






## Article

# Physicochemical Characterization and Functional Potential of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. Landrace Green Beans

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**Abstract:** The green bean is an important crop worldwide, because it is rich in protein, dietary fiber, vitamins, and minerals, as well as bioactive compounds that provide it with important functional properties; however, the composition of many landraces is still unknown. The purpose of this project was to characterize *Phaseolus vulgaris* and *coccineus* L. landrace green beans on pH, titratable acidity, total soluble solids, total sugars, color parameters, total phenols, monomeric anthocyanins, and in vitro antioxidant activity (DPPH and FRAP). Regarding the content of total sugars, differences were registered between both species, as opposed to results observed in total soluble solids. Color parameters showed higher reddish tones for *P. vulgaris* landraces, though *P. coccineus* had a higher total phenolic content, especially the reddish landraces, which correlated directly to a higher antioxidant activity by DPPH and FRAP. In the protein content, the species *P. vulgaris* registered the highest content. These results could contribute to a greater use and even promote the genetic improvement of the outstanding pods that serve as one of the main food products in rural regions for higher benefits.

**Keywords:** total phenols; monomeric anthocyanins; antioxidant activity; protein

## 1. Introduction

Legumes are among the most important crops in the world, since they represent one of the main sources of food in developing countries, in addition to being rich sources of vegetable protein and can be used to supplement the deficiency of different cereals, due to their content of essential amino acids, fiber, vitamins, and minerals such as iron, potassium, magnesium, and zinc, among others [1].

Among the cataloged legumes by the FAO is the genus *Phaseolus*, which includes up to 117 species, considering the Mesoamerican region as the main center of both origin and diversity [2]. Many of them, such as *P. vulgaris* and *P. coccineus* L., seem to have been initially domesticated in western Mexico and are among the five most cultivated and consumed species in Mexico by the number of commercial varieties and landraces [3], in addition to being two of the three most relevant species worldwide [4].

In addition to the seed, which would be the bean, the pod, known in Mexico as green beans, is also consumed. Prior to the senescence and dehiscence of the pods, they grow and reach their commercial maturity, in which they can develop shades from yellow and green to reddish and purple, depending on the variety to which they belong [5]. Regarding its

nutritional composition, it has a high-water content, which contributes to its characteristic turgor, in addition to minerals and vitamin A. To a lesser extent, it provides protein and fiber [6]. Green bean is classified as a vegetable and is part of the daily diet in many countries, even being considered one of the main sources of phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (flavonols, flavones, dihydroflavonols, flavanones, isoflavones, and isoflavanones), lignans, and other polar compounds (Kutkoside) with potential health benefits such as slowing the development of cancerous tumors and providing antibacterial, antiviral, antispasmodic, and anti-inflammatory properties [7–9].

It has been shown that the anticancer activity of green beans is mainly related to the presence of high amounts of chlorophyll, resistant starch, soluble and insoluble dietary fiber, and phenolic compound, which suggests that its regular consumption may reduce the risk of breast, colon, and prostate cancer. Likewise, it has been reported that due to its high fiber content, it could help control diabetes by reducing insulin generation and glucose levels that enter the bloodstream; besides, patients suffering from non-insulin dependent diabetes can prevent the need for insulin by up to 40% by eating green beans in their regular diet [10–13].

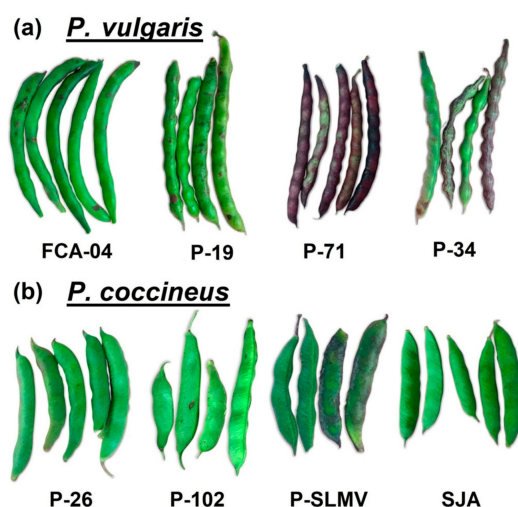
In addition to the nutritional aspect, endemic varieties represent a potential source of income for households in rural communities, since small farmers in Latin America and the Caribbean, Asia, and Africa contribute up to 77% of the world production of *P. vulgaris* [2]. However, a current trend has been observed in which endemic crops are being replaced by commercial outdoor varieties that tend to present a higher demand, harvest yield, and, therefore, commercial value. This leads to a gene pool loss since the landraces are limited to be cultivated in small plots or farmers backyards for self-consumption of the edible parts (flowers, leaves, fruits, and grains) as well as maintaining the germplasm for the next planting [14]. In addition to the biodiversity loss, there is a dependence on external resources that can compromise the economic and food security of these regions.

Part of the reason for the displacement of local varieties may be due to ignorance of their properties, so their study contributes to a better understanding and, in turn, to promoting their consumption. For this reason, the present work focuses on the physicochemical characterization and functional potential, measured through the phenolic compounds quantification and in vitro antioxidant activity, of various *P. vulgaris* and *P. coccineus* native varieties from rural regions of Oaxaca, Mexico, in order to know its nutritional and functional composition. This would contribute to promote both cultivation and consumption, with direct benefits in the food and economic security of rural populations, in addition to avoid the loss of unexplored germplasm with potential health benefits and maintaining the local gene pool.

