Chemical Composition, Tocopherol and Carotenoid Content of Seeds from Different Andean Lupin (*Lupinus mutabilis*) Ecotypes

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Abstract

Andean lupin (*Lupinus mutabilis*) seeds are appreciated for their high protein and lipid contents and have potential applications as ingredients in food, cosmetic, and pharmaceutical industries. Nevertheless, the information about the seed composition (especially in lipophilic antioxidants) of ecotypes from distinct cropping areas is currently limited. Thus, the aim of the present research was to assess the morphological characteristics, chemical composition, tocopherol and carotenoid contents of the seeds of 33 Andean lupin ecotypes from different Peruvian regions, along with three *L. albus*, one *L. angustifolius* and one *L. luteus* controls. Significant differences were noted among the Andean ecotypes for all analyzed features. The protein, lipid and ash contents were 32.0–46.9, 13.6–18.6 and 2.7–4.4 g/100 g dry matter (DM), respectively. The seeds were rich in tocopherols (172.1–249.8 mg/kg DM; γ-tocopherol was 98% of total tocols) and low in carotenoids (0.69–2.89 mg/kg DM). Debittering increased the tocopherol content (227.0–378.2 mg/kg DM), probably because of the soluble components loss, although the carotenoid concentration remained unchanged. The Andean lupins had higher protein, lipid and tocopherol contents than *L. albus* and *L. angustifolius;* the *L. luteus* values were within the *L. mutabilis* range. These results suggest that *L. mutabilis* harbors nutritional characteristics that are well suited to modern food trends.

Key words: color; HPLC; lipids; morphological traits; proteins; seed weight

Introduction

The worldwide growth in the population age is spurring healthy nutrition trends that aim to delay aging and prevent chronic diseases as well as reduce their risk factors. This shift in the food preferences is favored by governments interested in promoting healthy habits as a step toward reducing morbidity and its associated health-care expenditure [1]. Sugar and certain carbohydrates are considered as the main culprit of bad health, while unsaturated fats and proteins are highly required in a balanced diet. A strong interest has also been generated in natural functional foods, which are the products containing biologically active ingredients that improve health or lower the risk of diseases by supplying, besides macronutrients, bioactive compounds such as vitamins and minerals, antioxidants, pre- and pro-biotics, and enzymes, among others. In this context, plant-based alternatives are considered more resource-efficient and cost-effective when compared to animal products [1]. Accordingly, the search for natural food ingredients with highly nutritive composition and rich in bioactive compounds is constantly increasing [2], leading to the rediscovery of traditional, but under-utilized, crops from different regions. In addition, in developing countries these same crops, when properly managed, help to counter health-threatening problems such as malnutrition and obesity.

The Andean lupin *(Lupinus mutabilis* Sweet), locally called "chocho" or "tarwi", has been cultivated for millennia in temperate and cold climates of the Andes. Similar to other legumes, it fixes the atmospheric nitrogen in its roots (from 120 to 160 kg N/ha per year) [3] and thus does not require expensive fertilization treatments. Furthermore, after harvest the plant residues improve the soil and favor the growth of the succeeding crops. The seeds are appreciated for their high content in proteins (on average, 41.92 g/100 g dry matter (DM)) [4] with balanced amino acids composition [2] and in lipids (19.85 g/100 g DM) [4] rich in mono-and polyunsaturated fatty acids [2, 5]. Starch is usually absent, and the carbohydrates are mainly oligosaccharides (e.g., stachyose and raffinose) and cell wall storage polysaccharides [6]. Some information on antioxidant compounds (such as flavonoids, anthocyanins, phenolic acids, tocols, and carotenoids) of the Mediterranean lupins have been provided previously [7-13]. On the contrary, the data on *L. mutabilis* are extremely scarce: the total polyphenols content was reported to be 12.1 mg gallic acid equivalent/g in one sample [14] and 9.67–11.35 mg catechin equivalent/g in six samples [15], while total tocol (all γ-tocopherol) was 103.0 mg/kg in one ecotype [8].

