Nutritional, Antinutritional Compounds and Nutraceutical Significance of Native Bean Species (Phaseolus spp.) of Mexican Cultivars

Montserrat Alcázar-Valle, Soledad García-Morales, Luis Mojica, Norma Morales-Hernández, Ever Sánchez-Osorio, Lourdes Flores-López, Jhony Navat Enriquez-Vara and Eugenia Lugo-Cervantes

Abstract: Common beans (Phaseolus vulgaris), comba beans (Phaseolus lunatus), and ayocote beans (Phaseolus coccineus) are the most consumed beans worldwide. This work aimed to analyze the nutraceutical potential, antinutritional content, antioxidant activity, and physicochemical characteristics of 38 native bean accessions from South Pacific Mexico. Regarding size, the accessions collected from P. lunatus were the longest (15.31 ± 1.31 mm) and widest (11.04 ± 0.85 mm), while those of P. coccineus were of a greater thickness (6.40 ± 0.85 mm). In addition, it was observed that P. vulgaris species presented a higher percentage of protein and fat content. Moreover, an inverse correlation was found between the content of carbohydrates, fats, and proteins in all the collected accessions. The main free amino acid of P. lunatus and P. vulgaris was tryptophan with concentrations of 35.36 ± 0.37 mg/100 g and 47.41 ± 0.53 mg/100 g, respectively. While P. coccineus contained isoleucine as its main free amino acid with a concentration of 29.85 ± 1.06 mg/100 g. Furthermore, the collected accessions could be 100% correctly classified as P. vulgaris, P. lunatus, or P. coccineus according to the free amino acid content; this classification could serve as a marker to distinguish between Phaseolus species. In addition, principal component analysis of the phenolic compound content, nutritional composition, antinutritional factors, and antioxidant activity was performed, showing not only that P. vulgaris accessions can present nutraceutical potential but also that some accessions from P. lunatus and P. coccineus species can be promoted for the development of functional foods.

Keywords: Phaseolus spp.; amino acid content; antinutritional compounds; antioxidant activity; phenolic compounds; proximate composition

1. Introduction

Legumes are complex sources of carbohydrates, protein, fat, and dietary fiber, of which the main components are carbohydrates (55–60%) and proteins (17–40%) [1]. In addition, micronutrients such as vitamins, carotenoids, and phenolic compounds are essential to legumes’ sensorial and nutritional quality. These compounds have been reported as preservatives in natural foods. They play an important role preventing some chronic diseases such as diabetes, obesity, cardiovascular diseases, and some types of cancer [2].

However, legumes contain other minor compounds that have been considered antinutritional compounds, which can compromise the bioavailability and, consequently, the nutritional quality [3]. However, in the field of nutraceutical foods, the beneficial effects of these compounds are increasingly being recognized, mainly in soybeans, and common beans [4].
Common beans (*Phaseolus vulgaris* L.) are one of the most consumed legumes around the world [3,5], with the species *Phaseolus lunatus* and *Phaseolus coccineus* ranking in second and third place in terms of bean consumption, respectively [6]. Of note, other authors have reported *P. coccineus* and *P. lunatus* to be in second and third place worldwide distribution, respectively [7].

There are several reports addressing the nutritional content, phenolic components, and nutraceutical potential of some accessions of common beans (*P. vulgaris*), and some varieties of *P. coccineus* and *P. lunatus* [8–10]. Additionally, there is evidence that native species of common beans are essential because they contain important nutritional components for food safety and supplementation; for example, it has been shown that amino acid content in common beans varies according to genetic and environmental factors, genotype-environment interactions, seed management, and grain storage process [11]. In this context, it is reported that the predominant free amino acid in common beans are arginine, aspartic acid, asparagine, glutamic acid, and leucine [12]. Some amino acids, besides playing a key role in structural and physiological functions, are important precursors of metabolites, and can also act as antioxidants, that could help to decrease the oxidative stress, like tryptophan, phenylalanine, isoleucine or proline to mention a few, even few quantities of free amino acids could contribute to this antioxidant activity [13]. Moreover, free amino acids are the main constituents for a healthy skin like serine, glycine and alanine [12]. Therefore, some studies have focused on searching for the free amino acid profile in plants in order to identify alternatives of human health products in the food, pharmaceutical, and cosmetic industry [11–13].

With these points in mind, this work aims to analyze the nutraceutical potential of native beans (*P. coccineus*, *P. lunatus* and *P. vulgaris*) based on their nutritional content, phenolic compounds, antioxidant activity, and antinutritional factors to promote their propagation, increase their intake, and incentivize their conservation.

2. Materials and Methods

2.1. Plant Material

A total of 38 native beans were collected: 18 bean accessions in the state of Guerrero and 20 accessions in the state of Oaxaca from July to September 2019 (Figure 1).

