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INVESTIGACIÓN

Nutritional and functional characteristics of *Erophaca baetica* seeds, a legume endemic to the Mediterranean region

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RESUMEN

Características nutricionales y funcionales de semillas de *Erophaca baetica*, una leguminosa endémica de la región Mediterránea

Erophaca baetica es una leguminosa endémica de la región mediterránea. Aunque los frutos y las semillas son grandes, la presencia del alcaloide swainsonina causante de locoismo, ha impedido su uso como alimento para animales o para nutrición humana. El contenido proteico y su perfil cromatográfico, la composición de aminoácidos, de ácidos grasos, y contenido de polifenoles se han determinado con el fin de explorar el potencial de las semillas de E. *baetica* como una fuente de proteínas y de componentes funcionales de la dieta. El contenido de proteínas es del 36% (w / w), y el análisis de aminoácidos reveló deficiencia en aminoácidos azufrados, triptófano y lisina. El contenido de lisina bajo es probablemente debido a la abundancia de alcaloides metabólicamente derivados de este aminoácido. Los ácidos oleicos y linoleico son los principales ácidos grasos en las semillas. La actividad antioxidante de los extractos de polifenoles fue mayor que la actividad de los polifenoles extraídos de la mayoría de las semillas de leguminosas comestibles. Por lo tanto, las semillas de E. *baetica* representan una fuente interesante de componentes funcionales y nutricionales con la condición de que los alcaloides antinutricionales sean quitados antes.

PALABRAS CLAVE: Ácidos grasos – Aminoácidos – Erophaca baetica – Polifenoles – Proteínas.

SUMMARY

Nutritional and functional characteristics of *Erophaca baetica* seeds, a legume endemic to the Mediterranean region

Erophaca baetica is a legume endemic to the Mediterranean region. Although the fruits and seeds are large, the presence of the "locoism" which produces the alkaloid, swainsonine has prevented its use as animal feed or for human nutrition. Their protein content and chromatographic profile, amino acid composition, fatty acid composition, and polyphenol contents have been determined in order to explore the potential of the *E. baetica* seeds as a source of dietary protein with functional components. The protein content was found to be 36% (w/w), and an amino acid analysis revealed a deficiency in sulphur amino acids, tryptophane, and lysine. The low lysine content is probably due to the abundance of alkaloids metabolically derived from this amino acid. Oleic and linoleic acids are the major fatty acids in the seeds. The antioxidant activity of polyphenol extracts was higher than the activity of the polyphenols extracted from most edible legume seeds. Hence, *E. baetica* seeds represent a promising source of functional and nutritional components on the condition that the anti-nutritional alkaloids are previously removed.

KEY-WORDS: Amino acids – Erophaca baetica – Fatty acids – Polyphenols – Protein.

1. INTRODUCTION

Legumes are of nutritional interest not only because of their high quality protein, but also because they are rich in functional components such as polyphenols, fiber, carbohydrates, and unsaturated fatty acids. Polyphenols are minor components in fruits, vegetables, and legumes and they have become wellknown because of their health promoting properties (Ramos, 2007).

Erophaca baetica (L.) Boiss. the only species of the monotypic genus *Erophaca* Boiss, is a legume endemic to the Mediterranean Region. It has been historically included in the *Astragalus* genus under the denomination *A. lusitanicus* Lam. until it was transferred to *Erophaca* by Podlech (1993). It includes two subspecies which are distributed at opposite ends of the Mediterranean region, subsp. *baetica* in the west and subsp. *orientalis* in the east. *E. baetica* is locally well known due to its toxicity to animals because of the presence of swainsonine, an alkaloid responsible for the so called "*locoism"* syndrome. This legume and others containing this type of alkaloids are known as locoweeds (James *et al*., 2004). However, hepatoprotective, antioxidative, immunostimulant, and antiviral properties have also been described for these alkaloids (Ríos and Waterman, 1997) as well as anticancer effects (Sun *et al*., 2007; Sun *et al*., 2009). The presence of these alkaloids has prevented the use of *E. baetica* for animal and human nutrition. Nevertheless, removal of the anti-nutritional components responsible for toxicity could allow for the use of *E. baetica* for feeding animals or even for human consumption. The

goal of this work was to explore the potential nutritional and functional value of *E. baetica* seeds by determining their chemical composition, amino acid composition, fatty acid composition, and the antioxidant activity of polyphenol extracts.

