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Physico-Chemical and Nutritional Characterization of Three Traditional Spanish Chickpea Varieties

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Abstract

This study presents a physicochemical and nutritional characterization of three traditional Spanish chickpea (*Cicer arietinum L.)* varieties -*Pedrosillano* (PE), *Castellano* (CA), and *Blanco Lechoso* (BL)- together with the evaluation of a selected cultivar (*Garabito*, GA) derived from Pedrosillano, and the assessment of soil-type influence on PE seeds. Marked differences among varieties were mainly associated with morphometric traits. BL showed the largest seeds, with a 100-seed weight of 58.9 g, compared to 47.0 g in CA and 29.8 g in PE, as well as the highest fat (6.73 g/100 g) and total sugar contents (5.29 g/100 g). PE, despite its smaller size, exhibited high protein (20.3 g/100 g), fiber (23.7 g/100 g), and carbohydrate contents. All varieties showed high essential amino acid levels, with glutamate and aspartate as predominant amino acids. Mineral composition differed moderately, with potassium ranging from 10.1 to 11.8 g/kg and calcium from 1.11 to 1.38 g/kg. During cooking, BL absorbed the most water (138%) and produced the softest texture, whereas CA showed the highest level of hardness. The GA cultivar displayed physicochemical and nutritional values comparable to PE. No significant differences were observed in PE grown on contrasting soil types, indicating high compositional stability.

Keywords: *Cicer arietinum*; Castellano; *Pedrosillano*; Blanco Lechoso; traditional varieties; nutritional composition

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1. Introduction

Legumes stand out as one of the most nutritionally rich food sources, with their consumption linked to numerous health benefits [1,2]. Within the *Fabaceae* family, chickpeas (*Cicer arietinum* L.) are a traditionally cultivated legume found in many regions worldwide, contributing to their widespread use in both whole grain and flour forms. Chickpeas come in two main varieties: the desi-type, characterized by its small size and dark coat, and the kabuli-type, which is larger and has a beige coat [3].

Currently, there is a burgeoning interest in cultivating grain legumes, including chickpeas, due to their significant environmental contributions, serving as the linchpin of many sustainable agroecosystems [4]. This is primarily due to their ability to biologically fix atmospheric nitrogen in soils [4–6]. However, other benefits should also be acknowledged, such as their capacity to sequester carbon in the soil [7] and reduce herbicide usage by suppressing weed growth [8]. Moreover, legume cultivation, specially chickpea, requires less water and demonstrates greater resilience to drought conditions compared to other crops [9], offering considerable potential for reclaiming poor-quality land for agriculture [10].

From a nutritional perspective, chickpeas boast high nutritional value. Carbohydrates represent the most abundant compounds, constituting approximately 60% of their composition, chiefly due to their high starch content [11]. Traditionally, chickpeas have been esteemed as an excellent protein source, akin to all legumes, owing not only to their elevated protein content but also to their high bioavailability [12]. While its amino acid profile is well-balanced, chickpeas are deficient in sulphur amino acids such as methionine and cysteine [13]. Moreover, chickpeas are rich in dietary fiber and bioactive compounds [14], with phenolic compound concentrations ranging from 0.5 to 6.8 mg/g [15]. Mineral content in chickpeas includes calcium, phosphorus, and magnesium, with notably high iron levels compared to other legumes [16]. Despite a low-fat content (1-7%), chickpeas rank among the legumes with the highest fat content, with predominant unsaturated fatty acids such as linoleic acid and oleic acid [17].

However, chickpeas contain anti-nutritional substances that may hinder the availability of certain nutrients. Tannins, for instance, interact with proteins, reducing their digestibility and solubility [18], while phytic acid inhibits mineral absorption [19], and trypsin inhibitors interfere with protein absorption [20]. The nutritional composition of chickpeas varies by variety, with kabuli-type chickpea cultivars typically exhibiting higher protein contents [21]. Desi-type chickpeas, for instance, exhibit substantial differences in antioxidant activity among various indigenous varieties in Pakistan [22]. Studies on Ethiopian chickpeas have revealed significant variability in both nutritional and non-nutritional composition among varieties [17]. Additionally, factors such as environment, climate, agronomic practices, stressors, and soil composition influence chickpea composition [23]. For instance, the growing medium has been shown to affect starch, amylose, and protein concentrations in desi varieties but not in kabuli varieties grown in Canada [24]. Furthermore, drought and high temperatures have demonstrated influence over seed weight and sucrose and starch content [25].

