agronomy

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Nutritional Characteristics of the Seed Protein in 23 Mediterranean Legumes

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Abstract: The search for new sources of plant protein for food and animal feed is driven by an increasing demand in developing countries and the interest in healthy alternatives to animal protein. Seeds from 23 different wild legumes belonging to tribes *Gallegeae*, *Trifolieae*, and *Loteae* were collected in southern Spain and their total amino acid composition was analyzed, by reverse phase-high performance liquid chromatography (RP-HPLC), in order to explore their nutritional value. Protein content in the seeds ranged from 15.5% in *Tripodium tetraphyllum* to 37.9% and 41.3% in *Medicago minima* and *Medicago polymorpha*, respectively. Species belonging to tribe *Trifolieae*, such as *Melilotus elegans* and *Trifolium* spp., showed the most equilibrated amino acid composition and the best theoretical nutritional values, although all species were deficient in sulfur amino acids. The amino acid composition of the seeds from some of these legumes was characterized by high levels of the anticancer non-proteic amino acid canavanine This amino acid was found free in the seeds from some of the species belonging to each of the three tribes included in the present work. *Astragalus pelecinus* in tribe *Gallegea*, *Trifolium angustifolium* in tribe *Trifolieae*, and *Anthyllis vulneraria* in tribe *Loteae* have 3.2%, 3.7%, and 7.2% canavanine, respectively. Seeds from *Anthyllis vulneraria*, *Hymenocarpus lotoides*, and *Hymenocarpos cornicina* have the highest contents in canavanine overall. In conclusion, the seeds from some of these legumes could be used for human consumption and for feeding animals because they contain protein of good nutritional quality. These plants could be useful in domestication and breeding programs for production of new varieties with improved nutritional and functional properties. In addition, some of these species may be of interest as a source of the bioactive compound canavanine.

Keywords: seed protein amino acids; canavanine; protein efficiency ratio; biological value; amino acid score; essential amino acids

Citation: Elamine, Y.; Alaiz, M.; Girón-Calle, J.; Guiné, R.P.F.; Vioque, J. Nutritional Characteristics of the Seed Protein in 23 Mediterranean Legumes. *Agronomy* **2022**, *12*, 400. https://doi.org/10.3390/agronomy12020400

Academic Editor: Manosh Kumar Biswas

Received: 27 December 2021
Accepted: 2 February 2022
Published: 5 February 2022

1. Introduction

Plants represent an ever-growing source of protein for human nutrition because they are necessary to satisfy the increasing population of developing and third world countries. In addition, demand for plants as a source of quality protein is also increasing in developed countries because plants represent a healthier and more environmentally sound resource than animal protein. Thus, animal protein is more expensive to produce and implies a higher intake of saturated fats and cholesterol in comparison with plant protein. An inverse correlation between the consumption of plant foods and diseases—such as diabetes, stroke, cancer, and cardiovascular diseases (CVD)—is well established [1,2].

Legume seeds are in general rich in protein and fiber and poor in fat, with the exception of oilseeds such as soybean and peanut. Legumes and cereals constitute the main source of plant protein in human nutrition, although pulses have twice as much protein as cereals such as wheat, oat, and barley, and three times more than rice [3]. Pulses are also rich in minerals including magnesium, potassium, iron, and zinc. They are also rich in B vitamins and have a low content in sodium [3]. In addition, seed legumes are rich in secondary compounds with health promoting properties that include polyphenols, free amino acids, and proteins with antioxidant and antiproliferative activity [4,5]. Consumption of legumes also reduces the risk of suffering from CVD, which has been related with their benefical effect on risk factors such as cholesterol level, blood pressure, glycemic index, and insulin resistance [6].

Leguminous seeds are an important part of the human diet in most regions of the world, and both the seeds and green parts from many legumes are also used to feed animals. When used for human consumption, leguminous seeds, known as pulses, are usually subjected to softening by soaking in water before cooking [7]. These include pulses like beans, chickpeas, and lentils. Legumes are also grown to feed liverstock, including the green parts of species belonging to genera *Medicago*, *Trifolium*, and *Vicia* [8,9]. Soybean is a very important oilseed crop, and extraction of soybean oil renders a protein rich byproduct [10]. This protein byproduct can be subjected to alkaline extraction and purification for production of protein concentrates and isolates that have very good functional and nutritional properties and are extensively used in the food insustry. Leguminous seeds other than soybean can also be processed to make protein rich food products, facilitating the use of undervalued legumes for human consumption [11]. This is the case for instance of *Vicia sativa* L., which has been used for production of protein rich extruded products ready for human consumption [12].

Legumes in general have very good agronomic properties, and are able to thrive in harsh conditions that would not allow the use of many other crops. These properties include resistance to thermal and hydric stresses. In addition, nitrogen fixation by legumes reduces de need for fertilizers. Pulses also enjoy a very long shelf life, which greatly facilitates storage and distribution to consumers [13–15].

The 'Green Revolution' started a process of substituion of many local crops by comercial, more genetically uniform crops. Nevertheless, diversification of cultivars and protection of biodiversity is neccesary to preserve healthy agricultural systems, and greatly increases the chances of crops to survive climate change [16]. The goal of this work was to study the potential of 23 local legumes as new sources of seed quality protein. Amino acid composition was used to determine theoretical nutritional quality. Some of these species are still used, or have been used in the past, as sources of food and animal feed, or even have a history of use in traditional medine.