## 2. Materials and Methods

### 2.1. Plant Material

A total of 18 bean landraces were evaluated; nine from *Phaseolus vulgaris* L. (common bean) and nine from *Phaseolus coccineus* (scarlet runner bean), which were collected in different regions of the state of Oaxaca, Mexico where each seed lots correspond to a farmers variety. This means that each farmer preserves one or more native varieties because Oaxaca is part of the Mesoamerican region where *P. vulgaris* and *P. coccineus* have their origin center. Seed lots of each landrace were sown and cultivated in an open field plot in the Training Unit for Rural Development (UNCADER2) located in Coatepec, Veracruz, carrying out a basic fertilization (60:60:60 N:P:K). Green bean was harvested at stage 4 of maturity, when the color change was already observed in the dorsal suture and pedicel (Figure 1), since it has been reported that at this stage of development there is a higher phenolic compounds content and in vitro antioxidant activity [15]. The whole fruits in fresh state were used to perform analysis of the physicochemical parameters and compounds with in vitro antioxidant activity.



**Figure 1.** Landrace green bean samples evaluated of *P. vulgaris* (a) and *P. coccineus* (b).

## 2.2. Physicochemical Parameters

pH was measured according to the AOAC (Association of Official Analytical Chemists) [16] using a digital potentiometer (OAKTON, 510, Vernon Hills, IL, USA) and the titratable acidity (TA) was determined using the AOAC methodology [17] by acid-base titration, with some modifications. Using NaOH 0.1 N as a titrant agent and phenolphthalein as an endpoint indicator (pH 8.2), results were expressed as grams of citric acid per 100 g of dry weight (dw). Total sugar content (TSC) was determined by the phenol-sulfuric method [18]. Total soluble solids (TSS) were measured in a digital refractometer and expressed in °Brix (ATAGO, PR-32, Tokyo, Japan). Color parameters were determined by the CIEL\* a\* b\* system with a spectrophotometer for solids (Konica Minolta, CM-2600d, Osaka, Japan) and the values of L\*, a\*, b\*, chroma  $C^* = (a^{*2} + b^{*2})^{1/2}$  and hue angle  $h^\circ = \tan^{-1} (b^*/a^*)$  indexes were calculated according to McGuire [19]. Protein content was obtained of the homogeneous sample from each bean sample to measure the protein content according to the method described by Bradford [20]. The quantification was based on a standard curve obtained using samples of bovine albumin (Sigma-Aldrich, Saint Louis, MO, USA) at concentrations ranging from 0.006 to 0.018 mg mL<sup>-1</sup>.

## 2.3. Ethanolic Extracts Preparation

An ethanolic extract was obtained with a 5 g sample and 20 mL of ethanol 80% (v/v), which was homogenized for 30 s at 1 g × s (DAIHAN-brand HG-15-A, Gangneung, Korea). Subsequently, it was centrifuged at 35,000 g × s for 20 min at 10 °C (Hettich zentrifuge, Universal 32R, Tuttlingen, Germany) and the supernatant was brought to a volume of 25 mL with ethanol 80% (v/v).

## 2.4. Total Polyphenols

They were determined by the method described by Singleton and Rossi [21], and the reaction absorbance was measured at 750 nm in a UV-visible spectrophotometer (Jenway 6305, Bibby Scientific Ltd., Dunmow, Essex, UK). The quantification was performed based on a standard curve of gallic acid (0.021 to 0.165 mg mL<sup>-1</sup>), and the results were expressed in mg equivalents of gallic acid per gram of dry weight (mg GAE g<sup>-1</sup> dw).

## 2.5. Monomeric Anthocyanins

Anthocyanin content was determined by the differential pH method [22]. Two dilutions of the extract were made, one with potassium chloride buffer at pH 1.0 and the second with sodium acetate buffer at pH 4.5, diluting each by the previously determined dilution factor. Subsequently, a spectrophotometer was used to generate an absorption spectrum in the range of 460–710 nm to determine the maximum absorbance. The con-

centration of monomeric anthocyanins (MA) was calculated according to the following equation:  $MA = (A \cdot PM \cdot FD \cdot 1000) / (\epsilon \cdot I)$ , where the absorbance of sample A corresponds to (A<sub>λ510</sub>–A<sub>λ700</sub>) pH 1.0 – (A<sub>λ510</sub>–A<sub>λ700</sub>) pH 4.5; MW = 449.2 is the molecular weight of cyanidin-3-glucoside;  $\epsilon$  = 26,900 g/mol is the molar absorptivity of the cyanidin-3-glucoside; FD is the dilution factor used; and I is the cell length (1 cm). The results were expressed as mg of cyanidin-3-glucoside per gram of dry weight (mg C3G g<sup>−1</sup> dw).

