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Protein Content, Cooking Time, and Seed Color as Selection Criteria in Ayocote Bean (*Phaseolus coccineus* L.) for Consumption

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Abstract

Phaseolus coccineus L. is a legume characterized by wide phenotypic variability in seed color and size, traits associated with physical and nutritional grain attributes. The objective of this study was to evaluate the relationship between seed coat color and physical quality, cooking behavior, and chemical composition in ayocote bean materials. Nine F3 families selected based on color variability and yield were analyzed. Thousand-seed weight (TSW), cooking time (CT), broth density, and relative protein content using the Lowry method were determined, while chromatographic profiles were evaluated through paper chromatography. Analysis of variance showed significant differences in TSW among seed color groups ($F = 13.87$; $p < 0.001$). White mottled black (103.33 g), orange mottled black (102.94 g), and cream mottled black (102.42 g) materials presented the highest values, whereas white seeds showed the lowest TSW (92.01 g). CT ranged from 2.4 to 3.5 h, with purple mottled black materials exhibiting shorter cooking times, while white and cream seeds required longer softening periods. Broth density showed no significant differences among treatments ($p > 0.05$). Relative protein content ranged from 39.3 to 54.0%, with cream mottled black showing the highest values. Chromatographic profiles revealed between 4 and 5 spots with Rf values ranging from 0.04 to 0.45, indicating variability in the chemical composition among evaluated materials. The results suggest that seed coat color may be considered a useful criterion for selecting ayocote bean materials with attributes associated with physical quality, cooking performance, and chemical composition.

Keywords

Underutilized Legume, Secondary Metabolites, Paper Chromatography, Phenotypic Variability

1. Introduction

Phaseolus coccineus L., commonly known as ayocote bean, is a legume native to Mesoamerica. This species has been cultivated since pre-Hispanic times in the Mexican highlands, where it has adapted to diverse agroclimatic conditions [1]. As a result of this adaptation, it exhibits wide phenotypic variability, particularly in seed size and color, which can be black, purple, brown, cream, white, or mottled [2].

Currently, it is maintained as a traditional crop managed on a small scale by rural communities. Although it has high nutritional and cultural value, its production and consumption are limited, being concentrated in specific regions and local markets [3] [4]. Despite being relatively underutilized, it has notable nutritional potential and health benefits due to its high content of protein (185.3 mg/kg), carbohydrates (677.6 mg/kg), fiber (67.4 mg/kg), flavonoids (1612.9 mg QE/kg), and anthocyanins (1193.2 CGE/kg), which confer antioxidant and anti-inflammatory properties [5]. It also contains vitamin A, B-complex vitamins, and minerals such as iron and calcium [6].

Although its nutritional composition, bioactive compound content, and agronomic characteristics have been widely studied, it is still necessary to understand how attributes such as seed color and size are related to key quality parameters, including protein content and cooking time. These factors are determinant in the selection of ayocote beans for consumption, as they influence both their nutritional value and their sensory and processing properties [4]-[7].

Therefore, the present study focused on evaluating the relationship between seed color and variables of nutritional and technological importance, such as protein content and cooking time, in runner bean (*Phaseolus coccineus* L.) families. Furthermore, the results obtained contribute to a better characterization of their physicochemical properties and to the generation of selection criteria oriented toward consumption. Likewise, this information may be useful for breeding programs and for optimizing preparation processes, with the aim of maximizing the nutritional value and sensory quality of *P. coccineus* L.

2. Materials and Methods

2.1. Experimental Site Location

The study was conducted in the Molecular Genetics Laboratory at the Colegio de Postgraduados, Montecillo Campus, State of Mexico (19˚29'N, 98˚51'W, and 2250 meters above sea level).

2.2. Biological Material

The genetic material used in this study consisted of nine ayocote bean (*Phaseolus coccineus* L.) families, corresponding to an F3 population obtained through pedigree selection. Due to their origin, these families still present segregation of traits, which is reflected in the variability observed in seed color within some samples. Family selection was based on variability in seed color and yield. Different color tones were identified, including cream, black, purple, white, and mottled combinations. Detailed information on the families, plots, seed color, and yield is presented in Table 1. Each F3 family was considered an experimental unit for evaluations related to physical and cooking characteristics.