2. MATERIALS AND METHODS

2.1. Materials

Diethyl ethoxymethylenemanolate was purchased from Fluka. β-carotene, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fatty acid standards (ref. 1892, 1894, 1898) were from SIGMA. All other chemicals were of analytical grade.

2.2. Preparation of flour and oil. Polyphenol extraction

Fully mature *E. baetica* subsp. *baetica* seed samples were collected from a wild population located in Andalusia (southern Spain). The seeds were collected from ten different specimens and stored at –20 °C. Voucher specimens of this population are deposited at the Instituto de la Grasa (C.S.I.C.). Seeds of *Lathyrus cicera, Lathyrus sativus, Lens culinaris, Phaseolus vulgaris, Cicer arietinum, Glycine max*, and *Vicia faba* were purchased from a local market. Whole seeds were ground to powder and passed through a 0.2 mm-mesh sieve. Seed oil was extracted with hexane in a Soxhlet apparatus for 16 h. The extraction of polyphenols was carried out by shaking the defatted flour (10% w/v) in acetone 75% (v/v) at 4 °C for 1 hour three times. The supernatants resulting from centrifugation at 15.000 g for 15 min were taken to dryness under vacuum and dissolved in ethanol.

2.3. Extraction and fractionation of proteins

Defatted, acetone-extracted flour was used for the preparation of protein extracts by shaking a suspension of the flour (10% w/v) in 0.25% Na_2SO_3, 0.2 % NaOH pH 10.5 at 4 °C for 1 hour. The resulting extracts were centrifuged at 15.000 g for 15 min and the pellets were re-extracted twice. Supernatants containing solubilized proteins were pooled and acidified to the determined isoelectric point of globulins, pH 4. For the isoelectric point determination, an alkaline protein extract was titrated to various pH values using 0.5 N HCl. Nitrogen was determined in aliquots that were withdrawn from the extract and in supernatants after centrifugation of these aliquots at 15000 g for 15 minutes. Protein solubility was calculated as (nitrogen in supernatant / total nitrogen) × 100.

Precipitated globulins were recovered by centrifugation at 15.000 g for 15 min and freeze-dried after washing with water. The supernatant, containing albumins, was also recovered and freeze-dried.

2.4. Analytical methods

Moisture, fat, ash, and crude fiber were determined using AOAC (1997) methods 934.01, 920.39, 942.05, and 962.09, respectively. Protein was determined by elemental analysis as nitrogen content (%) × 6.25 using a LECO CHNS-932 analyzer (St Joseph. MI. USA) or by amino acid analysis according to Hidalgo *et al.,* (2001). Soluble sugars were measured according to Dubois *et al*., (1956), using a standard curve of glucose. Polyphenol content was determined using the Folin-Ciocalteou reagent as described (Singleton *et al*., 1999) using a standard curve of catechin.

2.5. Determination of seed oil fatty acid composition

Gas chromatography of fatty acid methyl esters was carried out according to the method of Garcés and Mancha (1993) with modifications. A methylation reagent (1 mL) consisting of 59% methanol, 3% sulphuric acid, 8% dimethoxipropane, and 20% toluene (v/v), and heptane (1 mL) was added to 50 mg seed flour in a screw-cap tube and incubated at 85 °C for 50 min. The upper phase containing the fatty acid methyl esters was taken to dryness under nitrogen and dissolved in 50 µL heptane. Two µL of this solution were injected into a gas chromatograph equipped with an HP-23 FAME capillary column (60 m length and 0.25 mm ID). Hydrogen at 20 psi was used as the carrier gas. Temperatures of injector, detector, and oven were 225 °C, 250 °C, and 180 °C, respectively. Fatty acid methyl esters were identified by comparing retention times with those of standards.