## 2.6. Antioxidant Assay Procedures

Antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [23]. A 100 µL sample of the extract was reacted with 2.9 mL of DPPH reagent and allowed to stand for 30 min at room temperature. The absorbance was measured using a UV-vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 517 nm using 80% (v/v) methanol as the target. To quantify the in vitro antioxidant activity, it was performed based on the inhibition percentage of a standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) curve in a concentration range of 0.13–0.79 µmol equivalents of Trolox (TE) per mL. The results were expressed in micromoles of Trolox equivalents per gram of dry weight (µmol TE g<sup>−1</sup> dw). The reducing power of the samples was also evaluated through the FRAP method [24]. The FRAP reagent was prepared by acetate buffer mixture (0.3 M pH 3.6), with a 10 mM TPTZ solution (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl and a solution of 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O, in a ratio 10:1:1. A 100 µL sample of the extract was reacted with 3 mL of FRAP and incubated at 37 °C during 30 min. The absorbance was measured using a UV-vis spectrophotometer (JENWAY 6305, Sttafordshire, UK) at 593 nm. The in vitro antioxidant activity quantification was performed using a standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) curve in a concentration range of 0.1 to 1 µmol equivalents of Trolox (TE) per mL. The results were expressed in micromoles of Trolox equivalents per gram of dry weight (µmol TE g<sup>−1</sup> dw).

## 2.7. Statistical Analysis

All samples were analyzed by quadruplicate in each determination, reporting the averages for the samples harvested at two different seasons on the same maturity stage. A database with all results was integrated, and an analysis of variance was performed using a completely random design with the nesting of landraces within species. Comparisons among species and landraces were made by the Tukey method ( $p \leq 0.05$ ). Pearson analysis was used to determine the correlation between bioactive compounds, in vitro antioxidant activity, and physicochemical parameters. All statistical analyses were performed using SAS software version 9.0 [25], and the values are reported as the mean of replicates ± standard deviation.

## 3. Results

In the variance analysis, significant differences were recorded in the genotype/species interaction in the physicochemical parameters, protein, and bioactive components, such as total phenolic compounds (TPC), total anthocyanin content (TAC), and in vitro antioxidant activity by the DPPH and FRAP method, evaluated with a coefficient of variation less than 26.5% ( $p < 0.01$ ; Table 1). With the exception of pH and titratable acidity, considering the mean squares value, it was estimated that the variance due to the species was greater than in the genotype/species interaction (Table 1). This indicates that, in general, the variation between species is greater than between landraces within each species and reflects part of the evolutionary differences between *P. vulgaris* and *P. Coccineus* [26,27]. In addition, statistical differences ( $p < 0.05$ ;  $p < 0.01$ ) were observed in the pH parameters, total sugars, L\*, protein and in vitro antioxidant activity evaluated by DPPH and FRAP between repetitions of the genotypes sown in the experimental plot (Table 1). This could be attributed to the heterogeneity between same genotype individuals, due to factors such as agronomic practices, sampling randomness, and typical variation of the physical characteristics (color, size, shape) between grains of each accession.

**Table 1.** Mean square values of the variance analysis of the physicochemical parameters in green beans.

Variable	Variation Sources				
	Repetition	Species	Genotype/Species	Error	CV (%) <sup>4</sup>
pH	0.1 **	0.0 ns	0.2 **	0.0	1.8
TA	0.0 ns,1	0.1 ns	1.0 **	0.1	23.8
TSC	5736.1 **,3	21802.8 *	7394.1 **	301.3	25.8
TSS	0.0 ns	84.7 **	59.4 **	1.8	16.6
Color parameters	L*	249.4 *,2	83.1 *	42.0	41.99
	a*	28.8 **	1.4 **	0.1	0.14
	b*	15.5 **	2.1 **	0.3	0.34
	C	12.1 **	1.8 **	0.3	0.33
	h°	23,406.2 **	1392.6 **	92.5	92.47
Protein	48.9 **	15.3 **	1.9	1.94	17.4
TPC	2918.3 **	422.9 **	7.2	7.19	26.5
TAC	10.3 **	7.2 **	0.1	0.14	15.4
DPPH	288.7 **	33.2 **	0.6 **	0.61 **	11.7
FRAP	458.5 **	33.3 **	0.8	0.76	14.0

<sup>1</sup>,ns not significant at  $p = 0.05$ , <sup>2</sup>,\* ( $p < 0.05$ ), <sup>3</sup>,\*\* ( $p < 0.01$ ), <sup>4</sup> CV% (Coefficient of variation).

### 3.1. Physicochemical Parameters

Regarding total sugars content, the *P. coccineus* species was 1.3 times higher (76.2 mg Glu g<sup>-1</sup> dw) compared to the *P. vulgaris* species (59.3 mg Glu g<sup>-1</sup> dw); on the contrary, *P. vulgaris* presented the highest content of TSS (Table 2). The interspecific variation for total sugars ranged from 47.9–125.5 mg Glu g<sup>-1</sup> dw in *P. coccineus* green beans, and for *P. vulgaris* from 36.8–81.8 mg Glu g<sup>-1</sup> dw, with this latter species being the one that presented the lowest variability (42%).

Among genotypes within each species, the SJA and P-69 landraces of *P. coccineus* green beans obtained the highest total sugars content (125.5 and 107.1 mg Glu g<sup>-1</sup> dw, respectively), while in the case of *P. vulgaris* green beans, P-19, P-71, P-68, and P-34 landraces showed the highest concentration, not presenting significant differences between them (Table 2). In the TSS content, the FCA-04 green beans landrace of *P. vulgaris* obtained the highest value (13.2 °Brix) followed by P-58 (10.4 °Brix) and SLMV (10.8 °Brix) of the *P. coccineus* species.

