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Nutritional characteristics of seed proteins in 15 *Lathyrus* species (fabaceae) from Southern Spain

Elena Pastor-Cavada [a], Rocio Juan [b], Julio E. Pastor [b], Manuel Alaiz [a], Javier Vioque [a,*]

[a] Instituto de la Grasa (C.S.I.C.), Avda Padre García Tejero 4, 41012-Sevilla, Spain
[b] Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, 41012-Sevilla, Spain

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ABSTRACT

The nutritional characteristics of seed proteins of 15 Spanish *Lathyrus* species have been analyzed. Protein contents in studied *Lathyrus* ranged from 17.7% in *Lathyrus sativus* to 25.6% in *L. tingitanus* with a 22.4% average protein content in studied *Lathyrus*. Among essential amino acids the most abundant were Leu, Lys, Phe, Thr and Val. Also, all species contained Lys above FAO recommendations. On the contrary, all species were limiting in Trp ranging from 0.5% to 0.8% content and were also limiting in sulphur containing amino acids, Met and Cys. In vitro protein digestibility (IVPD) in studied *Lathyrus* ranged from 76.0% in *L. pratensis* to 86.5% in *L. annuus*, with an average IVPD of 80.5%. Nutritional parameters, such as protein efficiency ratio, corresponded to high quality proteins. Protein digestibility corrected amino acid score was similar to the observed in other legumes such as lentil or peanut. An analysis of similarity based on the profile of seed protein amino acid composition showed, in the cluster generated, correlation with the taxonomic classification of these species according to morphological characters. Results confirm the interest of studying wild populations of cultivated and non-cultivated *Lathyrus* species as source of seeds with good nutritional characteristics.

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1. Introduction

Pulses are an important source of proteins and carbohydrates of high nutritional quality. Legume proteins are rich in lysine and complement the proteins in cereals, which are deficient in this amino acid. In addition to be a good source of nutrients, pulses reduce the risk of suffering cardiovascular diseases (Anderson & Major, 2002), diabetes (Rizkalla, Bellisle, & Slama, 2002) and some types of cancer (Mathers, 2002). The genre *Lathyrus* includes 187 species, and belongs to the *Fabeae* tribe together with other genres of great economical and nutritional importance such as *Lens*, *Pisum*, and *Vicia*. Archeobotanical studies show that *Lathyrus sativus* and *Lathyrus cicera* were cultivated in the Iberian Peninsula in the Neolithic (Peña-Chocarro & Peña, 1999). There is evidence suggesting that *L. sativus* is probably the oldest crop in Europe (Kislev, 1989). *Lathyrus* species are also well known for the presence of neurotoxic and osteotoxic non protein amino acids that have limited their use in human nutrition (Akalu, Johansson, & Nair, 1998; Yang et al., 2006).

Some of the 32 *Lathyrus* species in the Iberian Peninsula (Talavera et al., 1999) are of economical interest. Thus, *L. sativus*, *L. cicera* and *L. tingitanus* are seed crops, while *L. ochrus*, *L. latifolius* and *L. sylvestris* are used to feed animals. Although *Lathyrus* have been cultivated since ancient times, few data regarding protein composition and nutritional quality are reported in the literature and these are restricted to the most common *Lathyrus* species. Among *Lathyrus* species, *L. sativus* proteins are the best studied and characterized (Rosa, Ferreira, & Teixeira, 2000)

In this work the amino acid composition and nutritional quality of seed proteins have been studied in 15 *Lathyrus* species distributed throughout Southern Spain. Results may provide with useful information concerning which of the species possess better nutritional properties in their seed proteins, and which wild taxa could be of interest from a nutritional point of view.

2. Material and methods

2.1. Materials

Trypsin, chymotrypsin and peptidase were from Sigma (Tres Cantos, Madrid, Spain). Diethyl ethoxymethylenemanolate was purchased from Fluka. All other chemicals were of analytical grade.

* Corresponding author. Tel.: +34 954611550; fax: +34 959616790.
E-mail addresses: jvioque@cica.es, jvioque@ig.csic.es (J. Vioque).

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Table 1
Seed protein contents and in vitro protein digestibility in *Lathyrus* species studied. Data are the average ± standard deviation of indicated number of populations studied. Superscripts letters indicate significant differences between values in the column (Bonferroni's test, $^{**}P < 0.01$, $^{***}P < 0.001$).