Chickpea holds the third position globally among legumes in terms of production, trailing behind beans, with an average annual yield exceeding 11.5 million tons [26]. The bulk of this production is concentrated in India. However, there has been notable fluctuation in annual production in recent years, with climate change being cited as a significant factor contributing to these variations [27]. Within the European Union, Spain emerges as the leading country in chickpea cultivation, boasting over 38,200 hectares dedicated to its cultivation. This substantial area has yielded a production exceeding 45 thousand tons, as reported by the Spanish Ministry of Agriculture, Fisheries and Food. Despite Spain's prominence in chickpea cultivation, it predominantly occurs under rainfed conditions, resulting in relatively low yields.

Chickpeas constitute an integral component of the Spanish diet, finding application in a diverse array of traditional dishes across the nation. Spain boasts a multitude of chickpea varieties, characterized by variations in size, color, and flavor. This diversity stems from disparate edaphoclimatic and geographical conditions, fostering the development of varieties uniquely adapted to specific regions. Notably, most of the varieties cultivated in Spain belong to the kabuli chickpea type, characterized by beige or cream-colored seeds [28].

The region of Andalucía emerges as a focal point for chickpea cultivation in Spain, with cultivars such as "*Pedrosillano*" (PE) and "*Blanco lechoso*" (BL) prominently cultivated. Meanwhile, the regions of Castilla León and Castilla la Mancha favor the "Castellano" (CA) chickpea cultivar. Noteworthy is the significant inclusion of chickpea production within Protected Geographical Indications (PGIs), a quality designation regulated by European Council Regulation (EC) 2081/92. This certification ensures adherence to established quality standards and denotes association with a specific geographical area. For instance, the PGI "*Garbanzo de Escacena*" (Commission Regulation No 868/2013) in the Andalucía region and PGI "Garbanzo de *Fuentesauco*" (Commission Regulation No 1485/2007) in the Castilla y León region are notable examples. Within the "Garbanzo de Escacena," the BL cultivar predominates, while CA cultivar assumes prominence in "Garbanzo de *Fuentesauco*." Additionally, various quality-branded chickpeas, such as "Garbanzo *Pedrosillano*" from the province of Salamanca and "Garbanzo del *Oristán*" from Catalonia, both based on the PE cultivar, alongside "Garbanzo de *Valdelasec*a" from the province of Segovia, which references the CA cultivar, contribute to the diverse landscape of chickpea production in Spain. From among these three cultivars, namely BL, PE, and CA, researchers have derived new selections aimed at achieving greater homogeneity, enhanced yield, and improved resistance to fungal pathogens. One such instance is the development

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of a novel cultivar identified as "*Garabito*" (GA) in Salamanca province belongs to the Castilla y León region. This selection endeavors to improve the yield and refine the overall traits observed in the PE ecotype [29].

In light of the aforementioned considerations, the objective of this study is threefold: firstly, to characterize the composition of three traditional varieties of chickpea -BL, PE, and CA- cultivated in Spain; secondly, to compare the characteristics of the GA selected cultivar with those of the PE cultivar; and finally, to assess the influence of two different types of soil on the nutritional and morphometric characteristics of the PE cultivar. These varieties exhibit adaptation to their respective agronomic and production systems, and their comprehensive study and characterization contribute significant value.

This endeavor aligns with the goal of preserving genetic diversity and promoting the sustainability of food systems, as articulated in the 2030 European Agenda. Characterizing local varieties is paramount, not only to incentivize farmers to continue their cultivation but also to urge administrations to enact policies that bolster their production.

2. Materials and Methods

2.1. Plant Material, Growing and Edaphoclimatic Conditions

A total of 38 chickpea samples representing three varieties were analyzed: PE (Desi type; n = 26, including 4 samples from the GA-selected cultivar), CA (n = 10), and BL (n = 2), the latter two belonging to the Kabuli type. All samples were obtained from crops grown under rainfed conditions in the province of Salamanca. The number of samples per variety was proportional to the cultivated area of each variety within the province.

The cultivation area comprises a typical rainfed agricultural landscape situated in the northern sub-plateau of the Iberian Peninsula, featuring plains and peneplains. The edaphoclimatic conditions are emblematic of a continental Mediterranean climate, characterized by scanty and erratic rainfall, prolonged hot summers punctuated by occasional storms, and protracted cold winters. Given that the samples were sown in March 2022 and harvested in August 2022, the soil was managed using conventional tillage, and no pesticides or fertilizers were applied. The meteorological conditions specific to that period are detailed in Table 1.