![Figure 1. Native bean accessions were collected from South Pacific Mexico.](image-url)

2.2. Physicochemical Analysis

The 38 accessions collected were measured to determine their size (length, width, and thickness) and weighed by randomly selecting 100 seeds from each accession. The seeds were classified by weight into small (<25 g), medium (25–40 g), or large (>40 g) according to Singh et al. [14].
Furthermore, bean color evaluation was determined according to the following: luminosity (L), chromaticity (c), and hue (h) [15], using de CIE L*A*B scale spectrophotometry (CM-5, Konica Minolta Sensing Americas, Ramsey, NJ, USA).

In addition, proximate analysis of the various bean seeds was performed in order to determine the protein (AOAC, 960.52), moisture (AOAC, 945.03), lipid (AOAC, 920.30), raw fiber (AOAC, 985.28), and total carbohydrates content by differences [16].

Finally, free essential amino acid (leucine, phenylalanine, lysine, valine, threonine, isoleucine, histidine, methionine, and tryptophan) and nonessential amino acids (glutamic acid, aspartic acid, arginine, serine, glycine, tyrosine, alanine, proline, and cysteine) were quantified based on the official method from the AOAC [17].

2.3. Phenolic Compounds

Phenolic compounds were extracted for 16 h at room temperature by weighing out and macerating 15 g of bean flour with 150 mL of acetone, water and acetic acid (70:29:5.0:0.5 v/v/v/v). Next, the extracts were centrifuged (SL 40R, Thermo Scientific Waltham, MA, USA) and washed once with the solvent mixture; both supernatants were added and retained. The resulting extract was then concentrated using a rotatory evaporator at 45 °C and 450 mbar (R-210, Buchi, Flawil, Switzerland). Finally, the extracts were frozen at −20 °C until analysis [18,19].

The total phenol content was measured according to the Folin-Ciocalteu (F-C) spectrophotometric method [1], and the absorbance was measured at 765 nm (Infinite M200 Pro, TECAN, Männedorf, Switzerland) with gallic acid (mg GAE/G) as a reference.

Flavonoid analysis was carried out using the aluminum chloride test [20]. The flavonoid content was expressed as quercetin equivalents (mg QE/g), and the absorbance was measured at 410 nm (Infinite M200 Pro, TECAN, Männedorf, Switzerland).

Condensed tannins were analyzed using the vanillin test [21,22]. Based on catechin equivalents (mg CAE/g), the absorbance was read at 550 nm (Infinite M200 Pro, TECAN, Männedorf, Switzerland).

Anthocyanin analysis was performed with the bean seed coat according to Mojica et al. [23].

2.4. Antioxidant Activity

The antioxidant activity was determined according to ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Diroominium Salt) and DPPH (2,2 Diphenyl-1-picrylhydrazyl) assays, and both test reaction blanks and control samples were prepared. Accordingly, the radical scavenging activity was calculated [24].

2.5. Antinutritional Compounds

Phytic acid and oligosaccharide assays were performed using the commercial kits K-PHYT and K-RAFGL (Megazyme, Wicklow, Ireland), respectively, following the instructions provided by the supplier.

For lectin activity, the bean extracts were prepared following the methodology proposed by De Mejia et al. [25], with some modifications. Lectin activity was expressed as agglutination activity, which was the inverse of the maximum dilution at which agglutination was observed in mg of protein (HAU/mg protein).

Trypsin inhibitory activity was determined from prepared bean samples according to the methodology of Kakade et al. [26]. The trypsin activity methodology was performed using the commercial thypsin activity colorimetric kit MAK290-1KT (Sigma-Aldrich, St. Louis, MO, USA), with some modification. Trypsin activity was calculated, and trypsin activity formation was compared with that of the control to determine the TIU, which is defined as the amount of inhibitor that is required to inhibit one microgram of pure trypsin. The results were reported as TIU/g of the sample.
2.6. Statistical Analysis

The statistical software Statgraphics Centurion XVI version 16.1.03 (Statgraphics Technologies, The Plains, VA, USA) was used to determine Pearson’s correlation coefficients with protein, carbohydrates, lipids, moisture, ash, and raw fiber. Analysis of variance (ANOVA) was performed with a confidence level of α = 0.05. The difference between the mean values was analyzed with Tukey’s test with a significance level of p < 0.05.

Discriminant analysis of free essential and nonessential amino acids was performed to classify the accessions collected from the various species. Principal component analysis was performed to explain the variability of the collected accessions based on their phenolic content, nutritional and antinutritional components, and antioxidant activity.

3. Results

A total of 38 native beans were collected from South Pacific Mexico (Figure 1), of which 18 were collected in the state of Guerrero and 20 in the state of Oaxaca; two accessions belong to *P. coccineus* species; three accessions belong to *P. lunatus*; and 33 accessions belong to *P. vulgaris* (Table S1).

### 3.1. Physical Analysis

Based on the weight of 100 bean seeds, 66% of the accessions were small, 18% were medium, and 16% were large. Regarding size, the accessions collected from *P. lunatus* were the longest (15.31 ± 1.31 mm) and widest (11.04 ± 0.85 mm), while those of *P. coccineus* were of a greater thickness (6.40 ± 0.85 mm).