2.6. Assay of polyphenol antioxidative activity. β-carotene bleaching method

The antioxidant activity of polyphenol extracts was determined according to the β-carotene bleaching method (Marco, 1968) with modifications. The β-carotene reagent was prepared by vigorously mixing 1 mL β-carotene (10 mg/mL in chloroform), 20 mg linoleic acid, and 200 mg tween 20, followed by the removal of chloroform using a stream of nitrogen, and finally mixing into oxygen-sparged distilled water (50 mL) to obtain a clear solution. Polyphenol extracts (4 µg) were added to 200 µL of β-carotene reagent in 96-well flat-bottom microtitration plates and incubated at 50 °C using a microplate reader for the determination of absorbance at 450 nm at fixed time intervals. Four different methods were used to calculate antioxidative activity as previously described (Velioglu *et al.,* 1996).

The AOX value was calculated as the slope of the regression line resulting from plotting Log of absorbance vs. time.

The degradation rate (DR) (Al-Saikhan *et al.,* 1995) was calculated as:

DR = Ln (Absorbance at 0 min / Absorbance at 60 min) / 60

Using the DR the antioxidant activity (AA) was calculated as % inhibition relative to the control as:

$$AA = (DR_{control} - DR_{sample}) \times 100 / DR_{control}$$

Where $DR_{control}$ is the degradation rate of β-carotene in the absence of sample phenols.

Antioxidant activity was also expressed as the oxidation ratio (ORR):

$$ORR = DR_{sample} / DR_{control}$$

The antioxidant activity coefficient (AAC) was calculated according to Mallet *et al.,* (1994):

$$AAC = (Abs_{sample60} - Abs_{control60}) \times \times 1000 / (Abs_{control0} - Abs_{control60})$$

where $Abs_{sample60}$ and $Abs_{control60}$ are the absorbance of the sample and the control at 60 min, respectively, and $Abs_{control0}$ is the absorbance of control at time 0 min.

2.7. Amino acid analysis

Samples were hydrolyzed by incubation in 6 N HCl at 110 °C for 24 h. Amino acids were determined after derivatization with diethyl ethoxymethylenemanolate by RP-HPLC according to the method described by Alaiz *et al.,* (1992). using D-L α-aminobutyric acid as internal standard. Samples (20 µL) were injected into a reversed-phase column (Novapack C_{18}, 300 × 3.9 mm i.d., 4 µm; Waters). A binary gradient was used for elution with a flow of 0.9 mL min^{-1}. 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 determined by RP-HPLC chromatography after basic hydrolysis according to Yust *et al.,* (2004).

2.8. Determination of nutritional parameters

The amino acid composition of *E. baetica* seed proteins was used for the determination of several nutritional parameters as follows:

- Amino acid score (chemical score): % essential amino acids in sample / % essential amino acid as recommended by FAO (FAO/WHO/UNU, 1985).
- Protein efficiency ratio (PER) were calculated according to the following three equations (Alsmeyer *et al*., 1974):

  $PER_1 = -0.684 + 0.456$ x Leu $- 0.047 \times$ Pro
  $PER_2 = -0.468 + 0.454$ x Leu $- 0.105 \times$ Tyr
  $PER_3 = -1.816 + 0.435 \times$ Met $+ 0.78$ x Leu $+$ $0.211 \times$ Hys $- 0.944 \times$ Tyr
- Predicted biological value (BV) was calculated according to Morup, & Olesen (1976) using the following equation:
  $BV = 10^{2.15} \times Lys^{0.41} \times (Phe+Tyr)^{0.60} \times (Met+Cys)^{0.77} \times Thr^{0.24} \times Trp^{0.21}$

where each amino acid symbol represents:

% amino acid / % amino acid FAO pattern (1985), if % amino acid ≤ % amino acid FAO pattern. or:

% amino acid FAO pattern (1985) / % amino acid, if % amino acid ≥ % amino acid FAO pattern.