The Andean lupins are also characterized by the high content of bitter alkaloids (2.83 g/100 g DM) [4], which must be removed before consumption. This can be achieved by a treatment known as debittering or deamarization, which generally involves boiling and repeated soaking of the seeds in running water [16]. An alternative, permanent solution is breeding and/or selecting genotypes with low alkaloid content [2, 4], commonly labeled as "sweet" lupins. Once debittered, lupin seeds offer several potential applications, both as food for humans and for animals, as well as an ingredient for the cosmetic, pharmaceutical and medical industries [2, 4].

The spread of the Andean lupin beyond its ancestral borders is presently limited. Some studies performed in Europe on a small number of genotypes have shown low productivity when compared with other Old World lupin species because of indeterminate growth and drought susceptibility [4], but probably imputable to scarce adaptation of the tested genotypes to the new environments and to the dearth of improved varieties. To favor the expansion of this thrifty species to other cropping regions and to enhance the production of *L. mutabilis*-based foods, a better knowledge of the natural variation existing for the composition and the nutritional properties of its seeds is as crucial as the breeding of well-adapted varieties. Therefore, the aim of this research was to evaluate some morphological characteristics, the chemical composition, and the tocol and carotenoid contents of the seeds of 33 ecotypes of *L. mutabilis* from different ecogeographic regions of Peru. To properly appraise the results, five accessions of *L. albus*, *L. angustifolius* and *L. luteus* were analyzed as controls.

Materials and Methods

Materials

The seeds of the Andean lupin ecotypes from different regions of the Peruvian Andes at altitudes >2500 m above the sea level were kindly provided by the Leguminous Program of the National University of Agriculture, La Molina, Lima, Peru. The inventory of the 33 *L. mutabilis* tested, along with the information about their provenance, is reported in Online Resource 1. To better estimate their diversified origin, Online Resource 2 depicts a map of Peru showing the collection areas of the samples. One *L. albus* (Dulce 7) from Peru, two *L. albus*, one *L. angustifolius* and one *L. luteus* from Italy were analyzed as controls.

Morphological characteristics

The seed weight was determined from three independent 100-seed subsamples with a Europe 500 laboratory scale (PBI International, Milan, Italy). The seed dimensions (i.e., length, width and thickness) were measured on three independent ten-seed subsamples using a caliper.

Debittering and Milling

The lupin seeds debittering was performed as suggested by Córdova-Ramos et al. [16]. The complete elimination of the bitter alkaloids was assessed by sensorial analysis [17]. The bitter and debittered lupin samples (20-seed lots) were ground with the Grindomix GM 200 knife mill (Retsch GmbH, Germany) at 6000 RPM for 60 s, packed under vacuum in high-density polyethylene bags and stored at −20 °C until analysis.

Color

The color was assessed on three bitter wholemeal subsamples of each accession by the CIELAB method, using a Chroma Meter II tristimulus colorimeter (Minolta Italia SpA, Milan, Italy) with a standard white reflector plate and the illuminant C. Three parameters were assessed: L^* (luminosity), a^* (red-green), b^* (yellow-blue).

Chemical Composition

The chemical composition was assessed by the AOAC Official Methods 920.87 (proteins; conversion factor 6.25), 923.05 (lipids), 923.03 (ash) and 925.10 (moisture) [18]. The total carbohydrates were computed by the difference obtained by subtracting the measured protein, fat, ash, and water weights from the total weight. All tests were performed on two different batches of each sample.

Tocol and Carotenoid Contents

The tocols and carotenoids extracts were obtained from ground seeds after saponification [19] and quantified by NP-HPLC [20]. Briefly, 2 g of Andean lupin wholemeal were weighted in a screw-capped tube and saponified under nitrogen for 45 min at 70 °C, with the addition of 5 mL of ethanolic pyrogallol (60 g/L) as an antioxidant, 2 mL of ethanol (95%), 2 mL of sodium chloride (10 g/L) and 2 mL of potassium hydroxide (600 g/L). During the saponification process, the tubes were vortexed every 5–10 min. Later, they were cooled on an ice bath and 15 mL of sodium chloride (10 g/L) were added. The suspension was then extracted twice with 15 mL of hexane:ethyl acetate solution (9:1 v/v). The organic layer was collected and evaporated under vacuum, followed by nitrogen drying; the residue was dissolved in 2 mL hexane:isopropyl alcohol (99:1 v/v) and filtered through a 0.22-mm PTFE membrane.

