MS/MS. *Phytochemical Analysis, 19*, 444–452. https://doi.org/10.1002/pca.1073

Rees, A., Dodd, G. F., & Spencer, J. P. E. (2018). The effects of flavonoids on cardiovascular health: A review of human intervention trials and implications for cerebrovascular function. *Nutrients, 10*(12), Article 1852. https://doi.org/10.3390/nu10121852

Roland, W. S. U., Van Buren, L., Gruppen, H., Driesse, M., Gouka, R. J., Smit, G., & Vincken, J.-P. (2013). Bitter taste receptor activation by flavonoids and isoflavonoids: Modeled structural requirements for activation of hTAS2R14 and hTAS2R39. *Journal of Agricultural and Food Chemistry, 61*(44), 10454–10466. https://doi.org/10.1021/jf403387p

Roland, W. S. U., Vincken, J.-P., Gouka, R. J., Van Buren, L., Gruppen, H., & Smit, G. (2011). Soy isoflavones and other isoflavonoids activate the human bitter taste receptors hTAS2R14 and hTAS2R39. *Journal of Agricultural and Food Chemistry, 59*(21), 11764–11771. https://doi.org/10.1021/jf202816u

Shrestha, S., Hag, L., Haritos, V. S., & Dhital, S. (2021). Lupin proteins: Structure, isolation and application. *Trends in Food Science & Technology, 116*, 928–939. https://doi.org/10.1016/j.tifs.2021.08.035

Siger, A., Czubinski, J., Kachlicki, P., Dwiecki, K., Lampart-Szczapa, E., & Nogala-Kalucka, M. (2012). Antioxidant activity and phenolic content in three lupin species. *Journal of Food Composition and Analysis, 25*(2), 190–197. https://doi.org/10.1016/j.jfca.2011.10.002

Stobiecki, M., Staszków, A., Piasecka, A., Garcia-Lopez, P. M., Zamora-Natera, F., & Kachlicki, P. (2010). LC-MSMS profiling of flavonoid conjugates in wild Mexican lupine, *Lupinus reflexus*. *Journal of Natural Products, 73*(7), 1254–1260. https://doi.org/10.1021/np100139d

Tahara, S., Ingham, J. L., Nakahara, S., Mizutani, J., & Harborne, J. B. (1984). Fungitoxic dihydrofuranoisoflavones and related compounds in white lupin, *Lupinus albus*. *Phytochemistry, 23*(9), 1889–1900. https://doi.org/10.1016/S0031-9422(00)84936-5

Tian, Y., Laaksonen, O., Haikonen, H., Vanag, A., Ejaz, H., Linderborg, K., Karhu, S., & Yang, B. (2019). Compositional diversity among blackcurrant (*Ribes nigrum*) cultivars originating from European countries. *Journal of Agricultural and Food Chemistry, 67*(19), 5621–5633. https://doi.org/10.1021/acs.jafc.9b00033

Tian, Y., Liimatainen, J., Alanne, A. L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., & Yang, B. (2017). Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry, 220*, 266–281. https://doi.org/10.1016/j.foodchem.2016.09.145

Urrego-Pava, F., & Coy-Barrera, E. (2023). Isoflavone content and nutritional-related properties of debittered seeds from two Andean lupin (*Lupinus mutabilis* Sweet) ecotypes propagated in two soils. *Foods, 12*(9), Article 1841. https://doi.org/10.3390/foods12091841

Villacrés, E., Quelal, M. B., Fernández, E., Garcìa, G., Cueva, G., & Rosell, C. M. (2020). Impact of debittering and fermentation processes on the antinutritional and antioxidant compounds in *Lupinus mutabilis* sweet. *LWT, 131*, Article 109745. https://doi.org/10.1016/j.lwt.2020.109745

Waridel, P., Wolfender, J. L., Ndjoko, K., Hobby, K. R., Major, H. J., & Hostettmann, K. (2001). Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trap multiple-stage mass spectrometry for the differentiation of *C*-glycosidic flavonoid isomers. *Journal of Chromatography A, 926*(1), 29–41. https://doi.org/10.1016/S0021-9673(01)00806-8

Wojakowska, A., Piasecka, A., García-López, P. M., Zamora-Natera, F., Krajewski, P., Marczak, Ł., Kachlicki, P., & Stobiecki, M. (2013). Structural analysis and profiling of phenolic secondary metabolites of Mexican lupine species using LC–MS techniques. *Phytochemistry, 92*, 71–86. https://doi.org/10.1016/j.phytochem.2013.04.006

Yang, B., Liu, H., Yang, J., Gupta, V. K., & Jiang, Y. (2018). New insights on bioactivities and biosynthesis of flavonoid glycosides. *Trends in Food Science & Technology, 79*, 116–124. https://doi.org/10.1016/j.tifs.2018.07.006