Table 1. Characterization of genetic material in ayocote bean.

Sample	Plot	Seed color	Yield (Kg ha^{-1})
1	74	Cream Black	2258.33
2	17	Purple Cream, Black Mottled	2160.51
3	72	Orange, Black Mottled Black	2148.75
4	159	White	1741.27
5	103	Orange, Black Mottled Black, Orange Mottled Cream, Black Mottled	1579.78
6	1	Black, Orange Mottled	1556.03
7	28	Purple Black	1537.5
8	13	Cream Black	1492.89
9	32	Purple, Black Mottled Black Purple	1468.18

2.3. Physical Seed Quality

1000-Seed Weight

The weight of 1000 seeds was determined following the methodology of the International Seed Testing Association 2014-2015 [8]. Eight replicates of 100 seeds per treatment were randomly selected. The average weight of 100 seeds was used to calculate the 1000-seed weight using the following formula:

$$P1000S = (\bar{x}) * 10$$

where $\bar{x}$ is the average weight of 100 seeds. Variance, standard deviation, and

coefficient of variation were calculated as follows:

$$\text{Variance} = \frac{N\left(\sum x^2\right) - \left(\sum x\right)^2}{N\left(N-1\right)}, S = \sqrt{\text{Variance}}, CV = \frac{S}{\overline{x}} * 100$$

2.4. Sample Preparation for Cooking

Seeds (44 ± 0.3 g) were cooked in 300 mL of distilled water at a constant temperature of 94˚C using a controlled water bath. Cooking time was recorded from the moment the water reached the established temperature until the seeds achieved a soft texture, determined by manual compression using moderate pressure between the fingers, without evident resistance in the cotyledons. Three independent cooking evaluations were performed for each sample, and the obtained values were averaged. Additionally, differences observed in cooking behavior according to seed color were recorded.

Broth Density Determination

After cooking, the broth was allowed to cool to room temperature (approximately 20˚C). Once this temperature was reached, density was determined using a precision hydrometer.

2.5. Sample Preparation for Protein-Related Compound Analysis

For protein determination, a different sampling approach was used compared to the previous analyses. Instead of considering the nine F3 families described in Section 2.2 as experimental units, the seeds were grouped according to seed coat color, prioritizing the phenotypic variation observed within the population in order to evaluate protein content as a function of seed color. Due to segregation within the F3 families, some color groups included seeds derived from different families.

The seed groups defined according to color patterns, as well as the number of seeds analyzed per group, are presented in Table 2.

Table 2. Classification of seeds according to seed coat color for protein determination.

Group	Seed color	Number of seeds
1	Black mottled purple	10
2	Black mottled orange	10
3	Purple mottled black	10
4	Orange mottled black	10
5	Cream mottled black	10
6	White mottled black	10
7	Black	10
8	Purple	10
9	Cream	10
10	White	10

A total of 100 seeds were analyzed, distributed across 10 groups defined by seed coat color.

Subsequently, seeds were ground to obtain flour, which was sieved using a No. 325 mesh (0.044 mm). The sieved flour was weighed using an analytical balance (Ohaus Explorer™, sensitivity 0.0001 g) and dried at 70˚C for 4 days until constant weight was achieved. After drying, samples were reweighed to determine moisture loss.

2.5.1. Determination of Relative Protein-Related Compounds

Protein content was determined using the method described by Lowry *et al*. (1951), employing the Folin-Ciocalteu reagent.

Protein extraction was carried out using 0.2 g of flour per sample. Each sample was prepared in duplicate in 2 mL Eppendorf® tubes. Subsequently, 1 mL of 0.01 M sodium hydroxide (NaOH) was added to each tube, and samples were maintained under continuous agitation for 1 h 30 min at room temperature.

After extraction, samples were centrifuged at 12,000 × g for 17 min. Aliquots of the supernatant were collected for protein quantification, which was performed using a spectrophotometer (Bausch & Lomb Spectronic 20) at a wavelength of 500 nm.

2.5.2. Paper Chromatographic Analysis of Protein-Related Compounds

For protein visualization, ascending paper chromatography was performed using Whatman® No.1 paper. From each centrifuged sample, the supernatant was recovered and successive aliquots of 0.3 µL were applied onto the origin line until a total volume of 2.4 µL was reached.