For determining tocols content, the following system and operating conditions were used: Alltima SI column, 250 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); Alltima SI guard column 7.5 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); mobile phase, hexane:ethyl acetate:acetic acid (97.3:1.8:0.9, v/v/v); flow rate, 1.6 mL/min; pump L-2130 Elite LaChrom (VWR, Hitachi, Japan); and fluorimetric detector Jasco 821 FP Intelligent Spectrofluorometer (Japan) at excitation-emission wavelengths of 290 nm and 330 nm. The tocol standard curves, prepared from different concentrations of α-tocopherol (Fluka BioChemika, Buchs, Switzerland) and β-tocopherol (Supelco, Bellefonte, PA, USA) standards, were linear in the assessed intervals.

For the determination of carotenoid content, the following system and operating conditions were used: column Alltima SI column, 250 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); Alltima SI guard column 7.5 × 4.6 mm, 5 μm (Alltech Associates Inc., Deerfield, IL, USA); column oven at 20 °C L-2300 Elite LaChrom (VWR, Hitachi, Japan); mobile phase, hexane:isopropyl alcohol (5%); flow rate, 1.5 mL/min; pump L-2130 Elite LaChrom (VWR, Hitachi, Japan). The carotenoids were detected at 450 nm by using the Diode Array Detector L2450 Elite LaChrom (Merck, Hitachi, Japan). The HPLC system was controlled by the software EZChrom Client/Server (version 3.1.7). The carotenoids calibration curves,built using different concentrations of lutein standard (Fluka, St. Louis, MO, USA), β-carotene standard (Sigma, St. Louis, MO, USA), zeaxanthin (Extrasynthese, Genay, France) and seven β-cryptoxanthin (Extrasynthese, Genay, France) standards, were linear in the intervals assessed.

All analyses were performed on two different extracts per accession. The results were expressed in mg/kg on a dry matter basis (DM).

Statistical Analysis

To assess the existence of differences for the traits analyzed among the Andean lupins, among species or among their geographical area of origin, a one-way analysis of variance (ANOVA) was performed. In addition, to analyze the effect of debittering a two-way ANOVA was conducted considering the factors of ecotype and the treatment. The normal distribution of the data was verified and, when required, the log transformation was performed. When significant differences were detected, Fisher's least significant differences (LSD) at $p \leq 0.05$ were computed. All analyses were performed using the STATGRAPHICS® Centurion statistical program. The average values, standard deviations and standard errors were computed using the Excel program (Microsoft® Office Excel 2007).

Results and Discussion

Morphological characteristics

Online Resource 1 reports the weight and dimension of the seeds of the 33 Andean lupin ecotypes and of the five control varieties, while the color parameters (*L**, *a** and *b**) of the bitter and debittered seed flours are depicted in Online Resource 3. The ANOVAs (not shown) highlighted the significant differences among the Andean lupin ecotypes ($p \leq 0.001$) for weight, dimensions and color indices, while the species differed ($p \leq 0.001$) for all the traits except for *L** and *a**. The ANOVA comparing the results before and after debittering (Online Resource 4) indicated that ecotype and debittering, and, to a lower degree, their combination had significant effects on the color coordinates ($p \leq 0.001$). Ecotype was by far the most important parameter for L^* and a^*; treatment, instead, was the major factor for b^*, decreasing approximately 17% the yellow-blue coordinate of debittered *L. mutabilis* samples.

On average, *L. mutabilis* seeds were 9.64±0.11mm long, 7.91±0.11 mm wide, 4.95±0.11 mm thick and weighed 0.25±0.06 g. Chacas, Tauribamba Sihuas 3 N, Cheje Copani and Puno 2 blanquita had the lightest seeds, while Congona and SGC 22 had the heaviest ones. The shape varied from oblong in Puno 2 blanquita (length/width ratio: 1.34) to isodiametric in Huallanca (ratio: 1.00). Similar dimensions (9.3±0.6, 8.2±1.0 and 4.3±0.3 mm) and weight (0.205±0.042 g) for a seed sample from Tarma (Central Peruvian Andes) are reported [21]. Furthermore, a 0.16–0.32 g/seed weight range can be deduced from Figure 2 of a study on the domestication of the Andean lupin [22]. The weight of *L. angustifolius* and one *L. albus* (cv. Ares) fell within the interval of *L. mutabilis* (0.184-0.302 g), while the *L. luteus* and two *L. albus* were heavier. The seed dimensions of the controls were generally in the Andean lupin range, except for *L. albus* cv. Multitalia, which was longer and wider.