2. Materials and Methods

2.1. Materials

Diethyl ethoxymethylenemanolate and D, L α-aminobutyric acid were purchased from Fluka and Sigma, respectively. All other chemicals were of analytical grade. Legume seeds were collected from the wild during May and June 2014 in Huelva and Sevilla provinces, Andalucía, Spain. For the identification of legume plants, Flora Iberica (1999) [17] was employed. Seeds at full maturity were collected from several plants in each population and completely dried in the laboratory at room temperature before storage at −20 °C. Voucher specimens of each population are deposited at the Instituto de la Grasa, C.S.I.C., Sevilla, Spain.

2.2. Amino Acid Analysis

Seed flour samples (500 mg) were hydrolyzed by incubation in 6 N HCl at 110 °C for 24 h. Amino acids were determined, in duplicate, after derivatization with diethyl ethoxymethylenemanolate by reverse phase-high performance liquid chromatography (RP-HPLC), according to the method described by Alaiz et al. [18]. using D, L α-aminobutyric acid as internal standard.

The RP-HPLC system (Beckman-Coulter, Inc., Fullerton, CA, USA) consisted of a 126 solvent module, 166 detector, and IBM personal computer. Data acquisition and processing were carried out using 32 Karat 7.0 version software (Beckman-Coulter). Samples (20µL) were injected in a reversed-phase column (Novapack C_{18}, 300 mm × 3.9 mm i.d., 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 determined also by RP-HPLC after basic hydrolysis according to Yust et al., (2004) [19]. Protein content was based on amino acid analysis [20].

2.3. Determination of Nutritional Parameters

The amino acid composition of the seeds was used for determination of the following nutritional parameters:

- Amino acid score (chemical score):
  (1) (% essential amino acids in sample/% essential amino acid recommended by FAO [21]) × 100
- Protein efficiency ratio (PER) was calculated according to the following equations [22]:
  (2) $PER_1 = -0.684 + 0.456 \times Leu - 0.047 \times Pro$
  (3) $PER_2 = -0.468 + 0.454 \times Leu - 0.105 \times Tyr$
  (4) $PER_3 = -1.816 + 0.435 \times Met + 0.78 \times Leu + 0.211 \times Hys - 0.944 \times Tyr$
- Predicted biological value (BV) was calculated using the following equation [23]:
  (5) $BV = 10^{2.15} \times Lys^{0.41} \times (Phe + Tyr)^{0.60} \times (Met + Cys)^{0.77} \times Thr^{0.24} \times Trp^{0.21}$
  where each amino acid symbol represents:
- % amino acid/% amino acid FAO pattern [21], when % amino acid ≤% amino acid FAO pattern [21], or:
- % amino acid FAO pattern [21]/% amino acid, when % amino acid ≥% amino acid FAO pattern [21].

3. Results and Discussion

3.1. Protein Content

As shown in Table 1, protein content in the seeds ranged from 15.5% in *Tripodium tetraphyllum* (L.) Fourr. to 37.9% and 41.3% in *Medicago minima* (L.) L. and *Medicago polymorpha* L., respectively. This is consistent with previous reports for legumes in general [24] and for other wild Mediterranean legumes such as those belonging to genera *Vicia* [25] and *Lathyrus* [26]. The highest protein content was found in genus *Medicago*, and the average protein content in Tribes *Gallegeae*, *Trifolieae*, and *Loteae* were 29.1%, 32.5%, and 27.6%, respectively. The protein content in many of the species in Table 1 was even higher than those reported for commercial legumes such as chickpea (24.7%) [11] and *Vicia faba* L. (26.6%) [27] although lower than the reported for lupins [28].

Table 1. Protein content and amino acid composition in seeds. Data expressed as g/100 g protein are the average ± sd of two determinations.