In the luminosity parameter, significant differences were observed between species and landraces within each species, with *P. coccineus* being the one that presented the highest value with respect to *P. vulgaris* ( $p < 0.05$ ). Furthermore, between landraces of both species, the P-65 green beans (*P. vulgaris*) registered a lower average value (57.8) in contrast to SJA (*P. coccineus*) (59.2) (Table 2). In a\* and b\* color parameters, between species, *P. coccineus* green beans showed average values with a tendency to yellow-green, while *P. vulgaris* observed a tendency to red-purple, with variation between landraces of each species, since CAA green beans of *P. coccineus* obtained a negative value for a\* and positive for b\* corresponding to the yellow color, on the other hand, in *P. vulgaris*, beans of P-40 and P-71 presented positive values for a\* and b\* corresponding to the red color, without statistical differences between them ( $p < 0.05$ ). For chromaticity parameter (C\*), it was observed that *P. coccineus* species presented a higher average value (10.9) compared to *P. vulgaris* (8.5), with the landraces P-69 and FCA-04 being those that presented the highest values, respectively (Table 2). Regarding the hue angle parameter (h°), a wide interval (87.5–100.1°) was observed for *P. coccineus* green beans, where CAA obtained the highest value with a tendency to green; while, for *P. vulgaris* green beans, hue angle ranged from 57.9–96.5°, with P-71 (57.9°) being the one that presented a value with a tendency to red and P-65 (69.2°) and P-58 (72.5°) with a tendency to yellow ( $p < 0.05$ ).