Online Resource 5 depicts the images of all lupin samples. It is easy to perceive the broad variation existing for pericarp color, ranging from white to cream to brown and to black; two-color ecotypes (cream and light brown: Moteado beige and SGC 22; cream and gray: H6 INIA BP; cream and black: Tauribamba Sihuas 1BN, Huanuco 1BN, CD-Junin 7-2, CD-Junin 10-2, H6 INIA BN) are also present. The color of the pericarp influences the color of the wholemeal which, conversely, may have some relevance on the consumer/industry preference. Hence, the CIELab color parameters of the bitter whole meals displayed a broad variation. The luminosity ranged from 67.2 (Yana tarwi) to 87.5 (Yanamuclo 008-3); unsurprisingly, the three samples with brown or black pericarp (Tauribamba Sihuas 3N, Yana tarwi, Cheje Copani) showed the lowest brightness. The a^* coordinate ranged between 0.3 (Chacas and CD-Junin 10-2) and −3.4 (Congona); the ecotypes with pigmented pericarp generally had a larger red component. Finally, the b^* coordinate varied between 22.0 (Huallanca and Tauribamba Sihuas 3 N) and 33.3 (Compuesto blanco semiprecoz); as already observed for brightness, the dark color of the pericarp decreased *b**. The values recorded for the controls fell consistently within the variation range observed in *L. mutabilis*. The information about color measurement in lupin seeds is exiguous, and it is often difficult to compare different genotypes from different agronomic conditions. However, to the best of our knowledge, no information is available about *L. mutabilis*, *L**, *a** and *b** readings of 61.21±0.10, 2.22±0.10 and 11.47±0.04 [23], and of 65.2±0.03, 7.0±0.11 and 20.3±0.11 [24] are reported in *L. albus*.

Chemical Composition

Table 1 shows the average wholemeal chemical composition of the four species obtained from the bitter *Lupinus* seeds. The ANOVA (not shown) showed the existence of significant differences ($p \leq 0.001$) among the ecotypes. The moisture content in our Andean lupins ranged from 8.19 to 13.80 g/100 g, the protein content from 32.03 g/100 g DM (Cheje Copani) to 46.90 g/100 DM (SGC 22) and the lipid content from 13.60 g/100 g DM (Huanuco 2) to 18.55 g/100 g DM (Andenes INIA). The complete scores of the 33 ecotypes and the five controls are presented in the Online Resource 6. Our results are therefore alike those reported for protein and lipid content in Ecuadorian *L. mutabilis* (41.4–47.7 g/100 g DM and 15.0–20.1 g/100 g DM, respectively) [25] and in Peruvian ecotypes (34.6–50.2 and 14.3–23.6 g/100 g DM, respectively) [4], as well as to the values summarized by Carvajal-Larenas et al. [26] (32.0–52.6 and 13.0–24.6 g/100 g DM, respectively). The lowest ash content was observed in Yanamuclo 008-1 (2.70 g/100 g DM) and the highest in Puno 2 blanquita (4.40 g/100 g DM), a variation very similar to that (2.4–5.2 g/100 g DM) reported by Carvajal-Larenas et al. [26]. The carbohydrates, obtained by difference, ranged between 24.5 g/100 g DM (Andenes INIA) and 33.90 g/100 g DM (Lircay), which is in the lower end of the distribution (26.1–43.2 g/100 g DM) described by Carvajal-Larenas et al. [26].

The composition results of the different Old World lupins were similar to those presented by several authors [23, 24, 27, 28]. No significant differences were noted among species for ash content, although *L. angustifolius and L. albus* demonstrated lower protein and lipid concentrations ($p \leq 0.01$and $p \leq 0.001$, respectively) than *L. mutabilis*. The *L. luteus* values were within the Andean lupin range.

Tocol and Carotenoid Contents

Table 2 depicts the content of the tocopherols in the bitter and debittered samples of the four species, while no tocotrienols were detected. ANOVAs (data not shown) stressed the existence of significant differences ($p \leq 0.001$) among the *L. mutabilis* ecotypes as well as among the species.