Tribes	Gallegeae			Trifolieae				
Species	***Astragalus cymbaecarpos***	***Astragalus hamosus***	***Astragalus pelecinus***	***Ononis natrix***	***Medicago minima***	***Medicago polymorpha***	***Melilotus elegans***	**FAO [3]**
Aspartic a. [1]	10.13 ± 0.01	10.07 ± 0.07	10.03 ± 0.07	11.54 ± 0.08	11.71 ± 0.02	12.46 ± 0.16	12.18 ± 0.01	
Glutamic a. [2]	22.35 ± 0.14	22.82 ± 0.03	19.48 ± 0.03	19.50 ± 0.09	16.02 ± 0.01	16.54 ± 0.25	16.48 ± 0.01	
Serine	5.14 ± 0.00	5.56 ± 0.01	4.98 ± 0.01	5.54 ± 0.00	5.19 ± 0.02	5.32 ± 0.05	5.46 ± 0.03	
Histidine	2.74 ± 0.02	2.81 ± 0.19	2.86 ± 0.01	3.04 ± 0.04	2.75 ± 0.04	2.66 ± 0.04	3.27 ± 0.06	1.9
Glycine	5.51 ± 0.07	5.44 ± 0.12	6.20 ± 0.11	4.65 ± 0.12	5.45 ± 0.06	5.27 ± 0.07	5.68 ± 0.07	
Threonine	3.17 ± 0.02	3.26 ± 0.03	3.33 ± 0.01	3.73 ± 0.06	3.55 ± 0.08	3.66 ± 0.03	3.68 ± 0.02	3.4
Arginine	14.62 ± 0.06	14.65 ± 0.10	10.71 ± 0.07	12.71 ± 0.19	9.72 ± 0.17	9.93 ± 1.1	9.50 ± 0.07	
Alanine	4.17 ± 0.03	4.04 ± 0.13	3.83 ± 0.01	4.74 ± 0.03	4.54 ± 0.08	4.50 ± 0.06	4.27 ± 0.01	
Proline	3.11 ± 0.04	3.25 ± 0.11	3.76 ± 0.04	2.09 ± 0.21	2.69 ± 0.04	2.90 ± 0.15	4.84 ± 0.47	
Tyrosine	2.26 ± 0.01	2.09 ± 0.01	2.33 ± 0.01	2.39 ± 0.00	2.31 ± 0.00	2.34 ± 0.02	2.55 ± 0.02	6.3 [4]
Valine	4.11 ± 0.00	4.40 ± 0.19	5.57 ± 0.41	4.67 ± 0.27	7.61 ± 0.47	4.78 ± 0.49	4.48 ± 0.11	3.5
Methionine	1.03 ± 0.02	0.03 ± 0.01	0.80 ± 0.04	0.01 ± 0.01	0.69 ± 0.04	0.69 ± 0.06	0.42 ± 0.01	2.5 [5]
Cysteine	0.43 ± 0.00	0.22 ± 0.01	0.52 ± 0.01	0.40 ± 0.01	0.51 ± 0.01	0.63 ± 0.02	0.77 ± 0.00	
Isoleucine	3.61 ± 0.01	3.74 ± 0.01	3.92 ± 0.00	4.35 ± 0.02	4.36 ± 0.03	4.34 ± 0.05	4.21 ± 0.03	2.8
Tryptophan	2.12 ± 0.02	2.03 ± 0.09	3.06 ± 0.18	1.93 ± 0.04	2.42 ± 0.12	2.31 ± 0.01	2.92 ± 0.11	1.1
Leucine	6.13 ± 0.00	6.45 ± 0.09	6.13 ± 0.03	7.92 ± 0.03	7.86 ± 0.04	7.99 ± 0.06	7.51 ± 0.03	6.6
Phenylalanine	4.25 ± 0.00	4.38 ± 0.01	4.36 ± 0.03	5.15 ± 0.06	5.32 ± 0.12	5.36 ± 0.02	5.52 ± 0.73	
Lysine	4.47 ± 0.02	4.36 ± 0.00	4.95 ± 0.02	5.65 ± 0.08	4.94 ± 0.04	4.91 ± 0.06	6.28 ± 0.01	5.8
Canavanine	0.66 ± 0.01	0.42 ± 0.01	3.18 ± 0.03	0.00 ± 0.00	2.35 ± 0.03	3.43 ± 0.00	0.00 ± 0.00	
Protein	30.53 ± 0.5	31.58 ± 0.69	25.25 ± 0.12	36.53 ± 0.69	37.87 ± 0.52	41.29 ± 0.62	21.64 ± 0.21	

Tribes	Trifolieae					Loteae		
Species	***Trifolium angustifolium***	***Trifolium cherleri***	***Trifolium repens***	***Trifolium scabrum***	***Trifolium stellatum***	***Anthyllis vulneraria***	***Dorycnopsis gerardi***	***Ornithopus compressus***	**FAO [3]**
Aspartic a. [1]	11.12 ± 0.02	11.49 ± 0.13	11.99 ± 0.04	11.55 ± 0.02	11.08 ± 0.00	12.51 ± 0.08	12.86 ± 0.12	11.31 ± 0.10	
Glutamic a. [2]	16.96 ± 0.06	17.11 ± 0.03	16.01 ± 0.11	17.36 ± 0.15	16.43 ± 0.12	15.99 ± 0.02	15.52 ± 0.01	18.12 ± 0.06	
Serine	5.19 ± 0.02	5.45 ± 0.03	5.59 ± 0.05	5.32 ± 0.01	5.06 ± 0.05	5.63 ± 0.05	6.03 ± 0.00	5.47 ± 0.01	
Histidine	3.05 ± 0.02	3.17 ± 0.19	3.02 ± 0.01	3.18 ± 0.04	2.99 ± 0.02	1.77 ± 0.13	2.43 ± 0.01	2.61 ± 0.04	1.9
Glycine	5.39 ± 0.03	5.76 ± 0.00	5.74 ± 0.00	5.69 ± 0.04	4.85 ± 0.07	5.30 ± 0.04	5.47 ± 0.00	6.45 ± 0.10	
Threonine	3.54 ± 0.01	3.85 ± 0.03	3.75 ± 0.04	3.69 ± 0.02	3.29 ± 0.01	3.44 ± 0.02	3.74 ± 0.00	3.62 ± 0.05	3.4
Arginine	9.44 ± 0.01	10.79 ± 0.02	10.05 ± 0.03	11.57 ± 0.02	10.77 ± 0.04	9.77 ± 0.01	12.79 ± 0.07	12.75 ± 0.03	
Alanine	4.66 ± 0.02	4.89 ± 0.00	4.98 ± 0.01	4.89 ± 0.01	4.55 ± 0.03	4.30 ± 0.04	5.08 ± 0.05	4.73 ± 0.06	
Proline	3.67 ± 0.03	4.39 ± 0.05	3.84 ± 0.01	2.99 ± 0.22	3.96 ± 0.14	4.16 ± 0.00	4.57 ± 0.07	3.66 ± 0.08	
Tyrosine	2.41 ± 0.00	2.37 ± 0.01	2.40 ± 0.01	2.29 ± 0.01	2.38 ± 0.02	2.31 ± 0.01	2.00 ± 0.02	2.44 ± 0.03	6.3 [4]
Valine	4.70 ± 0.01	4.65 ± 0.00	4.92 ± 0.03	4.83 ± 0.02	7.35 ± 0.53	4.47 ± 0.06	4.74 ± 0.07	4.58 ± 0.01	3.5
Methionine	0.07 ± 0.01	0.63 ± 0.01	0.05 ± 0.01	0.66 ± 0.07	0.68 ± 0.00	0.60 ± 0.04	0.32 ± 0.09	0.58 ± 0.01	2.5 [5]
Cysteine	0.33 ± 0.00	0.46 ± 0.01	0.27 ± 0.01	0.38 ± 0.02	0.27 ± 0.00	0.26 ± 0.01	0.03 ± 0.01	0.58 ± 0.00	
Isoleucine	4.28 ± 0.01	3.89 ± 0.01	4.46 ± 0.04	4.14 ± 0.01	4.52 ± 0.04	3.82 ± 0.02	4.00 ± 0.02	3.92 ± 0.04	2.8
Tryptophan	2.26 ± 0.07	2.15 ± 0.04	2.71 ± 0.15	2.18 ± 0.03	2.36 ± 0.04	2.59 ± 0.05	2.40 ± 0.03	2.33 ± 0.09	1.1
Leucine	7.56 ± 0.14	7.37 ± 0.01	8.11 ± 0.15	7.43 ± 0.00	7.45 ± 0.06	6.72 ± 0.06	7.37 ± 0.12	7.28 ± 0.03	6.6