**Table 2.** Physicochemical, color parameters, and protein content in green beans from bean landraces.

Species and Genotypes	pH	TA	TS	TSS	L*	a*	b*	C*	h°	P
<i>P. coccineus</i> L.	6.4 ± 0.1 A <sup>1</sup>	0.9 ± 0.3 A	76.2 ± 27.1 A	7.4 ± 1.9 B	55.9 ± 1.9 A	−1.4 ± 0.7 B	10.8 ± 1.7 A	10.9 ± 1.7 A	96.3 ± 4.2 A	5.6 ± 2.3 B
Fat bean	6.4 ± 0.1 a–f <sup>1</sup>	0.8 ± 0.1 e–g	48.6 ± 6.4 f–i	6.8 ± 0.6 g–i	55.0 ± 7.1 ab	−1.7 ± 0.9 e–g	11.4 ± 3.5 a–d	11.6 ± 3.6 a–d	98.1 ± 2.1 abc	9.6 ± 0.8 c–e
SLMV	6.5 ± 0.2 a–e	0.9 ± 0.1 c–e	56.4 ± 11.3 e–i	10.8 ± 2.3 b	57.7 ± 4.2 ab	−0.8 ± 0.6 d–g	7.9 ± 2.2 b–g	7.9 ± 2.3 b–f	95.2 ± 2.8 a–d	6.2 ± 1.1 gh
SJA	6.3 ± 0.2 gh	1.3 ± 0.4 ab	125.5 ± 39.7 a	5.3 ± 0.5 ij	59.2 ± 8.0 a	−0.2 ± 1.6 d–f	9.6 ± 6.1 a–f	9.7 ± 6.1 a–f	87.5 ± 10.9 b–e	6.5 ± 1.0 gh
CAA	6.3 ± 0.1 f–h	1.2 ± 0.1 bc	93.5 ± 14.5 bc	5.0 ± 0.0 j	57.4 ± 5.7 ab	−2.2 ± 0.9 g	11.9 ± 4.0 ab	12.1 ± 4.1 ab	100.1 ± 1.8 a	3.0 ± 1.6 j
P-69	6.5 ± 0.2 a–d	1.4 ± 0.4 a	107.1 ± 11.6 ab	8.6 ± 2.0 d–f	53.9 ± 5.9 ab	−2.4 ± 0.7 g	13.6 ± 3.3 a	13.8 ± 3.3 a	99.8 ± 0.9 a	3.1 ± 1.7 j
P-26	6.3 ± 0.1 e–h	0.8 ± 0.1 d–f	66.8 ± 12.9 d–g	6.3 ± 1.0 h–j	55.6 ± 5.5 ab	−1.7 ± 0.9 e–g	10.4 ± 3.7 a–f	10.5 ± 3.8 a–e	98.8 ± 2.0 ab	8.1 ± 0.5 e–g
P-96C2	6.5 ± 0.1 a–d	0.5 ± 0.2 g	47.9 ± 7.8 f–i	9.1 ± 3.3 cd	56.3 ± 7.7 ab	−0.8 ± 1.5 d–g	9.4 ± 4.3 a–f	9.5 ± 4.4 a–f	92.0 ± 9.2 a–d	5.3 ± 0.3 hi
P-91C2	6.5 ± 0.0 a–f	0.7 ± 0.2 e–g	76.5 ± 14.2 c–e	8.0 ± 1.8 d–g	53.5 ± 6.9 ab	−1.3 ± 1.2 e–g	11.0 ± 3.3 a–e	11.1 ± 3.4 a–e	96.2 ± 4.9 a–d	5.4 ± 0.7 h
P-102	6.5 ± 0.1 a–e	0.8 ± 0.2 ef	63.6 ± 11.5 d–g	7.1 ± 0.1 f–h	54.5 ± 5.6 ab	−1.9 ± 0.7 fg	11.8 ± 3.2 abc	11.9 ± 3.3 a–c	98.8 ± 1.4 ab	3.3 ± 0.6 ij
<i>P. vulgaris</i> L.	6.4 ± 0.1 A	1.0 ± 0.2 A	59.3 ± 14.8 B	8.5 ± 2.0 A	54.1 ± 2.6 B	1.0 ± 1.4 A	8.2 ± 2.4 B	8.5 ± 2.2 B	78.7 ± 12.2 B	10.1 ± 1.8 A
P-34	6.4 ± 0.1 b–g	1.1 ± 0.2 b–d	69.8 ± 12.4 d–f	7.3 ± 0.9 e–h	55.4 ± 8.1 ab	0.0 ± 2.4 c–e	9.9 ± 3.7 a–f	10.1 ± 3.7 a–e	87.0 ± 14.0 b–e	10.1 ± 0.6 b–e
P-68	6.6 ± 0.1 a	1.1 ± 0.1 b–d	71.7 ± 23.2 c–e	8.2 ± 1.0 d–g	57.3 ± 6.4 ab	−1.5 ± 1.2 e–g	11.8 ± 3.2 a–c	11.9 ± 3.3 a–c	96.5 ± 5.1 a–c	8.7 ± 1.3 d–f
P-76	6.4 ± 0.1 b–g	1.1 ± 0.4 bc	59.3 ± 21.1 e–h	8.3 ± 1.1 d–g	56.8 ± 7.5 ab	−0.2 ± 1.0 d–f	7.5 ± 3.7 c–g	7.6 ± 3.7 c–f	86.6 ± 11.1 c–e	12.2 ± 2.5 ab
P-19	6.5 ± 0.1 ab	1.2 ± 0.1 ab	81.8 ± 25.1 cd	6.8 ± 0.4 g–i	52.6 ± 6.0 ab	0.4 ± 1.6 b–d	8.1 ± 2.4 b–g	8.3 ± 2.3 b–f	84.5 ± 14.1 d–f	9.1 ± 1.2 c–f
P-71	6.5 ± 0.0 a–c	0.6 ± 0.1 fg	72.2 ± 18.5 c–e	7.1 ± 1.0 f–h	53.9 ± 7.6 ab	2.6 ± 0.9 a	4.8 ± 2.4 g	5.6 ± 2.3 f	58.0 ± 13.4 h	10.7 ± 3.5 a–d
P-58	6.5 ± 0.1 a–e	0.9 ± 0.2 c–e	45.2 ± 3.8 g–i	10.4 ± 0.3 bc	52.5 ± 7.5 ab	1.9 ± 0.9 ab	6.5 ± 2.3 fg	6.8 ± 2.3 ef	72.6 ± 7.6 fg	12.3 ± 1.7 a
P-65	6.2 ± 0.1 h	0.8 ± 0.1 ef	36.8 ± 8.6 i	7.6 ± 0.4 d–h	57.8 ± 8.1 ab	2.0 ± 2.8 ab	7.4 ± 4.3 d–g	8.1 ± 4.3 b–f	69.2 ± 20.0 gh	11.8 ± 0.9 ab
P-16	6.4 ± 0.0 d–g	0.8 ± 0.2 d–f	41.5 ± 14.7 hi	7.1 ± 1.4 f–h	52.0 ± 6.0 ab	1.7 ± 0.7 a–c	6.9 ± 2.0 e–g	7.1 ± 2.0 ef	75.7 ± 5.7 eg	10.9 ± 1.1 a–c
P-40	6.4 ± 0.1 c–g	1.2 ± 0.5 ab	57.2 ± 13.9 e–i	8.8 ± 0.8 c–e	51.7 ± 5.7 ab	2.8 ± 2.4 a	6.5 ± 2.7 fg	7.3 ± 2.9 d–f	66.4 ± 16.1 gh	8.7 ± 0.4 d–f
FCA-04	6.5 ± 0.1 ab	0.9 ± 0.1 c–e	57.1 ± 23.1 e–i	13.2 ± 1.3 a	50.8 ± 4.5 b	−0.2 ± 1.0 d–f	12.1 ± 2.8 ab	12.2 ± 2.8 ab	90.6 ± 4.1 a–d	7.0 ± 0.9 f–h

TA = titratable acidity (g Citric acid 100 g<sup>−1</sup> dw), TS = Total sugars (mg Glucose g<sup>−1</sup> dw), TSS = Total soluble solids (°Brix), L\*: Luminosity, a\*: Red/green Coordinates, b\*: Yellow/blue Coordinates, C\*: Chromaticity, h°: Hue angle, P: Total protein (g 100 g<sup>−1</sup> dw). SLMV: Santa Lucia Monte Verde, SJA: San Juan Atepec, CAA: Cerro Amole Ayutla. <sup>1</sup> Between species (capital letter) and among landraces (lowercase letter), means with same letter indicate non-significant differences (Tukey's test,  $p < 0.05$ ).