The most abundant homologue in the Andean lupin bitter wholemeals was γ-tocopherol, representing almost 98% of total tocols, followed by β-tocopherol and δ-tocopherol (approximately 1% each) and by α-tocopherol (0.3%). A wide range of variation was also observed for each homologue (Online Resource 7) as well as for their total content (Figure 1). The minimum total tocopherol concentration (172.1 mg/kg DM) was recorded in Vicos, while the maximum (249.8 mg/kg DM) was recorded in Huanuco 1. These values were approximately double than those detected in a sample of wild *L. mutabilis* from Ecuador, which is rich only in γ-tocopherol (103.0 mg/kg DM) [8].

Among the tested Old World lupins, γ–tocopherol was also the most abundant compound, albeit far less abundant in *L. angustifolius* and *L. albus* (Table 2) than in *L. luteus*, which, on the other hand, showed γ-tocopherol and total tocopherol contents similar to that in *L. mutabilis*.

The total tocols values of the control cultivars were similar to those reported for *L. albus* (120.0–132.8 and 63.2–134.0 mg/kg DM) [7, 8], and for *L. angustifolius* (73.5–95.4 mg/kg DM) [8], but greater than those described for domesticated and wild *L. luteus* (14.5–22.7 mg/kg DM) [9]. Similarly, γ-tocopherol was the most abundant homologue as per the records of the abovementioned studies.

The total carotenoid content of the Andean lupins (Table 2) was low (average: 1.61 mg/kg DM) and mainly limited to the presence of lutein (average: 1.22 mg/kg DM, i.e., approximately 76% of the total). Other carotenoids detected were, in a decreasing order, (α+β)-carotene (0.27 mg/kg DM, i.e., 17% of the total), zeaxanthin (0.10 mg/kg DM, i.e., 6% of the total) and β-cryptoxanthin (0.02 mg/kg DM, i.e., 1% of the total). Nevertheless, a limited variation for total carotenoids (0.69–2.89 mg/kg DM; Figure 1) and carotenoids composition [e.g. (α+β)-carotene varied from 9% to 40% and lutein from 0.55% to 0.86% of total carotenoids; Online Resource 7] was observed.

Among the controls, *L. angustifolius* and *L. albus* contained the highest amounts of (α+β)-carotene, lutein, and zeaxanthin, while *L. luteus* had the lowest (similar to *L. mutabilis*). Very little information is available on carotenoid content and composition in *Lupinus* species. For instance, Fernández-Marín et al. [9] observed 4.1 and 7.5 mg/kg DM in domesticated and wild *L. luteus*, and the same compounds were recorded in identical decreasing concentrations, while El-Difrawi and Hudson [13] reported 4.7 mg/kg in *L. albus*.

The ANOVA (Online Resource 4) comparing the bitter and the debittered samples highlighted how debittering, ecotype, and their interaction affected the tocopherols content, while, among the carotenoids only, (α+β)-carotene and β-cryptoxanthin were influenced by the type of treatment. After debittering, a general increase in tocopherol content was recorded (Table 2) because of the effect of concentration on this process (which includes boiling and long water steeping) displays by washing away soluble compounds, such as carbohydrates and minerals, leading to a percentual increase in the protein and lipid fractions [16, 29]. The carotenoids content remained almost unchanged, possibly because the abovementioned concentration effect was offset by their partial degradation during debittering. Tocopherols and carotenoids are generally degraded during processing by the enzymatic activity of lipoxygenases; however, while tocopherols are stable at high temperatures, carotenoids are not [19, 30]. Lupin debittering included 12 h of hydration at the room temperature, with a probable partial enzymatic activation, followed by a 1-h boiling phase. Therefore, a limited enzymatic degradation can be envisioned only before enzyme inactivation at the boiling temperature. A negligible carotenoid loss during pasta cooking in boiling water has been reported [31], suggesting that the significant loss during lupin debittering should be attributed to the longer boiling step (10–16 min vs. 60 min, respectively).

