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Original Article

PROXIMATE ANALYSIS AND AMINOACID PROFILES OF LEAVES, FLOWERS, PODS, AND SEEDS OF *ERYTHRINA EDULIS* FROM PERU

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ABSTRACT

Objective: The study aim was to determine the proximate composition and amino acid profiles of seeds, leaves, pods, and flowers of *Erythrina edulis* that are consumed in Oxapampa, Perú.

Methods: Plant parts of *E. edulis* were analyzed for proximate composition (proteins, carbohydrates, fat, fiber, and ash) according to AOAC methods. Amino acid profiles were determined by HPLC analysis.

Results: Fat contents were low (<1.3%) in all four plant parts. The crude fiber was high in leaves and pods (19.9 and 15.1%, respectively). Protein contents in leaves (24.4%) and flowers (23.7%) were higher than that of the pods (19.3%). The limiting amino acids in the seeds were methionine and tryptophan, while the pods were deficient in four amino acids (methionine, tryptophan, histidine, and isoleucine). Of the four plant parts studied, the flowers turned out to be a promising source of protein because they meet most of the amino acid requirements for adults recommended by FAO.

Conclusion: The seeds and flowers of *E. edulis* have a high protein content and a good amino acid profile that makes them recommended for human consumption.

Keywords: Amino acids, *Erythrina edulis*, Proximate analysis

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INTRODUCTION

Erythrina edulis is one of the about 112 legume species belonging to the genus *Erythrina* [1]. The name of the genus comes from *erythros*, which means red in reference to the color of its flowers [2]. It is the only species with edible seeds and is widely cultivated in the highlands of the Andes from Venezuela to Bolivia as living fenceposts and to provide food for humans and domestic animals [3].

E. edulis is a tree that reaches 8-15 m in height and 24-45 cm in diameter, its fruit is a pod that measures 15-50 cm long, with 8-12 seeds per pod [4, 5].

This legume is distributed in tropical and subtropical areas of South America, in Peru it grows at altitudes of 900 to 3,200 meters above sea level, presenting for these reasons numerous vernacular names such as Sacha poroto, Inca bean, basul, pashuro, poroto, balú, chachafruto, among others [6, 7].

E. edulis is a perennial tree, long-lived (average 140 y), and multipurpose. It is a notable nitrogen fixer, controls soil erosion, and provides nectar from its abundant flowers. This species is one of the easiest trees to grow, does not require special care or phytosanitary treatment, and withstands long periods of drought. Sections of stem take root readily and become living and long-lasting fence posts. It can also be used for shading coffee, cocoa, and other sun-sensitive crops [6, 8].

This tree blooms in dark red and orange-red clusters 30-45 centimeters long and each cluster has an average of 190 flowers. The pods can have 8 to 12 dark brown kidney-shaped seeds, with two greenish-white cotyledons 3.5-7 cm long by 2-3 cm in diameter [9].

The leaves and immature pods of this legume are used to feed cattle, pigs, sheep, guinea pigs, and chickens [10]. The flowers are very beautiful so the tree can have an ornamental use. The flowers can also be used in salads and sweets and infusions taken for anxiety and urinary problems [9]. The large seeds are usually boiled in water with salt and are served as a side dish to corn, cassava, bread, or potatoes. The seeds are also consumed in the form of cake or dessert and the pods are sometimes used in the preparation of pickles [11, 12]. The seeds contain a high amount (18-25%) of proteins with high digestibility and quality higher than that of other legumes [6, 8, 11].

Although the use of different parts of the species *E. edulis* in human and animal nutrition is well known, their nutritional composition and amino acid profiles are not known in detail. The objective of the present work is to determine the proximal composition (proteins, fats, fiber, carbohydrates, and ashes) and the amino acid profiles of the seeds, pods, leaves, and flowers of *E. edulis* from Oxapampa, Peru, for a comprehensive benefit of this natural resource.

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals were of analytical grade. Amino acid standards, trifluoroacetic acid, and 2-mercaptoethanol were purchased from SIGMA-Aldrich; monobasic phosphate from MERCK, hydrochloric acid, methanol, acetonitrile, sodium bicarbonate, and milli-Q-Water. All reagents and solvents used were of analytical grade and HPLC grade respectively.

Plant material

The vegetable material was collected in Pozuzo, Pasco, Peru. Plant material was identified by one of us (A. P.), and a voucher specimen under the accession number Ee-126 was deposited at HOXA, Oxapampa, Peru (a Peruvian affiliate of the Missouri Botanical Garden, MO, USA). Samples were taken from seeds, leaves, pods, and flowers. Each sample was analyzed after the stove drying.

Proximate analysis

The proximal analyses of samples were performed according to the methodology established in AOAC 2010 [13]. Moisture contents of the samples were determined during desiccation at 105 °C until a constant weight was reached. Total fat was extracted in Soxhlet extractor with petroleum ether. Protein content was calculated by converting the nitrogen content determined by Kjeldahl (N x-factor

of 6.25). The total fiber was analyzed with alkaline and acid digestion, and the ash content was determined by incineration in a muffle furnace at 550 °C. Carbohydrate content was calculated as difference using protein, lipid, fiber, and ash content data.