2.9. SDS-PAGE electrophoresis

Protein extracts (2 mg mL^{-1}) were mixed (1:1 v/v) with a solubilization buffer (Tris 80 mM, 0.57% EDTA, 0.26% DTT, 3.3% SDS, 0.008% bromophenol blue, 20% sucrose, pH 6.8) and incubated at 100 °C for 10 min. Tricine - sodium dodecylsulphate - polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Schägger and von Jagow (1987) with slight modifications. The separating gel consisted of 15% T and 2.6% C, where the composition of the acrylamide mixture is defined by the letters T (total percentage concentration of acrylamide and bisacrylamide) and C (percentage concentration of the crosslinker relative to the total concentration T). The stacking gel consisted of 4% T and 3% C. The lengths of the separating and stacking gels were 6 and 2 cm, respectively, with a gel thickness of 1 mm. Electrophoresis was performed at a constant voltage of 60 V for the stacking gel and 120 V for the separation gel. Protein bands were fixed in a solution containing 20% methanol and 8% acetic acid for 15 min, before staining with 0.25% Coomassie Brilliant Blue G in 45% methanol, 10% acetic acid for 2 h. Destaining of the gel was performed in 10% acetic acid. Molecular masses were determined using the low molecular weight standards from Pharmacia LKB Biotechnology.

2.9.1. Gel filtration chromatography

Gel filtration was carried out in an AKTA purifier system equipped with a Superose 12 HR 10/30 column from GE as previously described (Lqari *et al.,* 2002) using blue dextran 2000 (2000 kDa), b-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa) as molecular weight standards.

3. RESULTS AND DISCUSSION

The chemical composition of *E. baetica* flour is shown in Table 1. The protein content, 35.6%, is higher than the protein content in most popular edible grain legumes, including *Lens culinaris* (25.9%), *Pisum sativum* (25.2%), *Glycine max* (24.2%), *Vicia faba* (23.7%), *Phaseolus vulgaris*

Table 1
Chemical composition of *E. baetica* flour. Data are the mean ± SE of two determinations

Compounds	% Contents
Ash	4.0 ± 0.09
Fiber	11.9 ± 0.25
Protein	35.6 ± 0.59
Lipids	10.3 ± 0.21
Soluble sugars	3.0 ± 0.02
Polyphenols	0.6 ± 0.01
Carbohydrates (by difference)	35.4

(20.7%) and *Cicer arietinum* (19.2%). It is also higher than the protein content in other wild legumes from Spain as previously described. Thus, the protein contents in six wild *Lupinus* species ranged from 23.8 to 33.6% (Pastor-Cavada *et al*., 2009a), the protein contents in 15 wild *Lathyrus* species ranged from 17.7 to 25.6% (Pastor-Cavada *et al.,* 2011a), and the protein contents in 28 *Vicia* species ranged from 20.1 to 32% (Pastor-Cavada *et al*., 2011b).

Protein from *E. baetica* seeds was extracted and fractionated into globulins and albumins as described in materials and methods. Table 2 shows the amino acid composition of total protein, globulins, and albumins. *E. baetica* protein is deficient in sulfur amino acids and tryptophan as compared to FAO recommendations (FAO, 1985), which is typical of legume seeds. Nevertheless, *E. baetica* seeds were also poor in Lys, an amino acid whose content is usually above FAO recommendations in legume seeds. This might be explained by the fact that Lys is the precursor of certain alkaloids that are found at high concentrations in *E. baetica* (Aniszewski, 2007). Thus, the use of high amounts of available Lys for synthesis of these alkaloids might have led to an adaptation consisting on having a relatively low Lys content in storage proteins. Interestingly, and supporting this hypothesis, seeds from *Lupinus*, which are also rich in alkaloids, have a low Lys content as shown by the amino acid analysis of six wild *Lupinus* species (Pastor-Cavada, *et al*., 2009a). The average Lys content in the seeds of these plants was 5.25%, well below FAO recommendations. However, the low Lys protein content is compensated in these plants by an accumulation of more protein in the seeds. Thus, Lys and protein contents in *E. baetica*, seeds are 4.0% and 35.6% (w/w), respectively, and the average Lys and protein contents in wild *Lupinus* species are 5.25% and 28.8% (w/w), respectively (Pastor-Cavada, *et al*., 2009a.). This is in contrast to other legume seeds belonging to taxa that are not rich in alkaloids, such as *Lathyrus*, with average Lys and protein contents of 7.6% and 22,2%, respectively (Pastor-Cavada *et al*., 2011a), and *Vicia* with average Lys and protein contents of 7.1% and 23.5%, respectively (Pastor-Cavada *et al*., 2011b). From these data, a clear negative correlation between Lys and total seed protein can be established, resulting in a Lys content in the seeds which is constant across different taxa regardless of the percentage of Lys composition in the seed protein.