Geographical Origin

The ANOVA performed after grouping the Andean lupins in geographical areas, viz. North (Online Resources 1 and 2, areas A and B), Center-North (C and D), Center-South (E and F) and South (G and H) suggested that significant differences existed only for β-tocopherol ($p \leq 0.05$), for the different carotenoids ($p \leq 0.05$ and $p \leq 0.01$), for total carotenoid content ($p \leq 0.001$) and for protein content ($p \leq 0.05$). In general, greater concentrations of β-tocopherol were detected in ecotypes coming from the Center-South and South regions of Peru, and the lutein and total carotenoids were more abundant in the samples collected from the South, while the protein content was slightly greater in those collected from the North. The Northern region (2700–3500 m a.s.l.) is characterized by dry, mild days and cold, frostless nights, as well as a rainy season between October and April, with a long vegetative period (between 7 and 9 months). The North-Central region (3000–3600 m a.s.l.) climate varies from dry and temperate (Huanuco) to warm and humid, with sporadic nightly frosts (Ancash) and a 6–8-months vegetative phase. The South-Central region (3200–3800 m a.s.l.) climate vary between temperate and cold, with unpredictable frost spells and a shorter growing cycle (5–7 months). The Southern region (3600–4000 m a.s.l.) climate varies from semi-dry and cold (Cuzco) to cold and very dry with frequent and sudden frosts, and the rainfall usually lasts from December to April, with a short vegetative period (4–6 months). Carotenoids and tocols are compounds produced by plants for their protection against external threats [32]; hence, the differences between the ecotypes coming from different locations are most likely a consequence of the selection pressure exerted by the harsher climatic conditions of the South-Central and Southern Andean environments.

Conclusions

The highly nutritive properties and the significant differences in the chemical composition and the tocopherol and carotenoid contents noted among the Andean lupin ecotypes as well as vs. Old world lupins stresses the importance of this little-exploited species for food production. Extremely relevant was that the tocopherol content was high even after the long (5 days) debittering treatment required to make lupins edible. In general, *L. mutabilis* showed higher protein, lipid and tocopherol contents than the control cultivars of *L. albus* and *L. angustifolius*. The *L. luteus* cv. Percoz was comparable to *L. mutabilis* for all these traits. These results suggest that *L. mutabilis* harbors nutritional characteristics, especially the high tocopherol content, which is well suited to the modern food trends with an emphasis on plant-based products that are rich in proteins, unsaturated fats and functional ingredients.