Protein content differs significantly between species and landraces within each species ( $p < 0.05$ ). The species *P. vulgaris* showed higher protein content ( $10.1 \text{ g protein } 100 \text{ g}^{-1} \text{ dw}$ ) compared to *P. coccineus* ( $5.6 \text{ g protein } 100 \text{ g}^{-1} \text{ dw}$ ) ( $p < 0.05$ ; Table 2). The protein content variation ranged  $7.0\text{--}12.2 \text{ g protein } 100 \text{ g}^{-1} \text{ dw}$  for beans of the species *P. vulgaris*, and for *P. coccineus*  $3.0\text{--}9.6 \text{ g protein } 100 \text{ g}^{-1} \text{ dw}$ , the latter being the one that presented the highest variability with 68.8%. Among genotypes, the landraces P-76, P-71, P-58, P-65, and P-16 of the species *P. vulgaris*, presented the highest protein contents, with respect to the other landraces and the *P. coccineus* species landraces, likewise, of the fat beans ( $p < 0.05$ ; Table 2).

### 3.2. Total Phenols, Anthocyanins, and In Vitro Antioxidant Activity

Total polyphenols content (TPC), total anthocyanins content (TAC), and antioxidant activity by DPPH and FRAP is shown in Table 3. TPC for *P. coccineus* species was significantly ( $p < 0.05$ ) higher than *P. vulgaris* with a value average of  $13.4 \text{ mg GAE g}^{-1} \text{ dw}$  and  $7.2 \text{ mg GAE g}^{-1} \text{ dw}$ , respectively, observing a high variability between results, with a range of  $6.0$  to  $26.5 \text{ mg GAE g}^{-1} \text{ dw}$  for *P. coccineus* and  $4.2$  to  $10.1 \text{ mg GAE g}^{-1} \text{ dw}$  for *P. vulgaris*, with the landraces with reddish tones being those that presented higher TPC concentrations, probably due to anthocyanins presence.

**Table 3.** Content of total phenols, anthocyanins, and in vitro antioxidant activity in green beans from bean landraces.

Species and Landraces	TPC	TAC	DPPH	FRAP
<i>P. coccineus</i> L.	$13.4 \pm 8.0 \text{ A}^1$	$0.7 \pm 0.0 \text{ B}$	$62.9 \pm 32.5 \text{ A}$	$60.2 \pm 32.3 \text{ A}$
Fat bean	$7.8 \pm 1.2 \text{ de-g}^1$	-	$37.9 \pm 12.6 \text{ f-j}$	$54.3 \pm 11.4 \text{ b-d}$
SLMV	$26.5 \pm 8.5 \text{ a}$	$0.7 \pm 0.1 \text{ c}$	$58.6 \pm 9.9 \text{ b-e}$	$53.9 \pm 17.7 \text{ b-d}$
SJA	$12.9 \pm 3.4 \text{ bc}$	-	$71.0 \pm 19.5 \text{ b}$	$65.6 \pm 33.3 \text{ b}$
CAA	$13.6 \pm 1.9 \text{ b}$	-	$67.5 \pm 9.5 \text{ bc}$	$57.4 \pm 5.1 \text{ bc}$
P-69	$23.7 \pm 4.9 \text{ a}$	-	$141.4 \pm 50.9 \text{ a}$	$141.0 \pm 43.8 \text{ a}$
P-26	$9.3 \pm 0.8 \text{ de}$	-	$51.6 \pm 6.9 \text{ c-f}$	$42.4 \pm 6.7 \text{ c-e}$
P-96C2	$6.0 \pm 0.6 \text{ f-h}$	-	$34.1 \pm 7.3 \text{ g-j}$	$34.3 \pm 8.6 \text{ e}$
P-91C2	$7.7 \pm 1.2 \text{ d-g}$	-	$39.3 \pm 7.8 \text{ f-j}$	$33.8 \pm 3.9 \text{ e}$
P-102	$12.9 \pm 3.0 \text{ bc}$	-	$65.2 \pm 18.5 \text{ b-d}$	$58.8 \pm 10.5 \text{ bc}$
<i>P. vulgaris</i> L.	$7.2 \pm 1.9 \text{ B}$	$2.2 \pm 1.0 \text{ A}$	$34.3 \pm 11.3 \text{ B}$	$27.0 \pm 10.9 \text{ B}$
P-34	$6.5 \pm 0.3 \text{ e-h}$	-	$31.5 \pm 10.8 \text{ g-k}$	$30.2 \pm 8.9 \text{ ef}$
P-68	$7.9 \pm 0.4 \text{ d-g}$	-	$40.1 \pm 2.7 \text{ f-j}$	$28.9 \pm 4.1 \text{ ef}$
P-76	$6.5 \pm 0.9 \text{ e-h}$	-	$45.6 \pm 7.5 \text{ e-g}$	$31.0 \pm 10.7 \text{ ef}$
P-19	$10.1 \pm 1.0 \text{ cd}$	-	$45.2 \pm 6.3 \text{ e-h}$	$44.8 \pm 7.4 \text{ c-e}$
P-71	$4.2 \pm 0.9 \text{ h}$	-	$14.3 \pm 1.9 \text{ k}$	$14.2 \pm 5.1 \text{ f}$
P-58	$9.0 \pm 4.0 \text{ d-f}$	-	$28.1 \pm 9.5 \text{ h-k}$	$15.2 \pm 3.7 \text{ f}$
P-65	$5.7 \pm 0.8 \text{ gh}$	-	$26.2 \pm 2.0 \text{ i-k}$	$16.1 \pm 3.0 \text{ f}$
P-16	$7.9 \pm 1.1 \text{ d-g}$	$2.9 \pm 1.6 \text{ a}$	$41.0 \pm 7.0 \text{ f-i}$	$34.9 \pm 7.0 \text{ e}$
P-40	$9.1 \pm 4.1 \text{ d-f}$	$1.2 \pm 0.5 \text{ b}$	$48.0 \pm 15.6 \text{ d-g}$	$37.8 \pm 13.7 \text{ de}$
FCA-04	$4.9 \pm 0.6 \text{ gh}$	-	$23.4 \pm 3.0 \text{ jk}$	$16.4 \pm 3.7 \text{ f}$