Separation was carried out using a solvent system of 2-butanol:acetic acid:water (45:11:9) for 4 h, until the solvent front reached an appropriate distance.

Subsequently, the chromatograms were air-dried and the spots were revealed by spraying with a 0.2% (w/v) ninhydrin solution in ethanol. Migration distances were recorded to calculate Rf values.

2.5.3. Comparative Chromatographic Analysis with Bean and Maize

For comparative purposes, two *P. coccineus* varieties were analyzed together with three common bean (*P. vulgaris* L.) varieties and three maize (*Zea mays* L.) varieties using the same chromatographic methodology. The common bean varieties included Oti bean, Flor de Mayo, and Black bean, while the maize materials corresponded to blue, yellow, and white maize.

Seed samples were ground and processed following the same extraction protocol described for *P. coccineus*. Briefly, 0.2 g of flour from each material was mixed with 1 mL of 0.01 M NaOH and maintained under constant agitation for 1 h 30 min at room temperature. After centrifugation at 12,000 × g for 17 min, the supernatants were used for ascending paper chromatography on Whatman® No. 1 paper using the solvent system 2-butanol: acetic acid: water (45:11:9). Chromatograms were revealed using 0.2% ninhydrin solution in ethanol, and Rf values were recorded for comparative analysis.

2.6. Statistical Analysis

Data obtained for cooking time, broth color, seed density, and seed weight were

analyzed using analysis of variance (ANOVA). A factorial design was considered to evaluate the effects of family and seed coat color, as well as their interaction.

Prior to analysis, assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests, respectively. When significant differences were detected ($p < 0.05$), means were compared using Tukey's honestly significant difference (HSD) test.

All statistical analyses were performed using RStudio (Posit Software, PBC, Boston, MA, USA), with R version 4.3.0.

3. Results and Discussion

3.1. Physical Seed Quality

1000-Seed Weight

The analysis of variance showed significant differences among seed colors for 1000-seed weight ($F = 13.87$; $p < 0.001$), as shown in Table 3, indicating that seed coat coloration influenced this physical quality trait.

Table 3. Analysis of variance for 1000-seed weight.

Source of variation	DF	SS	MS	F-value	p-value
Seed Color	9	1092.54	121.39	13.87	< 0.001
Error	70	612.74	8.75		
Total	79	1705.28			

The highest mean values were recorded in White mottled black (103.33 g), Orange mottled black (102.94 g), and Cream mottled black (102.42 g), with no significant differences among them (Table 4). These results suggest that certain mottled pigmentation patterns could be associated with greater reserve accumulation and larger seed size. Studies in *Phaseolus* have reported that seed coat coloration is related to genetic loci such as P, C, and T, which are involved in the biosynthesis of flavonoids and phenolic compounds that may also influence physical and nutritional seed characteristics [9] [10].

Table 4. Mean comparison of 1000-seed weight by seed color.

Seed color	Mean (g)	SD	CV (%)	HSD (Tukey)
Black mottled purple	96.81	3.20	3.30	c
Black mottled orange	101.34	3.41	3.36	ab
Purple mottled black	100.68	2.11	2.10	ab
Orange mottled black	102.94	2.79	2.71	a
Cream mottled black	102.42	2.86	2.79	a
White mottled black	103.33	3.98	3.85	a
Black	102.24	3.80	3.72	ab

Continued

Purple	98.65	2.94	3.19	bc
Cream	99.19	2.75	2.78	c
White	92.01	2.94	3.19	d

Means followed by the same letter are not significantly different according to HSD (Tukey) test at $\alpha = 0.05$.

In contrast, White showed the lowest 1000-seed weight (92.01 g), being statistically different from the remaining treatments. Studies in legumes have reported that light-colored seeds tend to present lower accumulation of phenolic compounds and structural differences in the seed coat compared to pigmented seeds [11] [12].

The coefficients of variation (2.10% - 3.85%) indicated low experimental variability and high precision of the measurements. Overall, the results suggest that seed color could be considered a useful selection criterion in ayocote bean for traits associated with consumption and physical grain quality.

3.2. Cooking Time

The obtained results showed notable differences in cooking time among the evaluated seed color categories (**Figure 1**).