Table 1. Meteorological conditions from March to August for 2022 growing season and long-term (30 years) averages in the cultivation area in the central plateau of the Iberian Peninsula.

Parameter	Growing season 1 March 2022–31 August 2022	Long term (1992-2022) 1 March–31 August
Mean temperature (°C)	16.3	15.1
Mean Max. temperature (°C)	24.3	22.9
Mean Min. temperature (°C)	8.2	7.3
Total precipitation (mm)	158.7	147.0

Data provided by a Spanish National Meteorological Agency weather station, located at Aldearrubia, Salamanca (41°00′53″N, 5°30′09″W, 811 m.a.s.l.).

Regarding edaphic conditions, while meteorological conditions remain largely consistent across the chickpea cultivation area, slight variations in soil characteristics must be noted. All CA and BL samples, along with half of the PE samples, were cultivated in slightly alkaline (pH 7.8) clay-loam soil (41° 4′ 20.2″N, -5° 38′ 26.9″W), classified by the USDA-NCRS edaphological taxonomy as a *Rhodoxeralf*. Conversely, the remaining PE (n = 13) samples were grown in slightly acidic (pH 6.5) sandy loam soil (40° 58′ 11.6″N, -5° 9′ 20.6″W), classified by the previous taxonomy systema as a *Xerochrept*.

Samples were cleaned by sieving and vacuuming to remove dirt, grit and/or broken grains and stored in big bags at room temperature (30 ± 2 °C).

2.2. Morphometric and Physical Seed Characteristics

The parameters of color, color intensity, shape, and ribbing were assessed on 70 seeds per sample following the methodology outlined in the International Union for the Protection of New Varieties of Plants, method TG/143/5 (UPOV) [30]. The weight of chickpeas was determined in triplicate, by analyzing the weight of 100 dried seeds.

Furthermore, chickpea volume, eccentricity coefficient, and surface area were calculated with the methodology and equations delineated by Chenoll et al. [31]. The volume (in mm^3) and surface area (in mm^2) were computed assuming an ellipsoidal shape. The eccentricity coefficient quantifies the degree of similarity between an ellipsoid and a sphere, with a value of zero indicating perfect spherical symmetry. All measurements were also conducted on 70 chickpeas per sample using a digital caliper (Atrium Enterprises GMBH, Lünen, Germany).

2.3. Proximal Composition

Moisture content was determined according to the gravimetric method AOAC 925.10 [32] by drying in a Binder FD 115 oven (Tuttlingen, Germany) at 105 °C for 12 h. The ash determination was assessed following the AOAC 923.03 [33] by incineration of the samples at 550 °C ± 10 °C in a Mufla Thernolyne (Thermo Scientific, Madrid, Spain). Protein content was determined by the Kjeldahl method according to AOAC 950.36 [34], and fat was analyzed by the Soxhlet AOAC 935.38 method [35], using petroleum ether. The starch content was determined with the enzymatic method described in AOAC 996.11 [36], and the total fiber content was determined following the guidelines of AOAC 991.43 [37], using an ANKOM analyzer (ANKOM technology, Macedon, NY, USA). The total amount of carbohydrates was then calculated as $100 - (Ash + Protein + Total\ Fat)$. The total sugar content was analyzed by volumetric analysis using the Luff-Schoorl method, after inversion with hydrochloric acid. The results were expressed as a percentage of glucose. Soluble solids content was calculated from the refractive index (Brix) measured in a RSD500 Brix 0-85% (0,1%) portable refractometer (Labbox, Barcelona, Spain) over a solution of ground chickpea (1g/ml). All determinations were carried out in triplicate, and the results were expressed in g/100g fresh weight.

2.4. Amino Acid Analysis

A Biochrom analyzer (Pharmacia, UK) was used for the determination of amino acids by liquid chromatography with an ion exchange column. Detection was performed after reaction with ninhydrin and quantification by using external standards. The analyses were carried out in duplicate, and the results were expressed in g/100 g.

2.5. Mineral Analyses

Mineral content was analyzed by ICP-MS according to the method described by Absi et al. [38]. First, 0.2 g of sample were digested with HNO_3 using a Milestone system. Subsequently, the mineral concentration was analyzed using an Agilent 7800 ICP mass spectrometer (Agilent, Santa Clara, CA, USA). Quantification was performed with certified standard solutions (1g/L) (Panreac, Spain). Analyses were performed in duplicate and mineral content was expressed as mg/kg fresh weight.