Table 1. Cont.

Tribes	*Trifolieae*					*Loteae*			
Species	***Trifolium angustifolium***	***Trifolium cherleri***	***Trifolium repens***	***Trifolium scabrum***	***Trifolium stellatum***	***Anthyllis vulneraria***	***Dorycnopsis gerardi***	***Ornithopus compressus***	**FAO** [3]
Phenylalanine	5.27 ± 0.00	5.13 ± 0.06	5.42 ± 0.00	5.26 ± 0.10	5.21 ± 0.09	4.94 ± 0.00	6.06 ± 0.04	4.87 ± 0.10	
Lysine	6.38 ± 0.02	6.46 ± 0.03	6.15 ± 0.02	6.56 ± 0.01	6.80 ± 0.05	4.27 ± 0.01	4.59 ± 0.02	4.58 ± 0.01	5.8
Canavanine	3.72 ± 0.01	0.00 ± 0.00	0.56 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	7.15 ± 0.04	0.00 ± 0.00	0.14 ± 0.02	
Protein	31.01 ± 0.23	28.81 ± 0.31	27.70 ± 0.00	31.94 ± 0.21	35.82 ± 0.28	23.09 ± 0.16	16.33 ± 0.13	33.41 ± 0.18	
Tribe	*Loteae*								
Species	***Hymenocarpos cornicina***	***Hymenocarpos hamosus***	***Hymenocarpos lotoides***	***Tripodium tetraphillum***	***Coronilla glauca***	***Hippocrepis ciliata***	***Scorpiurus sulcatus***	***Scorpiurus vermiculatus***	**FAO** [3]
Aspartic a. [1]	11.55 ± 0.31	11.30 ± 0.01	11.11 ± 0.03	10.92 ± 0.00	10.97 ± 0.13	12.14 ± 0.00	11.52 ± 0.06	11.91 ± 0.11	
Glutamic a. [2]	16.57 ± 0.02	18.37 ± 0.05	16.01 ± 0.02	18.25 ± 0.09	16.93 ± 0.07	19.94 ± 0.05	18.29 ± 0.05	18.41 ± 0.01	
Serine	6.08 ± 0.01	6.36 ± 0.01	6.12 ± 0.01	5.70 ± 0.02	5.70 ± 0.01	5.67 ± 0.03	5.93 ± 0.02	5.81 ± 0.01	
Histidine	2.45 ± 0.14	3.07 ± 0.03	2.44 ± 0.05	2.73 ± 0.06	3.36 ± 0.07	2.76 ± 0.04	2.95 ± 0.02	2.72 ± 0.02	1.9
Glycine	6.16 ± 0.02	6.69 ± 0.08	6.69 ± 0.24	6.65 ± 0.01	6.87 ± 0.12	6.08 ± 0.02	6.30 ± 0.02	5.10 ± 0.01	
Threonine	3.53 ± 0.02	3.68 ± 0.02	3.54 ± 0.00	3.68 ± 0.00	3.78 ± 0.01	2.89 ± 0.03	3.68 ± 0.01	3.39 ± 0.01	3.4
Arginine	10.66 ± 0.20	12.42 ± 0.03	10.09 ± 0.10	14.11 ± 0.03	10.47 ± 0.03	14.21 ± 0.02	13.05 ± 0.05	16.05 ± 0.01	
Alanine	4.43 ± 0.00	4.90 ± 0.02	4.64 ± 0.03	4.15 ± 0.06	4.93 ± 0.02	4.29 ± 0.08	4.90 ± 0.04	4.71 ± 0.03	
Proline	3.69 ± 0.09	1.54 ± 0.08	3.41 ± 0.25	4.26 ± 0.03	5.03 ± 0.16	0.43 ± 0.14	2.76 ± 0.12	1.94 ± 0.01	
Tyrosine	2.41 ± 0.01	2.26 ± 0.00	2.30 ± 0.02	2.35 ± 0.00	2.68 ± 0.03	2.28 ± 0.03	2.87 ± 0.00	2.89 ± 0.02	6.3 [4]
Valine	4.57 ± 0.10	4.64 ± 0.03	4.46 ± 0.03	4.23 ± 0.04	4.40 ± 0.01	6.69 ± 0.13	4.73 ± 0.01	4.54 ± 0.06	3.5
Methionine	0.22 ± 0.06	0.02 ± 0.00	0.29 ± 0.00	0.08 ± 0.00	0.10 ± 0.03	0.32 ± 0.01	0.09 ± 0.01	0.30 ± 0.05	2.5 [5]
Cysteine	0.45 ± 0.01	0.53 ± 0.01	0.52 ± 0.00	0.15 ± 0.01	0.47 ± 0.01	0.39 ± 0.06	0.32 ± 0.01	0.30 ± 0.00	
Isoleucine	3.69 ± 0.05	3.80 ± 0.01	3.65 ± 0.00	4.37 ± 0.01	3.92 ± 0.02	3.93 ± 0.03	4.17 ± 0.00	4.14 ± 0.01	2.8
Tryptophan	2.05 ± 0.02	2.35 ± 0.07	2.38 ± 0.06	1.84 ± 0.09	2.86 ± 0.06	2.42 ± 0.03	2.25 ± 0.06	1.68 ± 0.01	1.1
Leucine	7.01 ± 0.09	7.16 ± 0.16	6.65 ± 0.14	6.34 ± 0.03	7.33 ± 0.03	6.69 ± 0.04	6.83 ± 0.01	6.84 ± 0.05	6.6
Phenylalanine	5.29 ± 0.07	5.63 ± 0.01	5.32 ± 0.01	5.12 ± 0.07	4.63 ± 0.01	4.53 ± 0.09	4.36 ± 0.04	4.32 ± 0.03	
Lysine	4.74 ± 0.01	5.30 ± 0.02	4.95 ± 0.02	5.08 ± 0.00	5.57 ± 0.01	4.35 ± 0.02	5.01 ± 0.01	4.96 ± 0.03	5.8
Canavanine	4.46 ± 0.17	0.00 ± 0.00	5.44 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Protein	34.18 ± 0.22	29.57 ± 0.29	28.65 ± 0.19	15.53 ± 0.13	20.29 ± 0.00	31.25 ± 0.58	26.82 ± 0.47	34.66 ± 0.30	