The protein content in foods, including grain legumes, has been traditionally determined on the basis of total nitrogen as determined by Kjeldahl or an elemental analysis. A conversion factor of 6.25 results from assuming an average nitrogen content of 16%. The resulting equation (% protein (w/w) = % nitrogen (w/w) × 6.25) is also based on the assumption that most nitrogen is present in the form of free amino acids and protein. This is not the case of legume seeds containing high contents of alkaloids. Thus, a multiplication factor of 5.25 is recommended for the calculation of protein in the seeds from the *Lupinus* species. The protein content in *E. baetica* seeds as determined by a nitrogen analysis using a 6.25 conversion factor and by an amino acid analysis was 59.5% and 35.9%, respectively. This implies that the multiplication factor that should be used for the calculation of the protein content in these seeds based on nitrogen content is 3.9, a factor even lower than the factor recomended for *Lupinus* seeds, indicating that *E. baetica* seeds have an even higher amount of alkaloids than *Lupinus* seeds.

Table 2 shows several nutritional parameters that were calculated using the data on amino acid composition as described in materials and methods. The percentage of EAA in flour was higher than in globulins and albumins, but lower than the values reported for the seeds of other wild legumes such as *Lathyrus* spp. (41.2%) (Pastor-Cavada *et al*., 2011a), and *Vicia* spp. (40.1%) (Pastor-Cavada *et al*., 2011b). Interestingly, the percentage of EAA in flour was similar to the value reported for wild *Lupinus* spp. (34.1%), which are also rich in alkaloids (Pastor-Cavada *et al*., 2009a). Similarly, the amino acid score, a simplified model for predicting dietary protein quality (Moughan. 2005), was lower in *E. baetica* than in legumes lacking alkaloids. Three theoretical PER values that bear a good relationship with real PER values were calculated according to the concentration of Leu (PER_1), Leu and Tyr (PER_2), and Met, Leu, His and Tyr (PER_3). Low quality proteins are characterized as having PER values below 1.5 and high quality proteins are characterized as having PER values higher than 2 (Friedman 1996). Only the albumin fraction had PER theoretical values indicating poor protein quality. All other PER theoretical values were higher than those found in peanuts (1.45-1.76) (Ghuman *et al*., 1990). The theoretical biological value, which is an estimation of how much of the ingested protein would be incorporated into the organism, was higher in albumins (38.6) than in flour (30.3). In conclusion, *E. baetica* seed proteins are of lower nutritional value than the protein from other wild legumes such as *Lathyrus* and *Vicia* due

Table 2
Amino acid composition and nutritional characteristics of *E. baetica* seed flour total proteins, globulins and albumins

	Seed proteins	Globulins	Albumins	FAO[a]
Asp + Asn	11.7 ± 0.25	9.9 ± 0.03	13.4 ± 0.11	
Glu + Gln	23.9 ± 0.05	27.2 ± 0.41	23.9 ± 0.25	
Ser	6.5 ± 0.05	5.1 ± 0.06	5.1 ± 0.15	
His	2.7 ± 0.00	3.6 ± 0.31	2.7 ± 0.22	1.9
Gly	5.1 ± 0.05	4.2 ± 0.15	7.9 ± 0.00	
Thr	3.1 ± 0.05	3.4 ± 0.03	5.3 ± 0.08	1.4
Arg	11.7 ± 0.15	17.1 ± 0.01	11.6 ± 0.11	
Ala	3.8 ± 0.05	3.6 ± 0.01	5.6 ± 0.16	
Pro	3.1 ± 0.15	3.1 ± 0.23	2.0 ± 0.08	
Tyr	1.9 ± 0.05	1.8 ± 0.02	2.2 ± 0.05	6.3[b]
Val	3.7 ± 0.05	3.3 ± 0.00	4.4 ± 0.02	3.5
Met	0.4 ± 0.05	0.2 ± 0.01	0.5 ± 0.01	2.5[c]
Cys	0.3 ± 0.05	0.7 ± 0.05	0.9 ± 0.05	
Ile	4.1 ± 0.05	2.7 ± 0.02	2.0 ± 0.05	2.8
Trp	0.6 ± 0.05	0.2 ± 0.00	0.4 ± 0.00	1.1
Leu	7.9 ± 0.05	6.5 ± 0.27	4.2 ± 0.18	6.6
Phe	6.0 ± 0.05	4.2 ± 0.16	2.5 ± 0.03	
Lys	4.0 ± 0.05	3.1 ± 0.01	5.3 ± 0.28	5.8
% EAA	34.7	29.7	30.4	
AAS	108.8	93.1	95.3	
PER_1	2.77	2.3	1.14	
PER_2	2.92	2.29	1.21	
PER_3	3.3	2.4	0.17	
BV	30.3	27.9	38.6	