Amino acid profile

Five milligrams of the defatted sample were hydrolyzed in a mixture of trifluoroacetic acid and HCl 6 N (1:2) and 2-mercaptoethanol at 166 °C for 25 min. For amino acid derivatization, the solution was dried and dissolved in 1 ml of $NaHCO_3$ (0.15 M) buffer [14].

Derivatization of amino acids was carried out according to Ribeiro *et al.* [15], with some modifications. Dabsyl chloride was dissolved in acetonitrile and then filtered and store at-20 °C. The elution buffer consisted of a solution of potassium phosphate acid 0.025M, pH 6. A dilution buffer was prepared by mixing 50 ml of ethanol and 50 ml elution buffer.

260 µl of dabsyl chloride solution (12.4 mmol) was added to aliquots of 160 µl of standard solution or hydrolyzed solution. The vials were mixed and incubated at 70 °C for 10 min, the reaction was stopped by placing the vials in an ice bath for 5 min. 580 µl of dilution buffer was added followed by mixing and centrifugation (5 min at 5000 rpm, the clear supernatants were filtered and placed in 1 ml vials.

A reversed-phase supelcosil TMLC-DABS column (15 x 4.6 cm; particle size 3 µm) was used. Two eluents were used as the mobile phase: elution buffer (A) and 80% acetonitrile (B). Elution was performed at a flow rate of 1 ml/min, starting with 25% B up to 10 min and installing a gradient to obtain 35% B at 35 min, 50% B at 45 min and 100% from B at 56 min, keeping 100% B up to 66 min. Detection was accomplished with a UV-vis detector at 436 nm. Quantification of free amino acids was achieved using the absorbance recorded in the chromatograms in relation to the external standards.

RESULTS AND DISCUSSION

The chemical compositions of seeds, leaves, pods, and flowers of *E. edulis* are shown in table 1. Fat contents were low (<1.3%) in all four plant parts. The crude fiber was high in leaves and pods (19.9 and 15.1%, respectively). Fiber helps prevent and control cardiovascular disease by reducing total and low-density lipoprotein (LDL) cholesterol levels. It also helps regulate blood sugar levels and is beneficial from a nutritional point of view, since fiber helps the absorption of trace elements in the intestine [16]. The ash content in leaves, pods, and flowers was high (<9%), which could indicate that the plant is rich in minerals that can contribute a substantial amount of minerals to our diet [17].

Table 1: Proximate analysis of seeds, leaves pods, and flowers of *Erythrina edulis*[a]

Parameter	Percentage (%)			
	Seeds	Leaves	Pods	Flowers
Fat	0.3±0.1	0.6±0.0	0.9±0.1	1.3±0.0
Ash	5.5±0.1	9.6±0.1	12.8±0.3	14.1±0.5
Crude Protein	26.4±1.2	24.4±0.7	19.3±0.1	23.7±0.8
Crude Fiber	7.5±0.7	19.9±0.3	15.1±0.6	10.6±0.1
Total Carbohydrate	60.2±1.4	45.6±0.6	51.9±0.5	50.3±1.2

[a]Values are the means of three determinations±SD (n=3).

The seeds have high contents of carbohydrates (60.2%) and protein (26.4%). Other studies reported protein values ranging from 18% to 26.2% [6, 10, 18]. The content of protein in the pods in the studied sample (19.3%) is similar to those found by Arango *et al.* [18] and Barrera and Mejía [10] (18.4% and 21.0%, respectively). Protein contents in leaves (24.4%) and flowers (23.7%) were higher than that of the pods (19.3%). No previous reports were found for protein contents in leaves and flowers of *E. edulis*.

The limiting amino acids in the seeds were methionine and tryptophan, while the pods were deficient in four amino acids (methionine, tryptophan, histidine, and isoleucine). The flowers were deficient only in histidine (13.2 mg/g protein).

Table 2: Amino acid profiles of seeds, leaves pods, and flowers of *Erythrina edulis*[a]