[1] Aspartic acid + asparagine. [2] Glutamic acid + glutamine. [3] Suggested pattern of amino acid requirements (FAO/WHO/UNU, 1985). [4] Tyrosine + phenylalanine. [5] Methionine + cysteine.

3.2. Amino Acid Composition

The amino acid composition of the legume seeds was within the expected range [29]. All the analyzed species were deficient in the sulfur amino acids Met and Cys. In addition, other deficiencies were found in some of the seeds. Thus, seeds from *Astragalus* species within tribe *Galegeae* were deficient in Thr and Lys; *Ononis natrix* L. and *Medicago* species belonging to tribe *Trifolieae*, and species in tribe *Loteae* were deficient in Lys; *Trifolium stellatum* L. and *Hippocrepis ciliata* Willd. were deficient in Thr; *Anthyllis vulneraria* L. was deficient in His; and *Hymenocarpos lotoides* (L.) Vis. and *Tripodium tethraphyllum* (L.) Fourr. were deficient in Leu. The seeds from *Melilotus elegans* Salzm. ex Ser. and *Trifolium* species belonging to tribe *Trifolieae*, with the exception of *T. stellatum*, did not have any deficiency other than Met and Cys, and provided the amino acid compositions that more closly resembled FAO requirements. Most importantly, the content in Lys exceeded FAO requirements, ranging from 6.2% in *Trifolium repens* to 6.8% in *T. stellatum*. The contents in sulfur amino acids and Lys are characteristicaly low in legumes and cereals, respectively. Trp contents were high, in comparison, for example, with wild *Lathyrus*, ranging from 0.5% to 0.8% [26].

In addition to the 20 amino acids that are present in proteins, many legumes accumulate in their seeds important amounts of free non-proteinogenic amino acids (NPAA) [30]. These legumes are clustered in the NPAA clade that includes most of the herbaceous legumes and is subdivided in several additional subclades [31]. One of these subclades is the inverted-repeat (IR) lacking clade that includes the three legume tribes included in the present study. Species in the IR-lacking clade are caracterized by the absence of an inverted repeat sequence in their chloroplast DNA [32].

Canavanine, an analoge of arginine (Figure 1), is one of the most common NPAA in legume seeds [33].