The luminosity (L) of the 38 accessions collected ranged from 73.95 ± 0.13% to 17.05 ± 0.09%, and the accessions with the highest luminosity were GR-12 (73.95%), OX-12 (73.02%), OX-11 (71.60%), OX-14 (71.50%), and OX-07 (69.30%). The hue (H) ranged from 1.50 ± 0.01 to −1.50 ± 0.07, and the chromaticity (c) ranged from 22.8 ± 0.73 to −0.23 ± 0.01.

Regarding the chromaticity, hue, and luminosity, it was observed that the accessions of *P. coccineus* presented purple (GR-15), and black (OX-20) tones, and for *P. lunatus*, the color were black–purple (GR-01), light brown (GR-08), and white (GR-09). In the case of *P. vulgaris*, it was observed that the accessions presented different tones, which were white, light brown, dark brown, purple, red, black, black-purple, and black–blue (Table 1).

### Table 1. Physical characteristics of bean seeds (*Phaseolus* spp.) collected in the states of Guerrero and Oaxaca, Mexico.

| Sample        | L    | H    | c    | Length (mm) | Width (mm) | Thickness (mm) | Weight (g) | Color         |
|---------------|------|------|------|-------------|------------|----------------|------------|---------------|
| GR-01 P1      | 24.24| 0.14 | 1.27 | 1.05 ± 0.03 | 15.22 ± 1.25 | 11.56 ± 0.89 | 4.38 ± 0.31 | Black-purple  |
| GR-02 Pn      | 39.46| 0.76 | 0.89 | 22.38 ± 0.73 | 8.58 ± 0.65 | 6.44 ± 0.46 | 5.05 ± 0.38 | Brown         |
| GR-03 Pn      | 68.64| 0.22 | 1.44 | 14.00 ± 0.10 | 8.50 ± 0.75 | 5.86 ± 0.38 | 5.10 ± 0.42 | White         |
| GR-04 Pn      | 21.31| 0.13 | 1.45 | 1.39 ± 0.01 | 9.91 ± 0.27 | 5.94 ± 0.54 | 4.06 ± 0.40 | Black-purple  |
| GR-05 Pn      | 22.03| 0.21 | 1.50 | 2.23 ± 0.17 | 10.63 ± 0.69 | 6.46 ± 0.32 | 4.32 ± 0.25 | Black-purple  |
| GR-06 Pn      | 68.64| 0.20 | 1.33 | 19.60 ± 0.03 | 9.19 ± 0.54 | 6.38 ± 0.54 | 4.43 ± 0.29 | Black-purple  |
| GR-07 Pn      | 61.56| 0.25 | 1.25 | 16.53 ± 0.09 | 12.40 ± 0.98 | 7.21 ± 0.58 | 4.72 ± 0.47 | Light brown   |
| GR-08 Pn      | 57.20| 0.25 | 1.24 | 10.73 ± 0.25 | 15.13 ± 1.18 | 10.66 ± 0.79 | 4.13 ± 0.33 | Light brown   |
| GR-09 Pn      | 65.85| 0.13 | 1.31 | 13.88 ± 0.08 | 15.59 ± 1.50 | 10.91 ± 0.98 | 4.33 ± 0.70 | Light brown   |
| GR-10 Pn      | 22.10| 0.31 | 1.44 | 1.56 ± 0.05 | 8.70 ± 0.76 | 5.84 ± 0.58 | 4.01 ± 0.39 | Black-purple  |
| GR-11 Pn      | 32.31| 0.47 | 0.61 | 20.06 ± 0.16 | 10.27 ± 0.61 | 6.89 ± 0.51 | 4.63 ± 0.50 | Black-purple  |
| GR-12 Pn      | 73.95| 0.13 | 1.48 | 10.85 ± 0.03 | 8.83 ± 0.74 | 6.21 ± 0.46 | 5.11 ± 0.42 | Red           |
| GR-13 Pn      | 29.10| 0.39 | 0.33 | 17.00 ± 0.47 | 10.84 ± 0.94 | 5.70 ± 0.39 | 4.61 ± 0.43 | Purple        |
| GR-14 Pn      | 18.47| 0.07 | 0.73 | 11.65 ± 0.47 | 10.20 ± 0.91 | 6.28 ± 0.61 | 4.70 ± 0.44 | Black-purple  |
| GR-15 Pn      | 37.05| 0.66 | 0.73 | 11.37 ± 0.13 | 10.15 ± 1.21 | 9.60 ± 0.91 | 6.13 ± 0.75 | Black-purple  |
| GR-16 Pn      | 42.74| 0.29 | 0.94 | 13.37 ± 0.13 | 11.95 ± 1.28 | 7.89 ± 0.82 | 5.94 ± 0.79 | Red           |
| GR-17 Pn      | 23.18| 0.17 | 1.06 | 17.63 ± 0.10 | 14.17 ± 1.16 | 8.93 ± 0.70 | 6.63 ± 0.70 | Black-blue    |
| GR-18 Pn      | 55.06| 0.82 | 1.05 | 20.05 ± 0.24 | 10.30 ± 0.74 | 6.43 ± 0.42 | 5.45 ± 0.35 | Black-blue    |
| OX-01 Pn      | 19.82| 0.04 | 1.82 | 11.11 ± 0.89 | 9.48 ± 0.68 | 6.10 ± 0.38 | 4.15 ± 0.35 | Black-blue    |
| OX-02 Pn      | 17.47| 0.27 | 1.50 | 0.34 ± 0.03 | 9.53 ± 0.72 | 5.83 ± 0.43 | 4.21 ± 0.38 | Black-purple  |
| OX-03 Pn      | 19.22| 0.09 | 1.44 | 0.34 ± 0.03 | 9.56 ± 0.74 | 6.14 ± 0.44 | 4.58 ± 0.37 | Black-purple  |
| OX-04 Pn      | 17.05| 0.09 | 1.45 | 0.40 ± 0.15 | 8.82 ± 0.90 | 5.23 ± 0.46 | 3.67 ± 0.44 | Black-blue    |
| OX-05 Pn      | 18.93| 0.25 | 1.37 | 1.48 ± 0.13 | 10.40 ± 0.88 | 7.11 ± 0.70 | 5.26 ± 0.36 | Light brown   |
| OX-06 Pn      | 66.08| 0.29 | 1.32 | 21.79 ± 0.79 | 9.39 ± 0.64 | 5.97 ± 0.33 | 4.97 ± 0.30 | White         |
| OX-07 Pn      | 69.23| 0.16 | 1.43 | 14.37 ± 0.26 | 9.70 ± 0.06 | 5.66 ± 0.45 | 4.33 ± 0.57 | Light brown   |
| OX-08 Pn      | 49.47| 0.64 | 1.01 | 21.01 ± 0.18 | 8.70 ± 0.86 | 5.66 ± 0.45 | 4.33 ± 0.57 | Light brown   |
3.2. Nutritional Content