TPC: Total polyphenol content ( $\text{mg GAE g}^{-1} \text{ dw}$ ), TAC: Total anthocyanin content ( $\text{mg C3G g}^{-1} \text{ dw}$ ), DPPH: Antioxidant activity ( $\mu\text{mol TE g}^{-1} \text{ dw}$ ), FRAP: Antioxidant capacity ( $\mu\text{mol TE g}^{-1} \text{ dw}$ ). SLMV: Santa Lucia Monte Verde, SJA: San Juan Atepec, CAA: Cerro Amole Ayutla. <sup>1</sup> Between species (capital letter) and among landraces (lowercase letter), means with same letter indicate non-significant differences (Tukey's test,  $p < 0.05$ ).

Regarding TAC, only green beans of three genotypes presented monomeric anthocyanins detectable values, following an order from higher to lower concentration, purple beans of P-16 ( $2.9 \text{ mg C3G g}^{-1} \text{ dw}$ ) and red beans of P-40 ( $1.2 \text{ mg C3G g}^{-1} \text{ dw}$ ) from *P. vulgaris* species, and green beans from SLMV ( $0.7 \text{ mg C3G g}^{-1} \text{ dw}$ ) from *P. coccineus* (Table 3).

The close relationship between TPC and antioxidant activity has been documented in various fruits and vegetables, as observed in this study (Table 4). Most of the evaluated green bean landraces showed a strong correlation ( $p < 0.05$ ) between the phenolic compounds content and the antioxidant activity determined by the DPPH free radical reduction.

The P-69 landrace of the *P. coccineus* species that exhibited a high concentration of phenolic compounds also presented high antioxidant activity ( $141.4 \mu\text{mol TE g}^{-1} \text{ dw}$ ), as well as its ability to reduce iron ( $141.0 \mu\text{mol TE g}^{-1} \text{ dw}$ ). In contrast, P-71 which showed the lowest phenolic compounds concentration ( $4.2 \text{ mg GAE g}^{-1} \text{ dw}$ ) also showed the lowest antioxidant activity by DPPH and FRAP method ( $14.3 \mu\text{mol TE g}^{-1} \text{ dw}$  and  $14.2 \mu\text{mol TE g}^{-1} \text{ dw}$ , respectively). However, this relationship was not observed in all landraces, since SLMV presented the highest TPC average ( $26.5 \text{ mg GAE g}^{-1} \text{ dw}$ ), reporting an activity against DPPH of 58.6% lower compared to P-69, probably due to the variability in the individual polyphenols profile and that each compound can exert a different reducing activity.

**Table 4.** Correlation coefficients between bioactive compounds, in vitro antioxidant activity, and physicochemical parameters in green beans from bean landraces.

Variable	TPC	TA	TS	FRAP	DPPH	$h^{\circ}$	C*
TPC	1.0						
TA	0.4 **,2	1.0					
TS	0.3 **	0.5 **	1.0				
FRAP	0.7 **	0.5 **	0.5 **	1.0			
DPPH	0.8 **	0.5 **	0.5 **	0.9 **	1.0		
$h^{\circ}$	0.3 **	0.1 **	0.2 **	0.4 **	0.4 **	1.0	
C*	0.1 ns,1	0.1 ns	0.1 ns	0.2 **	0.2 **	0.6 **	1.0

<sup>1,ns</sup> not significant at  $p < 0.05$ , <sup>2,\*\*</sup>significant (Student's test,  $p < 0.001$ ).

A similar effect was observed for the antioxidant activity determined by the FRAP technique. Most of the landraces that presented a high DPPH radical reducing activity also showed an ability to reduce the ferric ion, although some landraces, mainly those of the *P. vulgaris* genus, showed an activity between 15% (P-16) and 46 % (P-58) lower in the FRAP technique compared to DPPH.

#### 4. Discussion

In general, regarding the physicochemical parameters of pH and titratable acidity, there were no differences between *P. vulgaris* and *P. coccineus*. Organic acids concentration determines the physiological and commercial fruits maturity such as green beans [28]. In this sense, the state of maturity chosen for this study shows pH values similar to those reported before [29] with a pH value of 6.2 and 6.4 for the Strike and Bina green bean varieties (*P. vulgaris* L.), respectively, and 5.8–6.4 for green beans (*Phaseolus vulgaris* L. var. Helda) in an immature state [30]. In titratable acidity, Garzón-García et al. [15] reported a higher value ( $1.6 \text{ g citric acid } 100 \text{ g}^{-1} \text{ dw}$ ) in green beans (*Phaseolus vulgaris* L.) in the same state of maturity as that used in this study. Regarding the sugars content, and color parameters  $L^*$ ,  $b^*$ ,  $C^*$ , and  $h^{\circ}$ , the *P. coccineus* species presented the highest average values. It has been reported that the main sugars in green beans are glucose, fructose, and sucrose [31], which could constitute the total sugar content in this study, presenting a lower average value ( $32.7 \text{ mg Glu g}^{-1} \text{ dw}$ ) compared to green beans (*Phaseolus vulgaris* L.) from a native bean common accession from Oaxaca [32].