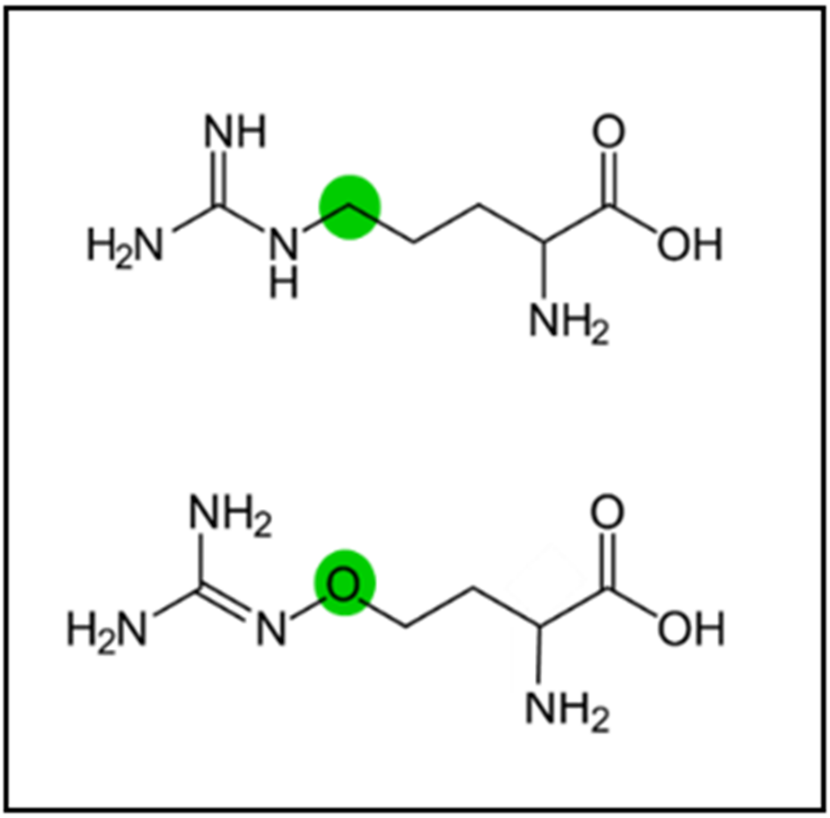

Figure 1. Chemical structures of arginine (**up**) and canavanine (**down**); green dots highlight the differential structural features.

Canavanine has traditionally been considered an antinutritional compound because it can subtitute for arginine in newly synthesized proteins [34]. It is also considered a source of nitrogen for seed germination and seedling growth for some species in which it is very abundant. More recently, some interesting bioactive properties have been attributed to canavanine—including antiproliferative, chemopreventive [35], chemosensitizing, and radiosensitizing [36] activities. Additionally, in vivo studies of the combined effect of canavanine and 5-fluorouracil have been developed [37]. Thus, canavanine is now considered to be a bioactive compound with potential health promoting and therapeutic properties. Canavanine was found in the seeds from some of the species belonging to each of the three tribes included in the present work, and many of them are actually very rich in this NPAA (Table 1). *Astragalus pelecinus* (L.) Barneby in tribe *Gallegea*, *Trifolium angustifolium* L. in tribe *Trifolieae*, and *Anthyllis vulneraria* in tribe *Loteae* have 3.2%, 3.7%, and 7.2% canavanine, respectively. Seeds from *Anthyllis vulneraria*, *Hymenocarpus lotoides* and *Hymenocarpos cornicina* (L.) Vis. have the highest contents in canavanine overall. Thus, the seeds from this group of local, mediterranean legumes include species that represent not only a good source of nutritious protein, but also a source of canavanine as a bioactive compound. Proper removal of canavanine can be readily accomplished in order to prevent possible anti-nutritional effects [38].

3.3. Nutritional Properties

The amino acid composition of proteins is a very good predictor of nutritional quality. It readily affords valuable nutritional information for a large number of samples that can be later confirmed using more time consuming in vitro and in vivo methods [39]. Four different parameters have being calculated using data on the amino acid compositions shown in Table 1. The theoretical protein efficiency ratio (PER) based on the amino acid composition shows a high correlation with real PER, which is determined by calculating weight gain in feeding trials. Theoretical PER values depend on the amount of Leu (PER_1); Leu and Tyr (PER_2); and Met, Leu, His, and Tyr (PER_3). PER values below 1.5 are indicative of low-quality protein and PER values above 2 are characteristics of high quality protein. Calculated PER values were higher in tribe *Trifolieae*, especially in *Trifolium repens* L., and the two species of *Medicago* (Table 2). In general, values in tribe *Trifolieae* were within the range reported for cultivated legumes such as soybean [40] and chickpea [41], and higher than those reported for wild lupinus [42], peanut [43], and *Vigna radiata* (L.) R. Wilczek [44]. PER values were also higher than those reported for cereals such as rice and wheat [45]. The lowest PER values were found in genus *Astragalus* and in *Tripodium tetraphyllum*. Additionally, genus *Scorpiurus* showed the lowest PER_3 values. In general, the PER values that have been determined in this study are lower than those reported for other wild legumes such as *Vicia* [25].

The biological value (BV) for a protein constitutes a theoretical calculation of the amount of ingested protein that is incorporated into the organism [46]. The highest BVs, 52.2 and 52.6, were observed in *Astragalus pelecinus* (L.) Barneby and *A. cymbaecarpos* Brot. respectively. These values are lower than BV reported for wild Vicia species such as *V. benghalensis* L. (67) [25]. The lowest BV was observed in *Tripodium tetraphyllum* (10.9).