In addition, the ash, protein, carbohydrate, fat, moisture, and raw fiber contents of the 38 accessions were analyzed (Table S2). In the present work, it was observed that *P. vulgaris* species presented a higher percentage of protein and fat content, but presented a low carbohydrate content (Table 2). Moreover, according to Pearson’s correlation analysis for all the accessions collected, there was an inversely proportional relationship between carbohydrates and fats (r = −0.49; p-value = 0.0017) and between carbohydrates and proteins (r = −0.76; p-value = 0.05).

Table 2. Proximate analysis by *Phaseolus* species (% w/w).

| Species      | Moisture  | Ashes   | Fat     | Protein | Carbohydrates | Fiber | Food Energy (Kcal/100 g) |
|--------------|-----------|---------|---------|---------|---------------|-------|--------------------------|
| *P. coccineus* | 11.20 ± 0.74 | 4.27 ± 0.50 | 1.87 ± 0.44 | 17.28 ± 2.06 | 65.39 ± 1.39 | 4.18 ± 0.33 | 424.54 ± 9.96 |
| *P. lunatus*  | 9.62 ± 0.77 | 3.48 ± 0.19 | 1.60 ± 0.08 | 19.68 ± 0.58 | 65.62 ± 0.85 | 5.21 ± 0.20 | 445.98 ± 8.13 |
| *P. vulgaris* | 10.16 ± 1.49 | 3.78 ± 0.25 | 2.22 ± 0.38 | 20.48 ± 2.78 | 62.57 ± 0.43 | 5.02 ± 1.76 | 444.78 ± 2.45 |

Mean values ± SD with different lowercase letters within the same column denote significant differences based on Tukey’s test (p < 0.05).

In addition, 18 free amino acids (essential and non-essential) were analyzed from the 38 accessions collected. In terms of free essential amino acids (leucine, phenylalanine, lysine, valine, threonine, isoleucine, histidine, methionine, and tryptophan), it was observed that *P. vulgaris* species showed a higher concentration, followed by the *P. lunatus* and *P. coccineus* species (Figure 2a).

The main free amino acid of *P. lunatus* and *P. vulgaris* was tryptophan, with concentrations of 35.36 ± 0.37 mg/100 g and 47.41 ± 0.53 mg/100 g respectively. While *P. coccineus* contained isoleucine as its main free amino acid, with a concentration of 29.85 ± 1.06 mg/100 g (Figure 2a).

Concerning non-essential free amino acids (glutamic acid, aspartic acid, arginine, serine, glycine, tyrosine, alanine, proline, and cysteine), the *P. vulgaris* species also showed the highest concentration of these amino acids, followed by the *P. coccineus* and *P. lunatus* species. In all three species, arginine was found to be the main free amino acid, with a concentration of 107.86 ± 0.18 mg/100 g for *P. coccineus*, 123.54 ± 0.16 mg/100 g for *P. lunatus*, and 177.89 ± 0.47 mg/100 g for *P. vulgaris* (Figure 2b).

Additionally, all the accessions were used to develop a model to discriminate among the three *Phaseolus* species (levels). In this model, 18 free amino acids were introduced as predictor variables, and two discriminant functions resulted (p < 0.05). When these discriminant functions were used to graph the 38 accessions, all of them were 100% correctly classified into three *Phaseolus* species (Figure 3).
3.3. Phenolic Compounds, Antioxidant Activity, and Anti-Nutritional Composition

The accessions of *P. lunatus* revealed a higher concentration of total phenols (Table 3). According to Tukey mean analysis, there were no statistically significant differences between the *P. vulgaris* and *P. coccineus* species.