Declarations

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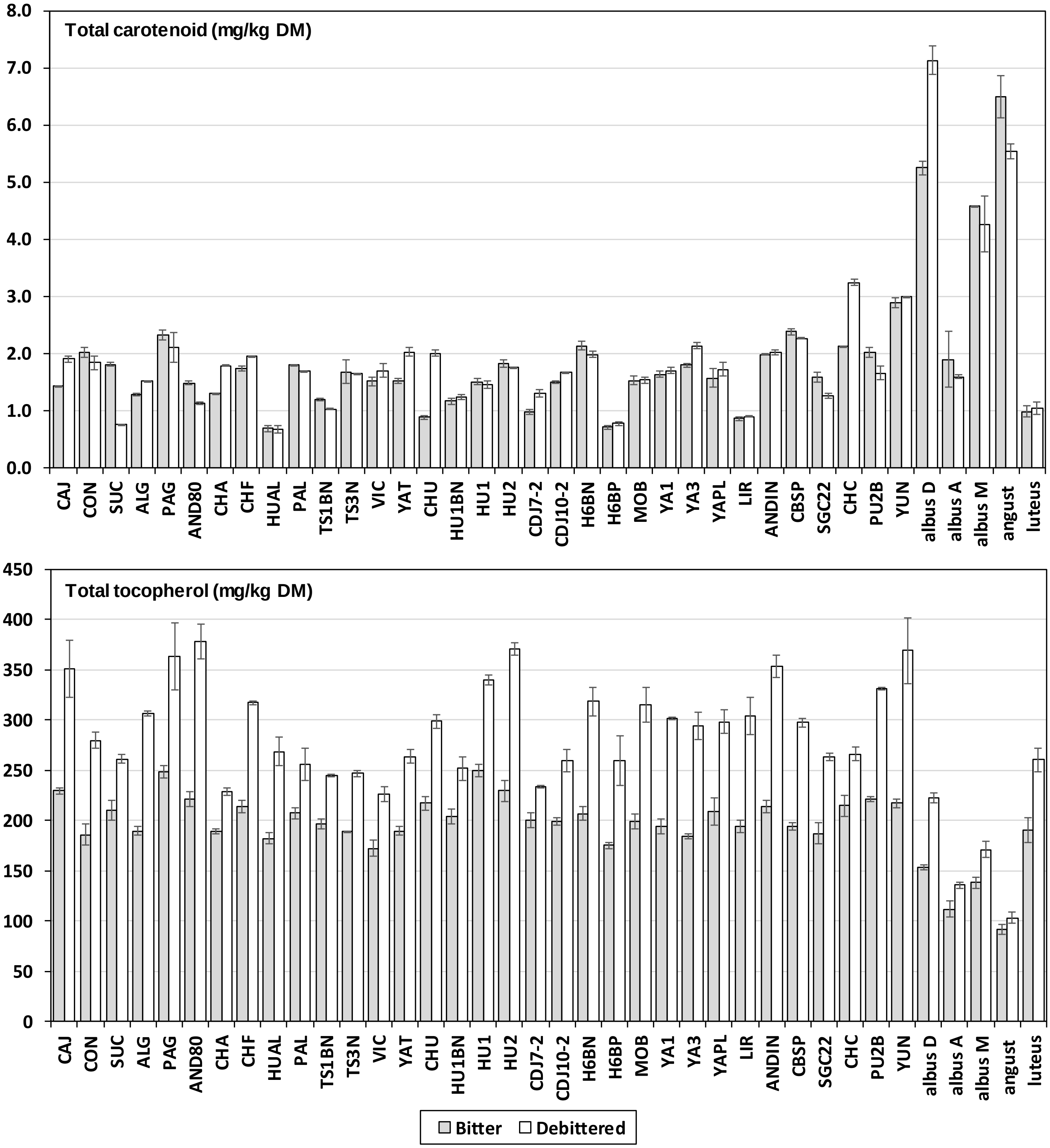
Captions to Figures

Fig. 1 Total tocopherol and total carotenoid contents of 33 *L. mutabilis*, three *L. albus*, one *L. angustifolius* and one *L. luteus* analyzed (ecotype codes are reported in Table 1). Bars represent the mean ± standard deviation of the mean.



Total carotenoid (mg/kg DM)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
CAJ
CON
SUC
ALG
PAG
AND80
CHA
CHF
HUAL
PAL
TS1BN
TS3N
VIC
YAT
CHU
HU1BN
HU1
HU2
CDJ7-2
CDJ10-2
H6BN
H6BP
MOB
YA1
YA3
YAPL
LIR
ANDIN
CBSP
SGC22
CHC
PU2B
YUN
albus D
albus A
albus M
angust
luteus
Total tocopherol (mg/kg DM)
450
400
350
300
250
200
150
100
50
0
Bitter
Debittered

385

Table 1. Mean value ± standard error and range (in parentheses) of moisture (g/100 g) and chemical composition (g/100 g DM) of bitter seeds from 33 *L. mutabilis*, three *L. albus*, one *L. angustifolius* and one *L. luteus* accessions.

	L. mutabilis	*L. albus*	*L. angustifolius*	*L. luteus*
Moisture	9.99 ± 0.14	9.79 ± 0.75	11.15 ± 0.06	8.06 ± 0.03
	(8.19 - 13.80)	*(7.98 - 11.04)*		
Protein	40.87 ± 0.40	31.02 ± 0.56	29.16 ± 0.39	39.03 ± 1.93
	(32.03 - 46.90)	*(29.93 - 32.20)*		
Lipid	16.12 ± 0.14	8.41 ± 0.71	5.15 ± 0.04	14.30 ± 0.12
	(13.60 - 18.55)	*(7.56 - 9.52)*		
Ash	3.58 ± 0.06	3.37 ± 0.03	3.22 ± 0.06	3.42 ± 0.07
	(2.70 - 4.40)	*(3.25 - 3.50)*		
Carbohydrates	29.45 ± 0.43	47.41 ± 0.52	51.31 ± 0.35	35.19 ± 1.85
	(24.85 - 33.90)	*(45.70 - 49.68)*		

Table 2. Tocopherol and carotenoid content (mg/kg DM; mean ± standard error) of bitter and debittered seeds from 33 *L. mutabilis*, three *L. albus*, one *L. angustifolius* and one *L. luteus* accessions. In parentheses, the ranges of the values observed.