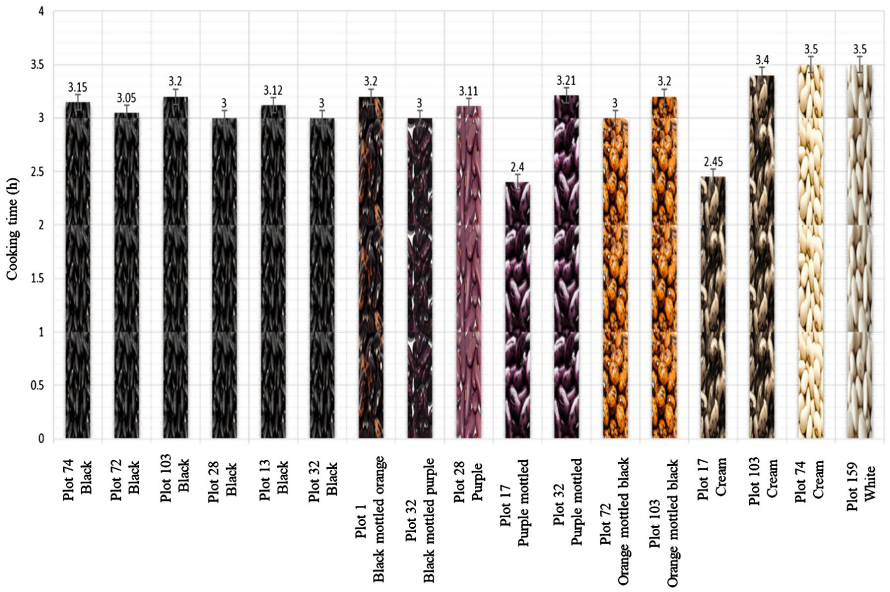

Figure 1. Variation in the cooking time of *P. coccineus* L. depending on the color of the seed coat.

White beans presented the longest cooking times (3.5 h), followed by cream and cream mottled genotypes, whereas purple mottled materials showed the shortest cooking times (2.4 h), indicating faster seed softening.

Seed coat color appears to be associated with the hydrothermal behavior of the

seed during cooking. In *Phaseolus*, several studies have reported that seed coat pigmentation is related to the accumulation of phenolic compounds, particularly flavonoids, tannins, and proanthocyanidins, which can modify seed coat permeability and affect water absorption [9]-[13]. The oxidation of these compounds promotes the formation of more hydrophobic and resistant structures, which may increase the time required for seed softening during cooking [14].

Although dark-colored seeds in common bean are generally reported to require longer cooking times, some light-colored materials in the present study also exhibited slow cooking behavior, suggesting that cooking time does not depend exclusively on pigmentation, but also on anatomical and structural seed characteristics. Recent studies have demonstrated that seed coat thickness, cotyledon cell wall composition, and the integrity of macrosclereid and osteosclereid layers significantly influence water imbibition rate and thermal softening of grains [15] [16].

Likewise, variations in insoluble polysaccharides, lignin, and pectin content may delay cell separation during the hydrothermal process, increasing resistance to cooking. Mujica *et al*. reported that changes in phenolic compounds and cell wall structure are associated with seed hardening in *Phaseolus* vulgaris. Similarly, Wang *et al*. reported that a higher degree of lignification and cell wall rigidity reduces the solubilization of middle lamella components during heating, which could contribute to longer cooking times [17] [18].

Broth Density Determination

Analysis of variance showed that seed coat color and cooking time did not have a statistically significant effect on broth density ($p > 0.05$) (**Table 5**). Seed coat color showed a moderate, although non-significant, effect on this variable ($F = 1.85$; $p = 0.125$), whereas cooking time showed no significant relationship with broth density ($F = 0.02$; $p = 0.882$).

Table 5. Effect of seed coat color and cooking time on broth density.

Source of variation	DF	MS	F-value	p-value
Seed Color	9	7.49	1.85	0.125*
Cooking time	1	0.08	0.02	0.882*
Error	19	4.05		

*not significant at $\alpha = 0.05$.

Despite the absence of significant differences, numerical variations were observed among the different seed color groups. The highest density values were recorded in Orange mottled black and Cream seeds, whereas the lowest values were observed in Purple seeds.