[a]FAO (1985). [b]Tyr + Phe. [c]Met + Cys.

to the lower content in Lys. Nevertheless, as pointed out before, this lower Lys content as determined by the amino acid analysis is compensated by a higher protein content in the seeds, so that the Lys content is similar in *E. baetica*, *Lathyrus* spp., and *Vicia* spp. when referring to seed weight.

The SDS-PAGE profile of total seed proteins, globulins, and albumins is shown in Figure 1A. Protein bands at around 60 and 20 kDa in lanes 1 and 2 correspond to the different isoforms of subunits α and β of legumin, respectively. Legumin is a globulin that represents the main storage protein in most legumes, and is formed by pairs of α and β chains linked by disulphide bridges that form trimmers. Two trimmers bind together by non-covalent forces forming an antiprisma. This protein receives different names depending on the plant source, i.e, glycinin from *Glycine max*, phaseolin from *Phaseolus vulgaris* and so on. Thus, we propose that the main globulin storage protein in *E. baetica* could be named erophachin.

The albumin fraction includes water soluble proteins, most importantly enzymes, protease inhibitors, and lectins. The SDS-PAGE analysis reveals two major bands at around 30 and 15 kDa (Figure 1A, lane 3). The 30 kDa band probably corresponds to a lectin subunit, as suggested by the presence of a similar subunit ina very close taxa, *Astragalus mongholicus*, where a lectin is formed by two of these subunits (Yan *et al*., 2005). The lower molecular weight bands correspond to the PA1 albumin fraction, which is found in most legumes and includes protease inhibitors. Gel filtration chromatography of the native albumin fraction revealed two peaks that correspond to the lectin (60 kDa) and PA1 (8 kDa) proteins (Figure 1B).

Extraction of the flour yielded 10% (w/w) oil with a fatty acid composition characterized by high concentrations of palmitic, oleic, and linoleic acids, which account for 90.6% of all fatty acids (Table 3). Data on fatty acid composition is presented in