Aminoacid	mg/g protein				FAO[4]
	Seeds	Leaves	Pods	Flowers	
Essential amino acids					
Phenylalanine	39.2±0.9	35.8±1.4	25.0±1.0	91.1±2.9	19[b]
Histidine	22.5±1.1	5.6±0.3	1.4±0.1	13.2±1.0	16
Isoleucine	16.1±0.9	15.2±1.6	5.1±0.5	31.2±1.9	13
Leucine	52.3±2.7	63.9±1.3	35.2±1.0	35.5±1.2	19
Lysine	47.1±1.6	56.1±1.7	22.2±0.6	77.8±2.7	16
Methionine	10.0±0.4	14.7±0.3	8.4±0.7	19.4±0.2	17[c]
Threonine	18.0±1.1	22.3±1.0	12.2±1.0	30.8±2.1	9
Tryptophan	4.9±0.0	7.5±0.8	4.3±0.5	13.8±1.0	5
Valine	13.3±0.4	16.4±0.4	13.6±1.2	22.7±1.2	13
Non-essential amino acids					
Alanine	40.0±3.7	52.1±0.9	28.0±1.0	55.5±1.6	
Arginine	35.3±2.3	30.4±0.6	36.1±0.9	29.7±1.7	
Asparagine	2.9±0.0	4.8±0.0	2.7±0.1	6.1±0.3	
Aspartic acid	63.6±1.3	53.2±0.2	32.0±0.5	48.3±1.0	
Cysteine	5.7±0.2	5.8±0.0	2.6±0.1	11.1±1.1	
Glutamic acid	53.5±0.5	47.9±0.2	26.6±1.3	42.5±1.3	
Glutamine	4.9±0.2	14.4±1.2	8.8±0.8	10.5±2.1	
Glycine	47.1±1.5	47.5±1.7	29.4±0.5	59.5±2.3	
Proline	30.9±1.3	25.5±1.1	24.9±1.4	27.3±2.7	
Serine	35.7±1.3	64.7±2.2	24.9±0.1	49.0±2.5	
Tyrosine	12.3±0.2	9.6±0.5	5.0±0.3	14.5±0.7	

[a]Values are the means of three determinations±SD (n=3)., [b]Phenylalanine+tyrosine; [c]Methionine+cysteine

According to Morales A. [19], the biological quality of proteins from *E. edulis* is superior to that of beans, lentils, peas, or chickpea. The biological value of the pajuro protein is 70.9% and, compared to other legumes such as lentil (44%), beans (58%), or peas (63.7%), is much higher, as mentioned by Acero [11].

The results are consistent with those reported by D´Amore [20], the seeds of *Erythrina edulis* comply with most of the amino acids required by the FAO except for methionine and tryptophan (10 and 4.9 mg/g of protein, respectively). Intiquilla *et al.* reported that in the seeds of *E. edulis* only four amino acids (histidine, leucine, lysine, and valine) meet the requirements. It should be mentioned that the values found by Intiquilla *et al.* are lower than those reported by us, except for histidine whose value is similar to 22 mg/g protein.

The most abundant essential amino acids in the seeds were leucine and lysine (52.3 and 47.1 mg/g of protein, respectively). Aspartic acid and glutamic acid were the major non-essential amino acids (63.6 and 53.5 mg/g protein, respectively), these findings were similar to those reported by Intiquilla *et al.* [21].

In the literature, no reports have been found of the amino acids present in the leaf, pods, and flowers. Thus, in the case of the leaves, they only present two deficient amino acids (histidine and methionine) concerning the requirements reported by the FAO for adults.

The most abundant essential amino acids in the leaves were leucine and lysine with values of 63.9 and 56.1 mg/g of protein, respectively, while aspartic acid and serine were the major non-essential amino acids (53.2 and 64.5 mg/g protein, respectively).

The chemical score referred to the FAO of the pods was evaluated observing that they are deficient in four amino acids: histidine (1.4 mg/g protein), isoleucine (5.1 mg/g protein), methionine (8.4 mg/g protein), and tryptophan (4.3 mg/g protein).

The most abundant essential amino acids present in the pods were leucine and phenylalanine (35.2 and 25.0 mg/g of protein, respectively). Aspartic acid and serine were the non-essential amino acids with the highest quantity, with values of 32.0 and 36.1 mg/g protein, respectively.

Regarding flowers, only histidine (13.2 mg/g protein) is deficient according to its chemical score referred by the FAO. The most abundant essential amino acids were phenylalanine and lysine (91.1 and 77.8 mg/g protein). The major non-essential amino acids were glycine and alanine (53.2 and 64.5 mg/g protein, respectively).

Erythrina edulis is a native species that, despite its high protein and good amino acid profile, is being underestimated and under-exploited. Like other indigenous crops, *E. edulis* is in danger of being replaced by other cultivated species and of suffering continuous genetic erosion, putting at risk a food resource of local populations [22].

CONCLUSION

The protein contents of seeds, pods, leaves, and flowers of *Erythrina edulis* were 26.4, 19.3, 24.4, and 23.7%, respectively. Of the four plant parts studied, the flowers turned out to be a promising source of protein because it meets most of the amino acid requirements for adults recommended by FAO. The limiting amino acids in the seeds were methionine and tryptophan, while the pods were deficient in four amino acids (methionine, tryptophan, histidine, and isoleucine).

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Nil

AUTHORS CONTRIBUTIONS

A. P. conceived the project and identified the plant specimen; J. G. collected the plant material and drafted the manuscript; R. P. carried out the proximate analysis and interpreted the data; C. R. determined the amino acid profiles of plant samples and analyzed the results; R. R. planned and directed the project, and drafted the manuscript with the input of all the authors. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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