Regarding the luminosity parameter, an interval of 36.5–50.3 lower than that registered in the two species evaluated in this study has been reported [29] in *P. vulgaris* L. green beans in an advanced stage of development. Regarding the TSS content, *P. vulgaris* species was higher than *P. coccineus*, whose values has been reported before [29] on a range of 9.5–20.1 °Brix, in the Strike and Bina green bean varieties (*P. vulgaris* L.) in different development stages, similar to those obtained in this study. Green beans (*Phaseolus vulgaris* L. cv. Paulista) irrigated by drip and furrows presented values of 4.37 and 4.60 °Brix, respectively [33], similar to *Phaseolus vulgaris* ssp. Volubilis grown in soils rich in organic matter and nitrogen fertilization with values of 4.9 to 5.7 °Brix [34], being much lower than that obtained in the two green beans species analyzed. Thus, the total soluble solids content variation, constituted by organic acids, but also total sugars, can be influenced by



agricultural practices, its post-harvest handling, as well as the state of maturity in which it is harvested [35,36].

Regarding protein content, Martínez et al. [37] reported a range of 16.9–17.2 g protein 100 g<sup>-1</sup> dw in the Cleo, Strike, and Sentry green bean varieties, while for beans of the species *P. vulgaris*, *P. coccineus*, Black Horse, Michigan, and Peruvian, a range of 12.6–21.3 g protein 100 g<sup>-1</sup> dw is reported [38]. For their part, Bhagya et al. [39], recorded a protein content in tender pods beans of *Canavalia cathartica* of 21.7 g protein 100 g<sup>-1</sup> dw. In contrast, in pea pod (*Pisum sativum* L.) and broad bean pod (*Vicia faba* L.), values of 10.8 and 13.6 g protein 100 g<sup>-1</sup> dw were obtained [40], respectively, similar to what was observed in this study in the species *P. vulgaris*.

Phenolic compounds concentration in the evaluated samples registered a high variability, showing the highest values those *P. coccineus* species reddish landraces, which coincides with that reported in Oaxaca landrace green beans of the *P. vulgaris* species [15]. Similar results have been observed before [41,42], where the reddish bean landraces presented higher phenolic compounds concentrations, compared to the green landraces. However, in this study, no correlation was found between the reddish landraces of the *P. vulgaris* species and the content of phenolic compounds, even though these species showed the highest anthocyanin content. Still, the TPC average results in both species were higher than the values reported [43] where concentrations of 3.6 mg GAE g<sup>-1</sup> dw were observed for varieties of green beans marketed in Turkey, as well as those recorded of 1.1–2.0 mg GAE g<sup>-1</sup> dw [44,45] for other *Phaseolus vulgaris* varieties, including Emerit, which showed a TPC of 2251 mg GAE g<sup>-1</sup> dw [46]. On the other hand, the evaluation of the phenolic compounds content and antioxidant activity for three varieties of green beans has shown values of 14.6 for the green variety and 40.8 µmol TE g<sup>-1</sup> dw for the reddish variety [41], which agrees with values reported in the present study. This great variability observed in the phenolic compounds content, anthocyanins, and antioxidant activity in the species *P. coccineus* and *P. vulgaris* green beans could be directly related to the phenolic compounds profile, which can vary widely due to the state of maturity, the accession, species, geographic location, and cultural practices within that region [47]. This not only promotes phenotypic differentiation, but also at the genetic level, in the identified phenolic compounds expression and synthesis (gallic acid, chlorogenic, epicatechin, rutin, and more than 72 polyphenols including 10 phenolic acids, 59 flavonoids, 2 lignans, and 1 iridoid) in different green beans landraces (*P. vulgaris* L. var Perona, Helda and Strike), as well as in different parts of this crop such as flowers [48], leaves and stems [49], and seeds [50,51]. Furthermore, the aqueous extract of *Phaseolus vulgaris* species green beans has been reported to have anti-obesogenic and hypoglycemic activity [52–56], which could help combat the obesity pandemic currently present in Mexico [57] and the world [58], as well as reduce the factors associated with metabolic syndrome, such as cardiovascular diseases, which are the main cause of mortality in the world [59].

## 5. Conclusions

Based on the two green beans species evaluated, we can conclude that there are significant differences between *P. coccineus* and *P. vulgaris* in the content of TS, TSS, and color, as well as in their content of polyphenols, flavonoids, anthocyanins, and antioxidant activity. *P. coccineus* was higher in phenolic compounds and antioxidant activity, while *P. vulgaris* was higher in anthocyanins. There was a high variability between the landraces evaluated, and, specifically, *P. vulgaris* landraces had more than double the anthocyanins compared to those of *P. coccineus*. Both species are a source of complementary polyphenols, flavonoids, and anthocyanins, and this suggests that their use can be considered a complementary food to improve the rural community's nutrition or implement a genetic improvement program towards a higher nutritional quality of the prominent pods where their food base is in conjunction with other native crops such as corn, squash, and chili. However, other studies are necessary to determine their potential in preventing some chronic degenerative diseases.

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