Regarding the content of flavonoids, the three species showed statistically significant differences, where the *P. coccineus* species showed the highest concentration. However, the *P. vulgaris* species showed the highest concentration of tannins and anthocyanins as well as antioxidant activity (Table 3).
Hence, the phenolic content and antioxidant activity showed a positive correlation between tannins and antioxidant activity ($r = 0.44, p = 0.005$) and with anthocyanins ($r = 0.49, p = 0.002$), while anthocyanins also showed a positive correlation with the antioxidant activity ($r = 0.50, p = 0.001$). In addition, there is a negative correlation between color parameters, phenolic compounds and antioxidant activity (Table 4), the above confirms that the lighter the bean color is, the lower concentration of phenol compounds are reached, and therefore the antioxidant activity decrease.

However, a negative correlation was observed between the oligosaccharide content and antioxidant activity ($r = -0.36, p = 0.029$). In particular, the *P. coccineus* species showed the highest concentration of oligosaccharides and the lowest antioxidant activity (Table 3). Additionally, this species showed the highest concentration of phytic acid.

Regarding trypsin inhibitory activity, the accessions of *P. lunatus* showed the highest activity, whereas the accessions of *P. vulgaris* showed the highest lectin content (Table 3).

Principal component analysis was then performed, generating four principal components, of 12 variables for analysis (total phenols, flavonoids, tannins, anthocyanins, ABTS, and DPPH radical scavenging activity). A reduced number of linear combina-

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**Table 3.** Total phenolic, flavonoid, anthocyanin, tannin, phytic acid, oligosaccharide, lectin, trypsin inhibitory activity, and radical scavenging activity from *Phaseolus* accessions collected.

| Variables Evaluated                          | *P. coccineus*   | *P. lunatus*    | *P. vulgaris*   |
|----------------------------------------------|------------------|----------------|----------------|
| Total phenolic content (mg GAE/g)            | 472.03 ± 0.17    | 604.85 ± 0.35  | 440.40 ± 0.61  |
| Flavonoid content (mg QE/g)                  | 12.38 ± 0.45     | 4.99 ± 0.94    | 8.82 ± 0.77    |
| Anthocyanin content (mg C-3G/g seed coat)    | 0.53 ± 0.06      | 1.71 ± 1.32    | 2.69 ± 1.24    |
| Tannin content (mg CAE/g)                    | 1.01 ± 0.65      | 0.59 ± 0.70    | 1.37 ± 0.98    |
| Phytic acid content (mg/100 g bean flour)    | 0.58 ± 0.22      | 0.43 ± 0.32    | 0.39 ± 0.56    |
| Oligosaccharid content (mg/100 g bean flour) | 6.44 ± 0.04      | 3.68 ± 0.11    | 3.92 ± 0.27    |
| Lectin (HAU/mg protein)                      | 1.33 ± 0.12      | 1.52 ± 0.03    | 1.63 ± 0.18    |
| Trypsin inhibitory activity (TIU/g bean flour) | 15.97 ± 0.03  | 18.05 ± 0.01  | 13.19 ± 0.32  |
| Radical scavenging activity (% ARSA)         | 80.57 ± 0.08     | 84.18 ± 0.01   | 86.43 ± 0.06   |
| Radical scavenging activity (% DRSA)         | 56.47 ± 0.08     | 88.08 ± 0.02   | 89.28 ± 0.04   |

Mean values ± SD with different lowercase letters within the same row denote significant differences based on Tukey’s test ($p < 0.05$). ARSA: (ABTS radical scavenging activity), DRSA: (DPPH radical scavenging activity).
tions was obtained that explained 66.83% of the data variability (Table S3). By plotting PC1 (26.30%) and PC3 (13.82%), the accessions could be grouped into the following: PC1 positive, phenolic compounds and antioxidant activity; PC1 negative, oligosaccharides, and phytic acid; PC3 positive, protein, raw fiber and lectin; or PC3 negative, trypsin inhibitory activity (Figure 4).

This analysis revealed 13 native accessions of *P. vulgaris* species. One accession of *P. lunatus* species was placed into the PC1 positive group. Therefore, these accessions had a high content of phenolic compounds and antioxidant activity (Table 5). Furthermore, four accessions form *P. vulgaris* species showed a high protein content, and these same accessions also exhibited a high lectin content (PC3 positive). Thus, there was a positive correlation between the protein and lectin content from all the species collected (r = 0.55, p-value = 0.0004).