Analysis of variance indicated that seed coat color and cooking time did not have a significant effect on broth density ($p > 0.05$). These results suggest that the color differences evaluated among the seeds were not associated with major changes

in the amount of compounds released into the cooking water under the experimental conditions used.

Despite this, differences were observed among some seed color groups, which could indicate a certain degree of variability in cooking behavior. Recent studies have reported that seed coat color in legumes may be associated with physical and compositional characteristics of the seed, which can influence properties observed during cooking [19].

Likewise, it has been reported that some dark-pigmented seeds exhibit differences in seed coat-associated compounds, which may modify broth properties and cooking characteristics [20]. However, in the present study these differences were not statistically significant, suggesting that the choice of seed color may respond primarily to consumer preferences without causing important changes in broth density.

On the other hand, cooking time showed no significant relationship with broth density. This could be due to the relatively similar cooking intervals evaluated among treatments. In addition, other physical seed traits, such as water absorption and seed coat integrity, may influence seed behavior during cooking [21].

3.3. Determination of Relative Protein-Related Compounds

Protein content showed variation among the evaluated *P. coccineus* varieties (**Table 6**).

Table 6. Protein content of ayocote beans.

Seed color	Mean absorbance	SD	Relative protein (%)
Black mottled purple	0.0673	0.0095	51.0
Black mottled orange	0.0573	0.0118	43.5
Purple mottled black	0.0575	0.0097	43.8
Orange mottled black	0.0615	0.0088	47.2
Cream mottled black	0.0720	0.0064	54.0
White mottled black	0.0577	0.0052	44.2
Black	0.0540	0.0026	41.3
Purple	0.0630	0.0137	48.1
Cream	0.0515	0.0095	39.3
White	0.0583	0.0160	44.4

SD = standard deviation.

The Cream mottled black variety exhibited the highest relative protein value (54.8%), followed by Black mottled purple (51.0%) and Purple (48.1%). In contrast, the Cream variety presented the lowest relative protein content (39.3%).

Mean absorbance values ranged from 0.0515 to 0.0720, with moderate variability observed among replicates for some varieties. The highest standard deviation

values were recorded in the White and Purple varieties, whereas the Black variety showed the lowest experimental variability.

The numerical differences observed in relative protein content among the evaluated varieties suggest the existence of compositional variability among the analyzed *P. coccineus* materials. Recent studies have reported that protein content in legumes may vary depending on genotype and intrinsic seed characteristics [22].

Likewise, factors related to seed coat composition and grain structure may indirectly influence the nutritional properties of seeds [23]. In the present study, the Cream mottled black variety exhibited the highest numerical value of relative protein content, followed by Black mottled purple and Purple, suggesting differences in protein behavior among the evaluated varieties.

The variability observed among some replicates may be associated with differences in sample homogenization, protein extraction efficiency, or instrumental sensitivity during spectrophotometric readings [24]. Nevertheless, the obtained results allowed the identification of general trends in the protein behavior of the evaluated varieties.

3.3.1. Paper Chromatographic Analysis of Protein-Related Compounds

Paper chromatography of *P. coccineus* extracts using the solvent system 2-butanol:acetic acid:water (45:11:9) allowed the separation of four to five spots per sample (Figure 2). Retention factor (Rf) values ranged from 0.04 to 0.45 (Table 7).

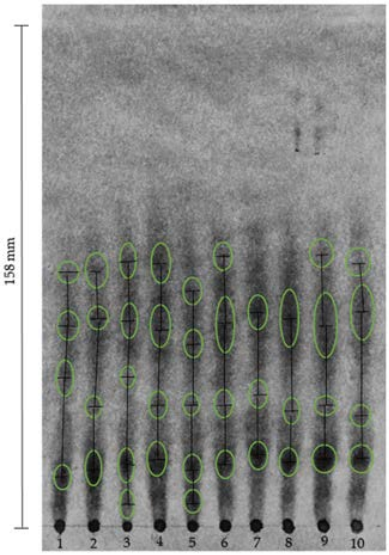

Figure 2. Paper chromatogram of extracts from different *P. coccineus* varieties.

Table 7. Retention factor (Rf) values of *P. coccineus* extracts obtained by paper chromatography.