The amino acid score (AAS) and the ratio of essential amino acids to total amino acids (% EAS) are considered to be better indicators of protein quality than BV, even though they are also approximations to the real nutritional value that do not account for amino acid digestibility and availability [47]. In general, tribe *Trifolieae* showed the highest AAS and % EAS values (Table 2). These two parameters were highest in Trifolium stellatum (123 and 43.3, respectively). On the contrary, *Astragalus hamosus* L. and *A. cymbaecarpos* had the lowest AAS and % EAS. These results are similar to those reported for other mediterranean wild legumes such as *Vicia* [25] and *Lathyrus* [26]. In summary, species belonging to tribe *Trifolieae* have the best nutritional profile according to PER, BV, AAS, and % EAS. Species in tribe *Gallegeae* had the worst PER, AAS, and % EAS values.

Table 2. Theorethycal nutritional parameters of seed protein based on amino acomposition.

Tribes	Species	PER_1	PER_2	PER_3	BV	AAS	%EAA	Uses
Gallegeae	*Astragalus cymbaecarpos*	2.0	2.1	1.9	52.6	97.5	34.3	
	Astragalus hamosus	2.1	2.2	1.8	13.1	95.9	33.8	Food (seedpods), medicine.
	Astragalus pelecinus	1.9	2.1	1.7	52.2	107.5	37.8	Forage.
Trifolieae	*Ononis natrix*	2.8	2.9	2.8	16.4	111.5	39.2	
	Medicago minima	2.8	2.9	3.0	41.8	120.3	42.3	Forage.
	Medicago polymorpha	2.8	2.9	3.1	44.3	112.7	39.7	Food (flowers, leaves, seeds), medicine.
	Melilotus elegans	2.5	2.7	2.5	37.7	118.2	41.6	Food (leaves, condiment).
	Trifolium angustifolium	2.6	2.7	2.5	16.0	113.2	39.9	Forage.
	Trifolium cherleri	2.5	2.6	2.6	33.6	114.0	40.1	
	Trifolium repens	2.8	3.0	2.9	13.8	117.2	41.3	Food (flowers, leaves, root), medicine.
	Trifolium scabrum	2.6	2.7	2.8	32.9	115.4	40.6	Forage.
	Trifolium stellatum	2.5	2.7	2.7	31.1	123.0	43.3	Forage.
Loteae	*Anthyllis vulneraria*	2.2	2.3	1.9	32.7	100.0	35.2	Food (leaves), forage, medicine.
	Dorycnopsis gerardi	2.5	2.7	2.7	15.3	107.1	37.7	
	Ornithopus compressus	2.5	2.6	2.4	41.3	106.2	37.4	Forage.
	Hymenocarpos cornicina	2.3	2.5	2.0	26.0	103.4	36.4	
	Hymenocarpus hamosus	2.5	2.5	2.3	21.4	109.2	38.4	
	Hymenocarpos lotoides	2.2	2.3	1.8	30.9	103.7	36.5	
	Tripodium tetraphillum	2.0	2.2	1.5	10.9	102.2	36.0	
	Coronilla glauca	2.4	2.6	2.1	23.6	111.1	39.1	
	Hippocrepis ciliata	2.3	2.3	2.0	30.7	105.8	37.2	
	Scorpiurus sulcatus	2.3	2.3	1.5	36.7	105.8	37.3	
	Scorpiurus vermiculatus	2.3	2.3	1.5	24.0	102.5	36.1	Food (seedpods), forage.

PER: Protein efficiency ratio. AAS: Amino acids score. EAA/TAA: Essential amino acids/total amino acids. BV: Biological value.

3.4. Multivariate Analysis

A multivariate analysis was carried out in order to clasify the legumes according to the seed amino acid composition. As shown in Figure 2, this clasification partially matches taxonomic clasification, and resulted in clades that resemble the distribution in tribes and genera. Nevertheless, the match with taxonomic classification was not perfect. For example, *Astragalus* species, included in tribe *Gallegeae* clustered in a single clade, while species in tribe *Trifoleae* clustered in a single clade that also includes *Coronilla glauca* L. and *Ornithopus compressus* L. Several species of the same genera also clustered together, including *Astragalus*, *Scorpiurus*, *Hymenocarpos*, and *Medicago* species. It is northworthy that Hymenocarpos is correctly resolved from *Anthyllis* and *Dorycnopsis*. These genera were until recently included in genus Anthyllis [48]. Hence, our results support the division of Anthyllis in these three genera. In summary, multivariate analysis indicates that data on amino acid composition is consistent with the taxonomic classification of the wild legumes included in the present study.