**Table 4.** Correlation analysis, of bean color and nutraceutical compounds with a confidence level of 95%.

| Correlation                      | r   | p    |
|----------------------------------|-----|------|
| Luminosity and hue               | 0.47| 0.003|
| Luminosity and chromaticity      | 0.64| 0.000|
| Luminosity and phenols           | -0.53| 0.001|
| Luminosity and flavonoids        | -0.72| 0.000|
| Luminosity and tannins           | -0.67| 0.000|
| Luminosity and DPPH              | -0.49| 0.000|
| Luminosity and anthocyanins      | -0.66| 0.000|
| Hue and flavonoids               | -0.39| 0.017|
| Hue and tannins                  | -0.56| 0.000|
| Chromaticity and tannins         | -0.49| 0.002|
| Chromaticity and DPPH            | -0.59| 0.000|
| Chromaticity and anthocyanins    | -0.80| 0.000|

**Figure 4.** Principal component analysis of total phenolic (TPH), flavonoid (FLV), anthocyanin (ANT), tannin (TAN), phytic acid (PHY), oligosaccharide (OSD), lectin (LET), trypsin inhibitory activity (TRY), raw fiber (FBR), protein (PTN) and radical scavenging activity (ARSA, DRSA) from *Phaseolus* accessions collected.
Table 5. Phaseolus accessions group by principal component analysis.

| Sample | Specie          | PCA1  | PCA3  |
|--------|-----------------|-------|-------|
| OX-01  | P. vulgaris     | 1.83  | -1.79 |
| OX-02  | P. vulgaris     | 1.47  | -1.72 |
| OX-03  | P. vulgaris     | 1.63  | -1.40 |
| OX-04  | P. vulgaris     | 4.52  | 0.76  |
| OX-05  | P. vulgaris     | 2.54  | -0.94 |
| OX-09  | P. vulgaris     | 2.51  | 0.06  |
| OX-10  | P. vulgaris     | 3.05  | -1.22 |
| OX-13  | P. vulgaris     | 1.90  | 1.30  |
| OX-16  | P. vulgaris     | 1.31  | 0.85  |
| GR-01  | P. lunatus      | 0.50  | -0.46 |
| GR-04  | P. vulgaris     | 1.63  | 1.47  |
| GR-05  | P. vulgaris     | 1.74  | 1.43  |
| GR-10  | P. vulgaris     | 0.98  | 0.33  |
| GR-14  | P. vulgaris     | 1.45  | -0.09 |

Phenolic compounds and antioxidant activity

| Sample | Specie          | PCA1  | PCA3  |
|--------|-----------------|-------|-------|
| OX-06  | P. vulgaris     | -1.31 | 1.22  |
| OX-07  | P. vulgaris     | -0.91 | 0.11  |
| OX-08  | P. vulgaris     | -1.76 | 0.86  |
| OX-12  | P. vulgaris     | -1.12 | -0.02 |
| OX-14  | P. vulgaris     | -2.39 | 0.33  |
| OX-19  | P. vulgaris     | -1.45 | 0.20  |
| OX-20  | P. coccineus    | -1.45 | -0.88 |
| GR-03  | P. vulgaris     | -1.78 | -0.61 |
| GR-06  | P. vulgaris     | -2.36 | 1.61  |
| GR-07  | P. vulgaris     | -1.88 | -0.98 |
| GR-08  | P. lunatus      | -1.06 | -0.51 |
| GR-09  | P. lunatus      | -1.20 | 0.03  |
| GR-15  | P. coccineus    | -2.93 | -1.75 |
| GR-17  | P. vulgaris     | -0.70 | -0.01 |
| GR-18  | P. vulgaris     | -1.98 | -1.49 |
Table 5. Cont.

| Sample  | Specie      | PCA1   | PCA3   |
|---------|-------------|--------|--------|
| OX-11   | *P. vulgaris* | −0.54  | 0.80   |
| GR-02   | *P. vulgaris* | −0.98  | 2.52   |
| GR-12   | *P. vulgaris* | −1.32  | 2.98   |
| GR-13   | *P. vulgaris* | 1.11   | 2.61   |

Protein, fiber, lectin

| Sample  | Specie      | PCA1   | PCA3   |
|---------|-------------|--------|--------|
| OX-15   | *P. vulgaris* | 0.90   | −1.63  |
| OX-17   | *P. vulgaris* | −0.10  | −0.85  |
| OX-18   | *P. vulgaris* | 0.00   | −0.05  |
| GR-11   | *P. vulgaris* | −0.47  | −1.52  |
| GR-16   | *P. vulgaris* | −1.38  | −1.52  |

Inhibitory trypsin activity
4. Discussion

According to Tukey mean comparison analysis (p < 0.05), the collected accessions of *P. lunatus* were the largest, while the accession of *P. coccineus* showed the greater thickness and *P. vulgaris* the smallest (Table 1). The above agrees with some previous reports, in which *P. vulgaris* varieties are noted to be the smallest in the central and south Mexico regions, especially in the states of Guerrero and Oaxaca, where, phenotypically, weight and size predominate among the Mesoamerican beans varieties [27,28]. Additionally, according to Singh et al. [14], common beans grow from northern Mexico to northwestern Argentina; in this wide region, the Mesoamerican varieties are characterized by small seeds (<25 g/100 seeds).