Seed color	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5
1) Black mottled purple	0.06	0.12	0.22	0.33	
2) Black mottled orange	0.04	0.12	0.22	0.35	

Continued

3) Purple mottled black	0.04	0.10	0.21	0.35	
4) Orange mottled black	0.04	0.10	0.20	0.32	
5) Cream mottled black	0.04	0.13	0.23	0.35	0.45
6) White mottled black	0.05	0.11	0.21	0.34	
7) Black	0.05	0.10	0.22	0.37	
8) Purple	0.05	0.14	0.22	0.32	
9) Cream	0.05	0.11	0.22	0.37	
10) White	0.05	0.12	0.25	0.39	

Spots with lower Rf values showed lower chromatographic mobility and were consistently present in all varieties. In contrast, spots with higher Rf values exhibited greater migration across the chromatographic paper. The Cream mottled black variety was the only one that presented five distinct spots, including a fifth spot with an Rf value of 0.45.

The chromatographic profiles obtained revealed differences in the mobility of compounds present in the extracts of *P. coccineus*, suggesting compositional variability among the evaluated varieties. Since the extraction was performed using NaOH and the spots were visualized with ninhydrin, the detected compounds mainly correspond to substances containing free amino groups, such as amino acids, peptides, or protein-derived compounds [25] [26].

Ninhydrin is widely used for the detection of amino acids and nitrogen-containing compounds in chromatographic techniques due to its ability to react with primary and secondary amino groups, forming colored complexes visible on the chromatographic support [26] [27]. In this regard, the differences observed in the number of spots and Rf values may be associated with variations in the composition and mobility of amino acid-related compounds present in the analyzed seeds.

Spots with low Rf values suggest the presence of compounds with higher polarity and greater affinity for the stationary phase, whereas spots with higher Rf values indicate greater affinity for the mobile phase and, consequently, higher chromatographic mobility [25]. These differences may be associated with variations in the polarity, molecular size, or chemical interactions of the compounds present in the extracts.

The Cream mottled black variety exhibited the most complex chromatographic profile, being the only one to display five distinct spots and a maximum Rf value of 0.45. This behavior may indicate a greater diversity of extractable nitrogen-containing compounds compared to the other evaluated varieties. In addition, this same variety showed the highest relative protein content in the spectrophotometric analysis, suggesting a possible relationship between chromatographic profile complexity and seed protein composition. Although the methodology employed does not allow the individual identification of the detected compounds, it does reveal differences in the mobility and distribution of nitrogen-containing frac-

tions among the evaluated materials.

On the other hand, some varieties exhibited relatively similar chromatographic profiles, particularly those sharing close Rf values in the first chromatographic spots. This may indicate the presence of chemically similar compounds or comparable patterns of protein-related composition among certain materials. However, slight variations in Rf values also suggest differences in compound concentration or in their interaction with the solvent system employed.

The solvent system used (2-butanol: acetic acid: water) promoted adequate separation of polar and nitrogen-containing compounds, allowing differentiation among varieties. The efficiency of this solvent system has previously been reported for the separation of amino acids and peptides in paper chromatography due to the differences in affinity generated between the mobile and stationary phases [25]. In this study, the observed differences in chromatographic mobility indicate that the evaluated varieties possess distinct compositions of soluble nitrogen-containing compounds, which is consistent with the variability observed in relative protein content.

Overall, the results suggest that the *P. coccineus* varieties exhibit differences in the composition and mobility of compounds containing free amino groups, reflecting biochemical variability among the analyzed materials. These differences may be associated with seed protein composition and with genetic characteristics linked to each variety.

3.3.2. Comparative Chromatographic Analysis with Bean and Maize

Additionally, two *P. coccineus* varieties were compared with three common bean (*Phaseolus vulgaris* L.) varieties and three maize (*Zea mays* L.) varieties using the same chromatographic system (**Table 8**). This comparison allowed the evaluation of similarities and differences in chromatographic migration patterns among species analyzed under the same extraction and visualization conditions.

Table 8. Retention factor (Rf) values of bean and corn extracts by paper chromatography.