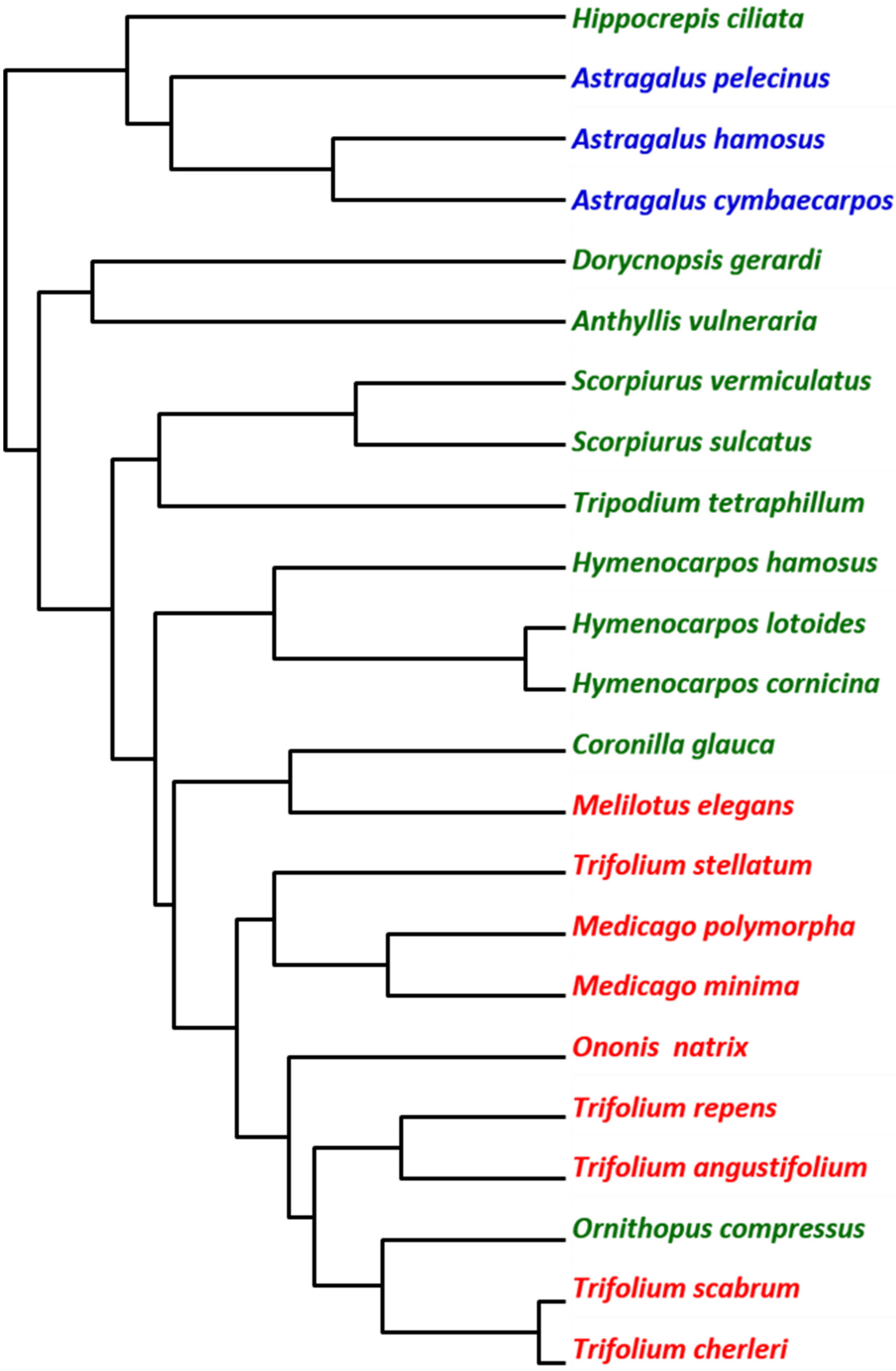

Figure 2. Dendrogram based on the seed amino acid composition.

4. Conclusions

The analysis of the amino acid composition of the seeds from 23 wild legumes show interesting characteristics from a nutritional and functional point of view. Hence, protein in the seeds of some of these plants is of good nutritional quality and may constitute a valuable resource for human consumption and as animal feed. Species belonging to tribe *Trifoliae* have the highest content in protein, and also the best nutritional quality in terms of theoretical nutritional parameters. Although some of the species included in the present work have a record of being used as animal feed or for human consumption (see the last column in Table 2), their potential is far greater than currently acknowledged. Some of the species also have a high content in canavanine, and may represent an interesting source of this NPAA with potential pharmacological applications. Further characterization of the nutritional value of the protein by determination of digestibility in vitro (protein corrected amino acids score) or in vivo would be of great interest. These results may constitute the basis for revalorization of some of these legumes, which are a valuable resource for domestication and breeding programmes, as constituents in foods and animal feeds, and for production of canavanine.

Author Contributions: Conceptualization, R.P.F.G. and J.V.; Methodology, Y.E., M.A. and J.V.; Software, Y.E. and J.V.; Formal analysis, Y.E. and J.V.; Investigation, Y.E. and J.V; Resources, Y.E. and J.V.; Data curation, Y.E. and J.V; Writing—original draft preparation, Y.E., J.G.-C. and J.V.; Writing—review and editing, Y.E., M.A., J.G.-C., R.P.F.G. and J.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by C.S.I.C. (Spain). The APC was funded by FCT—Foundation for Science and Technology, I.P. (Portugal), within the scope of the project ref. no. UIDB/00681/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: This work was supported by the FCT—Foundation for Science and Technology, I.P. (Portugal), within the scope of the project ref. no. UIDB/00681/2020. Furthermore, we would like to thank the CERNAS Research Centre and the Polytechnic Institute of Viseu for their support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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