Moreover, *P. coccineus* has two domestication centers, the Mesoamerican and the Andean, in terms of the morphological characteristics of the Andean seed, which is characterized by large or medium size, whereas the Mesoamerican seed varieties are small. In addition, color is yet another important characteristic for *P. coccineus* varieties since the purple seed color is exclusive to the Andean varieties. In contrast, pink, brown, or black colors predominate in the Mesoamerican pool [6]. In the present work, of the two accessions collected, one had a purple tone (GR-15), and the other had a black tone (OX-20).

For the collected varieties of *P. lunatus*, in terms of their morphological characteristics, it is known that Mesoamerican varieties predominate in southern Mexico since this species is highly accepted by the population [29].

Regarding the coloration of the collected bean seeds, some reports mention that common bean red varieties predominate in the state of Guerrero, while in Oaxaca, a mix of seed colors dominates [27,30]. However, in the present work, several colors are found in the accessions collected in both states of Guerrero and Oaxaca (Table 1). This finding is consistent with the results of Castillo-Mendoza et al. [31], who reported that there is a greater diversity of colors in *P. vulgaris* accessions than in those of *P. coccineus*, which could be attributed to their culinary and commercial value in the region where the seeds were collected.

Nutritional analysis of the accessions collected showed that the collected species were within the ranges reported in other legume studies, including those reporting on common beans [32–35]. According to Tukey’s mean comparison analysis (p < 0.05), there were no statistically significant differences in terms of protein content between the species *P. lunatus* and *P. vulgaris* (Table 2). High content of proteins in legumes, mainly in common beans, could serve as a protein to help supplement people’s diets [35]. However, the protein quality of foods is defined in terms of the variety and amount of amino acids, as well as their bioavailability. Thus, protein absorption is dependent on a balance of amino acid content [11]. In addition, the structural amino acid profile and the nutritional score as a source of proteins must be taken into account.

The collected accessions of *Phaseolus* species, from the South Pacific region of Mexico, have potential as a source of protein (17.28–20.48%). Since in this region of the country, bean grains are the main dietary source of protein in rural communities due to their accessibility and without considering the protein quality of beans in relation to their amino acid composition and protein digestibility [11,36].

There were statistically significant differences in the fat content among the collected accessions of *P. lunatus* and *P. vulgaris*. The accessions of *P. lunatus* had the lowest fat content (Table 2). This finding is in agreement with the report by Farinde et al. [10], who found that the raw seed of *P. lunatus* contains a low percentage of fat, which can be beneficial for health since it reduces the risk of cardiovascular diseases. Additionally, regarding the carbohydrate content, there were no statistically significant differences between the varieties of *P. coccineus* and *P. lunatus*, and both species exceeded the carbohydrate content compared to that found in *P. vulgaris* (Table 2). Thus, one may assume that the collected accessions of *P. coccineus* and *P. lunatus* could be good energy sources.

In addition, there were significant differences in the moisture content between *P. coccineus* and *P. lunatus* species, with the accessions of *P. coccineus* revealing a higher
moisture content (Table 2), possibly due to environmental characteristics, type of soil, and crop varieties [9]. Since no significant differences in raw fiber content were found among the three species evaluated (Table 2), it could be assumed that all the accessions collected have a similar potential to reduce cholesterol levels and colon diseases [10].

Finally, some studies have reported that there is an inversely proportional relationship between the fat and protein content in native species of *P. vulgaris*, and in some, cases, a proportional correlation between the protein content and cysteine and methionine content [11,32]. However, our work indicates that there is an inversely proportional correlation between the carbohydrate content and protein content \( r = -0.76 \) and between fats and carbohydrates \( r = -0.49 \); the above may be due to the type of fertilization and the local growing conditions [9] or a plant genotype-dependent characteristic [36].

This issue has been widely addressed, but little is known about the importance and use of free amino acids to obtain nutritional supplements with potential benefits for human health. Thus, in this work, the content of total proteins and free amino acids was determined since some of them are considered bioactive compounds [37].

On the other hand, the content of 18 free amino acids was obtained (Figure 2), of which nine are considered essential (tryptophan, methionine, histidine, isoleucine, threonine, valine, lysine, phenylalanine, and leucine) and nine non-essential (cysteine, proline, alanine, tyrosine, glycine, serine, arginine, aspartic acid, and glutamic acid). The importance of free amino acids lies in the fact that they can be metabolized or stored as a nutritional source, some of them function as signaling molecules during the regulation of cellular functions, including enzymatic activity, gene expression and oxide-reduction homeostasis; some free amino acids are also considered bioactive compounds [37].

As isoleucine was found to be the main free amino acid in *P. coccineus* species, it could be assumed that human consumption of these accessions (GR-15 and OX-20) could provide energy to muscles, regulate blood sugar and hemoglobin formation, and improve blood clotting [37]. In *P. vulgaris* specie, the main free amino acid was found to be tryptophan. This essential amino acid, as a bioactive molecule, appear to have the potential to play a role in autism therapy, as well as in the treatment of cardiovascular disease, cognitive function, chronic kidney disease, depression, and some inflammatory diseases [38,39]. However, the highest valine content was found in *P. lunatus*. Valine, along with leucine and isoleucine, could be involved in promoting anabolic pathways, preventing signs of hepatic encephalopathy, attenuating fatigue during exercise, and promotes wound healing [38].