Seed color	Scientific name	Spot 1	Spot 2	Spot 3	Spot 4
7) Black	*Phaseolus coccineus* L.	0.0892	0.1529	0.2994	0.4331
8) Purple	*Phaseolus coccineus* L.	0.0828	0.1911	0.3248	0.4777
11) Oti bean	*Phaseolus vulgaris* L.	0.0955	0.1847	0.2866	
12) Flor de Mayo	*Phaseolus vulgaris* L.	0.0892	0.1401	0.1847	
13) Black bean	*Phaseolus vulgaris* L.	0.0955	0.1401	0.2229	
14) Blue maize	*Zea mays* L.	0.1401	0.2420		
15) Yellow maize	*Zea mays* L.	0.3312			
16) White maize	*Zea mays* L.	0.231	0.380		

The maize varieties exhibited simpler chromatographic profiles and fewer detectable spots compared with *P. coccineus*. In particular, blue maize and white

maize reached maximum Rf values of 0.231 and 0.380, respectively, indicating lower chromatographic complexity under the solvent system employed. In contrast, the ayocote varieties showed a broader distribution of Rf values and greater migration variability, especially the purple and black materials, which reached maximum Rf values of 0.4777 and 0.4331, respectively. These differences suggest that *P. coccineus* possesses a greater diversity of soluble compounds containing free amino groups compared with the maize materials evaluated.

Similarly, the Phaseolus vulgaris varieties presented chromatographic profiles mainly concentrated within intermediate Rf regions and showed fewer detectable spots than *P. coccineus*. This behavior may indicate differences in the composition, polarity, or molecular interactions of nitrogen-containing compounds among species of the genus Phaseolus. Although both species belong to the same genus, the broader distribution of Rf values observed in *P. coccineus* suggests greater compositional variability in the extracted compounds.

Because the extracts were obtained using NaOH and visualized with ninhydrin, the detected spots mainly correspond to compounds containing free amino groups, such as amino acids, peptides, or protein-derived compounds [26] [27]. Therefore, the differences observed among species are more likely associated with variability in soluble nitrogen-containing compounds than with pigments or secondary metabolites. In this regard, the greater chromatographic complexity observed in *P. coccineus* may reflect differences in protein composition or in the diversity of soluble peptide fractions extracted from the seeds.

The recurrent presence of spots within similar Rf regions among some maize, common bean, and ayocote materials suggests that certain compounds with comparable chromatographic behavior may be shared among species. However, the differences observed in the number of spots and maximum Rf values indicate that each species possesses distinct compositional characteristics affecting chromatographic mobility. Similar variation in chromatographic separation patterns has been associated with differences in protein composition and soluble nitrogen fractions among legume genotypes and cereal grains [26].

Particularly, the purple and black ayocote materials exhibited the highest chromatographic mobility and the broadest distribution of Rf values among all evaluated materials. This behavior may indicate the presence of compounds with lower polarity or greater affinity for the mobile phase, resulting in increased migration across the chromatographic paper. In contrast, the more restricted chromatographic profiles observed in maize and common bean suggest lower diversity of detectable nitrogen-containing compounds under the extraction conditions used.

Overall, the obtained results demonstrate that *P. coccineus* exhibits greater chromatographic complexity compared with the analyzed maize and common bean varieties. These differences suggest compositional variability in compounds containing free amino groups among the evaluated species and support the existence of biochemical variation associated with seed composition in ayocote bean.

4. Conclusions

The obtained results demonstrated that seed coat color in *Phaseolus coccineus* L. is associated with variations in physical characteristics, cooking behavior, and chemical composition of the grain. Mottled materials presented higher 1000-seed weight values and, in some cases, higher relative protein content compared to uniformly colored seeds.

Differences in cooking time were also observed among the evaluated color groups, with purple mottled black materials showing shorter cooking times, whereas white and cream seeds exhibited slower softening behavior. In contrast, broth density did not show significant differences among the evaluated treatments.

Chromatographic analysis revealed variability in separation profiles and Rf values among the analyzed materials, indicating differences in the composition of compounds present in the seeds. The cream mottled black material exhibited the most complex chromatographic profile and the highest relative protein content.

Overall, the results suggest that seed coat color may be considered a useful criterion for the characterization and selection of ayocote bean materials with attributes related to physical quality, cooking performance, and chemical composition, providing relevant information for their utilization, conservation, and use in breeding programs.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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