Species	Number of populations studied	Seed protein content (g/100 g flour)***	Number of populations studied	In vitro protein digestibility (g/100 g protein)**
L. amphicarpos	3	19.9 ± 2.6^{ab}	3	81.3 ± 0.6^{abc}
L. angulatus	4	23.8 ± 2.0^{ab}	3	80.3 ± 1.5^{ab}
L. annuus	5	21.0 ± 1.7^{ab}	3	86.5 ± 2.8^{c}
L. aphaca	6	20.8 ± 0.5^{ab}	3	79.0 ± 3.0^{bc}
L. cicera	6	21.5 ± 1.0^{ab}	3	84.0 ± 0.0^{ab}
L. clymenum	7	21.6 ± 1.8^{ab}	3	80.3 ± 1.5^{a}
L. filiformis	2	21.2 ± 1.1^{ab}	2	76.6 ± 0.0^{abc}
L. hirsutus	3	25.1 ± 2.9^{ab}	3	80.6 ± 0.6^{ab}
L. latifolius	4	24.4 ± 1.8^{ab}	3	79.0 ± 1.7^{ab}
L. ochrus	4	20.2 ± 1.1^{ab}	4	79.2 ± 1.7^{ab}
L. pratensis	4	23.2 ± 0.9^{ab}	3	76.0 ± 1.0^{a}
L. sativus	2	17.7 ± 2.4^{a}	2	80.2 ± 0.3^{abc}
L. setifolius	4	22.7 ± 3.8^{ab}	3	80.6 ± 0.8^{abc}
L. sphaericus	5	23.5 ± 1.6^{ab}	3	81.0 ± 0.0^{abc}
L. tingitanus	7	25.6 ± 1.9^{b}	3	84.7 ± 2.9^{bc}

The samples of *Lathyrus* seed were taken from wild populations. Voucher specimens of the populations studied are deposited in the Herbarium of the Department of Plant Biology and Ecology of the University of Seville.

2.2. Amino acid analysis and protein content

Samples (10 mg) were hydrolyzed with 4 ml of 6 N HCl. The solutions were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz, Navarro, Girón, and Vioque (1992), using D, L-α-amino-butyric acid as internal standard. The HPLC system consisted of a Model 600E multi-system with a 484 UV–Vis detector (Waters) equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C_{18}, 4 μm, Waters). A binary gradient was used for elution with a flow of 0.9 ml/min. The solvents used were (A) sodium acetate (25 mM) containing sodium azide (0.02% w/v) pH 6.0 and (B) acetonitrile. Elution was as follows: time 0.0–3.0 min, linear gradient from A/B (91:9) to A/B (86/14); 3.0–13.0 min, elution with A/B (86/14); 13.0–30.0 min, linear gradient from A/B (86:14) to A/B (69/31); 30.0–35.0 min, elution with A/B (69:31). The column was maintained at 18 °C. Tryptophan was analyzed by HPLC after basic hydrolysis according to Yust et al. (2004). Protein contents of studied samples were determined after amino acid analyses.

2.3. In vitro protein digestibility (IVPD)

In vitro protein digestibility was determined according to the method of Hsu, Vavak, Satterlee, and Miller (1977). Samples containing 62.5 mg of protein were suspended in 10 ml of water and the pH was adjusted to 8.0. An enzymatic solution containing 1.6 mg trypsin (17.7 BAEE U mg^{-1}), 3.1 mg α-chymotrypsin (43 U mg^{-1}) and 1.3 mg peptidase (50 U g^{-1}) per ml was added to the protein suspension in a 1:10 v/v ratio. The pH of the mixture was measured after 10 min and the in vitro digestibility was calculated as a percentage of digestible protein using the equation: % digestible protein = 210.464–18.103 × pH.

2.4. Determination of nutritional parameters

The amino acid composition of studied *Lathyrus* species was used for the determination of several nutritional parameters of *Lathyrus* seed proteins:

- Amino acid score (chemical score) was calculated as:
  % sample essential amino acids contents/% recommended essential amino acids (FAO/WHO, 1985).