These differences in free amino acid content between species could be attributable to the conditions under which the crops were grown [33–35]. In other crops, free amino acids composition was related to other factors such as crop conditions, geographic location of the production system, harvest time, variety, and soil conditions [37].

In the case of nonessential free amino acids (Figure 2b), in the three *Phaseolus* species, the content of arginine, glutamic acid, aspartic acid and cysteine was higher compared to the rest of the non-essential amino acids, although statistical differences were obtained between species. Some of these free amino acids are determinants of food flavor. For example, glycine, alanine and proline provide sweetness; while glutamate (the ionized form of glutamic acid) provides a characteristic flavor to certain foods. In addition, it has been reported that free amino acids are used for the biosynthesis of aromatic compounds during fruit ripening [37]. Others, such as proline, play an important role in the regulation of plant response to abiotic stresses [37]. Thus, free amino acids are crucial precursors of plant secondary metabolism, mainly for the synthesis of phenolic compounds and glucosinolates. These secondary metabolites have an essential role in the interaction of plants with the environment; as well as they are also directly or indirectly involved in human health, as many of these compounds and some free amino acids can contribute to decrease oxidative stress [40]. Since all the accessions collected were 100% correctly classified into three *Phaseolus* species, the specific content of free essential and non-essential amino acids is characteristic of each species collected. Therefore, these accessions have high potential in the field of functional foods (Figure 3).
Additionally, the phenolic compounds, antioxidant activity, and antinutritional content were assessed (Table 3). It was confirmed that the species of P. lunatus and P. coccineus might contain a higher or equal concentration of phenolic compounds [7,19].

Additionally, according to these results, the antioxidant activity was positively correlated with the anthocyanin and tannin content [4,41]. Thus, the highest content of phenolic compounds and antioxidant activity was shown in the dark-colored seeds, being mostly P. vulgaris species as well as one accession of the P. lunatus (GR-01) PC1 positive group (Table 5), regardless of species type [28].

Although the dark-colored accessions of P. vulgaris continue to have the best results in terms of phenolic compound content, some species of P. lunatus and, to a lesser extent, accessions of P. coccineus species may also have some health benefits since the composition could also provide antioxidants [7,19]. Furthermore, some accessions of P. vulgaris whose colors were lighter may exhibit some nutraceutical potential as anticarcinogens, inhibitors of lymphomas, or as alternatives to animal proteins, given the high concentrations of protein and lectin (PC3, positive) [4].

The antinutritional components and the concentration of oligosaccharides and phytic acid were all associated with lighter color accessions and grouped as PC1 negative. Moreover, the remaining two accessions of P. lunatus (GR-08 and GR-09), as well as the two accessions of P. coccineus (GR-15 and OX-20), were located in this group (Table 5).

Finally, the trypsin inhibitory activity (PC3, negative) of all the accessions belonged to P. vulgaris species, whose color ranged from dark to light tones (Table 5).

In addition to the dark accessions of P. vulgaris having nutraceutical properties, the accessions of P. coccineus and P. lunatus may have potential for the development of functional foods to help prevent diseases that have been increasing in the world population, such as obesity, diabetes, or even cancer.

5. Conclusions

In the present study, 38 native bean accessions were collected in two southern Mexico-producing states. These accessions were classified into three different species based on their physical and morphological characteristics. P. lunatus accessions were characterized by having the largest size; P. coccineus accessions showed the greatest thickness, and P. vulgaris was the smallest. Additionally, the accessions collected showed a great diversity of color tones, which could be related to local consumer preference, use, and intake.

Correlation analysis showed that, while the carbohydrate content was low, protein and fat content increased, regardless of the bean species. The analysis of 18 free amino acids allowed the 38 accessions collected to be 100% correctly classified into the three Phaseolus species (P. lunatus, P. coccineus, and P. vulgaris), supporting the morphological classification. The bean accessions have a specific free amino acid content that allowed distinguishing between each of the three species. The content of these free amino acids should also be considered as a bioactive ingredient.

Principal component analysis showed that the dark-colored species had the highest concentration of phenolic compounds and antioxidant activity, regardless of Phaseolus species. In contrast, the light-colored beans had the highest content of antinutritional factors.

All native bean accessions analyzed showed nutraceutical potential, and their intake could bring health benefits to consumers, ranging from protecting against oxidative stress to the prevention of some inflammatory diseases. Future research should focus on the analysis of free amino acids as bioactive compounds, as well as their biological activity for use in the food industry, especially those related to health-promoting attributes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11111031/s1, Table S1. Phaseolus species were collected in the states of Guerrero and Oaxaca, Table S2. proximate analysis from Phaseolus species collected (% w/w). Table S3. Component weight of total phenolic (TPH), flavonoid (FLV), anthocyanin (ANT), tannin (TAN), phytic acid (PHY), oligosaccharide (OSD), lectin (LET), trypsin inhibitory activity (TRY), raw fiber (FBR), protein (PTN) and radical scavenging activity (ARSA, DRSA) from Phaseolus accessions collected.
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