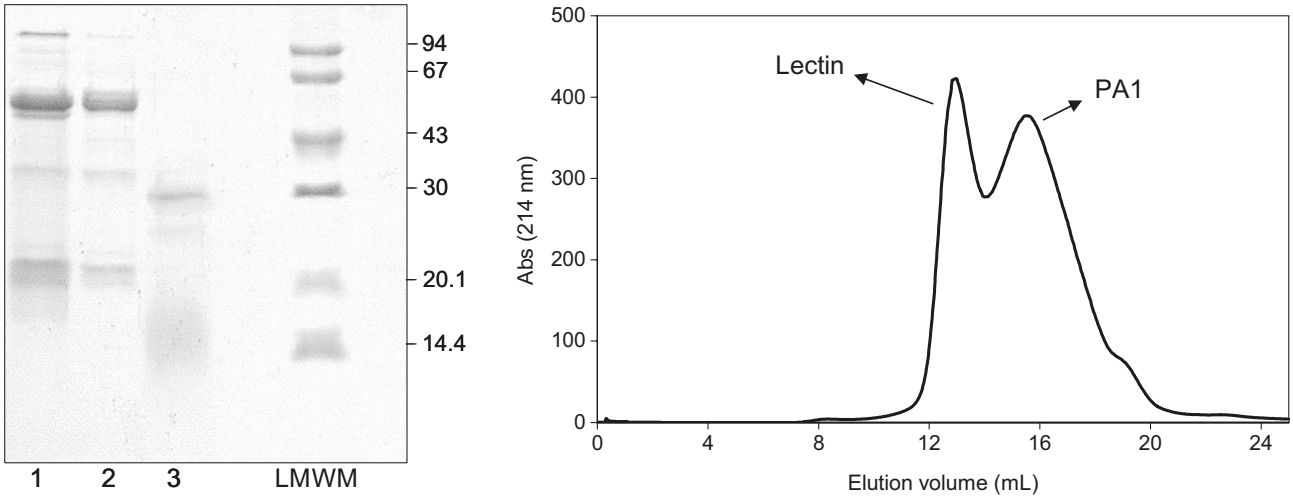

Figure 1
Characterization of *E. baetica* seed proteins. A) SDS-PAGE of *E. baetica* seed proteins (1), globulins (2), and albumins (3). LMWM: low molecular weight markers. B) *E. baetica* gel filtration profile of seed protein albumins.

table 3 according to the degree of unsaturation and different ratios that are indicative of nutritional value. As compared to *Lathyrus*, *Lens, Pisum* (Pastor-Cavada *et al*., 2009b), and *Vicia* (Pastor-Cavada *et al*., 2009c), *E. baetica* has a higher concentration of oleic acid and a lower concentration of linoleic acid.

Table 3
Fatty acid composition and nutritional characteristics of *E. baetica* seed oil

Fatty acids	% content
$C_{12:0}$	0.05 ± 0.01
$C_{14:0}$	0.09 ± 0.00
$C_{16:0}$	17.04 ± 0.03
$C_{16:1}$	0.17 ± 0.01
$C_{18:0}$	3.88 ± 0.02
$C_{18:1}$	35.82 ± 0.01
$C_{18:2}$	37.76 ± 0.01
$C_{18:3}$	3.47 ± 0.01
$C_{20:0}$	0.73 ± 0.01
$C_{20:1}$	0.26 ± 0.00
$C_{22:0}$	0.76 ± 0.00
$C_{14} + C_{16} / C_{18}$	4.42
TUFA	77.45
TSFA	22.55
TUFA / TSFA	3.43
PUFA	41.21
MUFA	36.24
PUFA / MUFA	1.14
$C_{18:1} / C_{18:2}$	0.95
$C_{18:2} / C_{18:3}$	10.88

E. baetica seeds have 5.43 mg polyphenols / g flour, which is similar to the content found in other wild legumes such as *Lathyrus* spp. (3.8-29.2 mg/g) (Pastor-Cavada et al., 2009d) and *Vicia* spp. (1.9-21.3 mg g^{-1}) (Pastor-Cavada *et al.,* 2011). Data on antioxidant activity as determined using the β-carotene bleaching method was used for the calculation of different antioxidant parameters as described in materials and methods. As shown in Table 4, the antioxidant activity of the extracts from *E. baetica* was almost twice the activity in polyphenol extracts from commercial grain legumes, indicating that *E. baetica* represents a good source of polyphenols with a high antioxidant activity.

These results show that *E. baetica* is a good source of nutritional and functional components, and its use for the production of these components is only limited by the presence of anti-nutrional alkaloids. Thus, procedures for the removal of these alkaloids would have to be established to allow for the use of this plant. *E. baetica* is a long-lived perennial plant that produces abundant pods up to 7 cm long in spring which contain seeds up to 1 cm long. These are good agronomic characteristics that would facilitate using this plant for the production of nutritional and functional components.

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Table 4
Antioxidant activity of polyphenolic extracts from *E. baetica* seeds and six different commercial legumes

	DR[a]	AA[b]	ORR[c]	AAC[d]	AOX[e]
Erophaca baetica	0.0042	0.0031	66.43	0.336	965.85
Lathyrus cicera	0.0085	0.0079	46.01	0.540	507.99
Lathyrus sativus	0.0097	0.0069	52.93	0.471	560.27
Lens culinaris	0.0098	0.0052	57.03	0.430	548.39
Phaseolus vulgaris	0.0074	0.0059	54.42	0.456	424.02
Cicer arietinum	0.0096	0.0067	50.87	0.491	355.87
Vicia faba	0.0112	0.0072	29.18	0.709	412.57

[a]Degradation rate. [b]Antioxidant activity. [c]Oxidation rate ratio. [d]Antioxidant activity coefficient. [e]Antioxidant value.

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