Table 2
Seed protein amino acids composition of *Lathyrus* species studied. Data expressed as g/100 g protein are the average ± standard deviation of indicated number of populations studied.. Superscripts letters indicate significant differences between values of the same column (Bonferroni's test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Species	n	[1]Asp	[2]Glu***	Ser*	His***	Gly	Thr***	Arg***	Ala**
L. amphicarpos	3	12.5 ± 0.7	19.7 ± 0.2^{ab}	6.2 ± 0.1^{ab}	2.8 ± 0.1^{ab}	4.5 ± 0.1	4.5 ± 0.2^{a}	9.8 ± 0.4^{ab}	4.7 ± 0.0^{ab}
L. angulatus	4	12.4 ± 0.7	19.1 ± 0.7^{ab}	6.1 ± 0.2^{ab}	2.5 ± 0.1^{ab}	5.0 ± 0.2	4.3 ± 0.1^{a}	10.9 ± 2.9^{ab}	4.7 ± 0.1^{ab}
L. annuus	5	11.8 ± 0.8	21.5 ± 0.5^{b}	5.8 ± 0.2^{ab}	2.6 ± 0.2^{ab}	4.4 ± 0.2	4.5 ± 0.2^{a}	8.9 ± 0.4^{ab}	6.0 ± 1.0^{b}
L. aphaca	6	12.3 ± 1.0	17.9 ± 1.0^{a}	6.1 ± 0.3^{ab}	2.7 ± 0.1^{ab}	4.7 ± 0.8	4.5 ± 0.3^{a}	9.6 ± 1.2^{ab}	4.8 ± 0.2^{ab}
L. cicera	6	12.6 ± 0.7	19.7 ± 0.4^{ab}	6.2 ± 0.2^{ab}	2.7 ± 0.1^{ab}	4.5 ± 0.1	4.4 ± 0.1^{a}	9.5 ± 0.3^{ab}	4.8 ± 0.1^{ab}
L. clymenum	7	12.2 ± 0.7	19.3 ± 0.5^{a}	6.4 ± 0.2^{b}	2.5 ± 0.2^{a}	4.3 ± 0.2	4.2 ± 0.1^{a}	8.7 ± 0.3^{a}	4.9 ± 0.1^{ab}
L. filiformis	2	12.3 ± 2.0	17.5 ± 0.1^{a}	6.3 ± 0.7^{ab}	3.3 ± 0.4^{b}	5.0 ± 0.4	5.0 ± 0.3^{ab}	9.3 ± 0.6^{ab}	6.1 ± 0.2^{ab}
L. hirsutus	3	12.3 ± 1.1	20.0 ± 0.3^{ab}	5.9 ± 0.2^{ab}	2.5 ± 0.1^{ab}	4.4 ± 0.1	5.8 ± 0.2^{b}	10.3 ± 2.0^{ab}	4.9 ± 0.2^{ab}
L. latifolius	4	10.6 ± 1.3	18.8 ± 1.0^{a}	6.4 ± 0.3^{b}	3.0 ± 0.1^{ab}	4.9 ± 0.5	4.4 ± 0.1^{a}	12.2 ± 1.1^{ab}	5.0 ± 0.2^{ab}
L. ochrus[A]	2	12.7 ± 1.1	19.8 ± 0.5^{ab}	6.5 ± 0.0^{b}	2.5 ± 0.0^{ab}	4.3 ± 0.1	4.3 ± 0.1^{a}	8.9 ± 0.3^{ab}	4.7 ± 0.0^{ab}
L. ochrus[B]	2	12.8 ± 0.8	19.3 ± 0.5^{ab}	6.5 ± 0.1^{b}	2.7 ± 0.1^{ab}	4.4 ± 0.2	4.2 ± 0.1^{a}	8.8 ± 0.2^{ab}	4.9 ± 0.0^{ab}
L. pratensis	4	11.4 ± 1.3	19.0 ± 0.7^{a}	6.0 ± 0.1^{ab}	3.1 ± 0.5^{b}	4.9 ± 0.1	4.7 ± 0.1^{a}	9.9 ± 1.0^{ab}	5.1 ± 0.3^{ab}
L. sativus	2	12.1 ± 0.1	19.3 ± 0.1^{ab}	6.4 ± 0.0^{ab}	2.7 ± 0.1^{ab}	4.9 ± 0.1	4.6 ± 0.3^{a}	10.8 ± 1.4^{ab}	5.0 ± 0.1^{ab}
L. setifolius	4	12.6 ± 0.5	19.6 ± 0.4^{ab}	6.2 ± 0.2^{ab}	2.5 ± 0.2^{ab}	4.5 ± 0.2	4.5 ± 0.2^{a}	8.6 ± 0.2^{ab}	5.1 ± 0.2^{ab}
L. sphaericus	5	12.5 ± 1.6	18.3 ± 0.5^{a}	5.9 ± 0.4^{ab}	2.5 ± 0.3^{ab}	4.4 ± 0.6	4.4 ± 0.1^{a}	12.6 ± 1.7^{ab}	4.4 ± 0.1^{a}
L. tingitanus	7	11.1 ± 0.9	19.4 ± 1.2^{ab}	5.6 ± 0.5^{a}	2.4 ± 0.1^{a}	5.3 ± 1.4	4.2 ± 0.4^{a}	12.9 ± 2.5^{b}	5.3 ± 1.3^{ab}
FAO[3]					1.9		3.4		

[1]Asp + Asn. [2]Glu + Gln. [3]Suggested pattern of amino acid requirements (FAO/WHO/UNU, 1985). [4]Tyr + Phe. [5]Met + Cys. *L. ochrus*[A]: populations from Sevilla and Jaen. *L. ochrus*[B]: populations from Cadiz.

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