	L. mutabilis		*L. albus*		*L. angustifolius*		*L. luteus*	
	Bitter	Debittered	Bitter	Debittered	Bitter	Debittered	Bitter	Debittered
α-tocopherol	0.63 ± 0.03	0.84 ± 0.05	1.07 ± 0.20	1.58 ± 0.06	2.08 ± 0.01	1.60 ± 0.08	0.83 ± 0.01	0.50 ± 0.05
	(0.27 - 1.24)	*(0.18 - 1.72)*	*(0.88 - 1.22)*	*(1.45 - 1.76)*				
β-tocopherol	2.08 ± 0.11	2.38 ± 0.14	0.42 ± 0.06	0.74 ± 0.25	0.24 ± 0.03	0.97 ± 0.75	1.07 ± 0.21	0.89 ± 0.00
	(0.70 - 3.77)	*(0.75 - 5.06)*	*(0.34 - 0.50)*	*(0.29 - 0.99)*				
γ-tocopherol	199.7 ± 2.4	288.0 ± 5.5	131.49 ± 11.09	172.06 ± 15.84	88.9 ± 3.7	100.4 ± 4.7	187.4 ± 8.5	257.2 ± 8.5
	(168.6 - 244.8)	*(222.7 - 369.6)*	*(108.5 - 150.7)*	*(131.0 - 218.4)*				
δ-tocopherol	1.86 ± 0.10	3.41 ± 0.13	1.61 ± 0.20	2.25 ± 0.45	0.67 ± 0.02	0.27 ± 0.01	1.26 ± 0.11	2.26 ± 0.09
	(0.43 - 3.36)	*(1.33 - 5.01)*	*(1.38 - 1.94)*	*(2.08 - 2.44)*				
Total tocopherol	204.3 ± 2.4	294.6 ± 5.5	134.60 ± 11.15	176.63 ± 16.22	91.95 ± 3.7	103.2 ± 4.0	190.5 ± 8.6	260.8 ± 8.3
	(172.1 - 249.8)	*(227.0 - 378.2)*	*(111.7 - 153.5)*	*(135.9 - 222.7)*				
(α+β)-carotene	0.27 ± 0.02	0.36 ± 0.03	0.56 ± 0.08	0.71 ± 0.02	1.10 ± 0.11	0.86 ± 0.02	0.14 ± 0.01	0.22 ± 0.05
	(0.06 - 0.60)	*(0.09 - 1.22)*	*(0.39 - 0.82)*	*(0.39 - 0.89)*				
β-cryptoxanthin	0.02 ± 0.00	0.04 ± 0.00	0.01 ± 0.001	0.04 ± 0.004	0.02 ± 0.00	0.05 ± 0.00	0.01 ± 0.00	0.03 ± 0.00
	(0.00 - 0.03)	*(0.02 - 0.07)*	*(0.011 - 0.017)*	*(0.025 - 0.057)*				
Lutein	1.22 ± 0.05	1.19 ± 0.05	2.49 ± 0.25	2.84 ± 0.21	3.93 ± 0.16	3.39 ± 0.04	0.75 ± 0.05	0.73 ± 0.03
	(0.43 - 2.20)	*(0.43 - 2.28)*	*(0.85 - 3.74)*	*(0.84 - 5.07)*				

Zeaxanthin	0.10 ± 0.00	0.10 ± 0.00	0.85 ± 0.13	0.76 ± 0.12	1.45 ± 0.01	1.25 ± 0.03	0.09 ± 0.01	0.07 ± 0.01
	(0.03 - 0.21)	*(0.04 - 0.19)*	*(0.65 - 1.02)*	*(0.34 - 1.17)*				
Total carotenoid	1.61 ± 0.06	1.68 ± 0.07	3.91 ± 0.46	4.34 ± 0.32	6.49 ± 0.26	5.55 ± 0.09	0.99 ± 0.07	1.05 ± 0.07
	(0.69 - 2.89)	*(0.68 - 3.25)*	*(1.90 - 5.26)*	*(1.60 - 7.14)*				