2.6. Soaking Behavior

The method described by Bidkhori and Mohammadpour [39], with a seed/water ratio of 1:30 (w/w) was used. Six grams of chickpeas were soaked in tap water at 25 °C for 10 hours, then they were superficially dried by paper towel, and their final weight was determined. Water absorption was expressed as the percentage of weight gain after soaking.

2.7. Cooking Behaviour

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Chickpeas were boiled for 2.5 hours at 100 °C. After cooking, the chickpeas were superficially dried by paper towel and cooled to 25 °C. The weight gain of the chickpeas was determined, and the number of detached skins was counted. The soluble solids remaining in the chickpea after cooking were determined. The sample (1 g ± 0.002) was mixed with 5 ml of distilled water in a Falcon tube and homogenized with an Ultraturrax (IKA Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany). The supernatant liquid after centrifuging (Sigma 4K15, Osterode am Harz, Germany) for 5 minutes at 10,000 rpm was collected and the Brix degrees were measured with a portable refractometer ZUZI 300 (Barcelona, Spain).

Chickpea texture after cooking was determined using a TA-XT2i texturometer (Texture Technologies Corp, Stable Micro Systems, Godalming, UK). A penetration test with a 0.2 mm diameter probe and a 5 kg load cell was used. The test conditions were pretest speed 2 mm×s^{-1}, test speed 1 mm×s^{-1}, posttest speed 2 mm×s^{-1}, penetration distance 4 mm. The test was carried out at 25 °C with 10 replicates.

2.8. Statistichal Analysis

Statistical analyses were performed using IBM-SPSS Statistics 27 software (IBM, Armonk, NY, USA). For each of the three experimental objectives pursued in this study, i.e., characterization of the three Spanish chickpea cultivars, assessment of potential differences between PE variety and its GA selected cultivar, and evaluation of the impact of the type of soil on the characteristics of the PE variety, significant differences were assessed using a one-way analysis of variance. Means and standard errors of the mean (sem) were computed for all variables, with statistical significance evaluated at a 95% confidence level ($\alpha = 0.05$) using Snedecor's F as the contrast statistic. Subsequently, distinct homogeneous subsets were discerned by employing post hoc Tukey's honest significant difference test [40]. To assess the independence or interrelation among the values of the categorical variables, i.e., color, intensity of color, shape, and ribbing, we employed the Chi-square test (χ^2), assuming an equal distribution of the expected frequencies, and evaluated the statistical significance with the aforementioned confidence level.

For visualizing potential Mahalanobis separation between samples arising from the distinct characteristics of each cultivar, a discriminant coordinate analysis was conducted [41] for the characterization of the three main cultivars and for the comparison of PE variety and its GA selected cultivar.

3. Results

3.1. Characterization of the Three Main Spanish Chickpea Cultivars

3.1.1. Physical and Morphometric Parameters

The main qualitative and quantitative physical and morphometric characteristics of the studied Spanish chickpeas varieties are depicted in Table 2 and Figure 1.

Table 2. Morphometric parameters and weight of seeds of the Spanish chickpea varieties analyzed.

Seed Parameters	Chickpea variety			Signification
	PE *	CA *	BL *	
Color **	Yellow	Greyed brown	Whitish	< 0.001
Intensity of color **	Medium	Light	Medium	0.005
Shape **	Round	Round to angular	Angular	< 0.001
Ribbing**	Weak	Medium	Strong	< 0.001
Seed weight (g) ***	29.80±0.52 c	47.00±1.01 b	58.89±1.56 a	< 0.001
Volume (mm3)	268.28±3.83 c	403.31±11.70 b	522.37±3.11 a	< 0.001
Eccentricity coefficient	0.57±0.01 b	0.64±0.01 a	0.63±0.01 ab	< 0.001
Surface (mm2)	436.90±9.60 b	500.47±27.81 ab	608.77±28.17 a	< 0.001

Data are mean±sd. Within a row, means followed by different letter indicate significantly different (P < 0.05) according to Tukey's test. * PE: *Pedrosillano*, CA: *Castellano*, and BL: Blanco lechoso. ** Based on the methodology of the International Union for the Protection of New Varieties of Plants (UPOV). *** Weight of 100 seeds determined in triplicate.

Significant differences among varieties were observed in all the analyzed variables. Firstly, all the varieties displayed different colors, each with distinct intensities. Notably, the PE variety exhibited smaller dimensions in terms of seed weight, surface area, and volume, contrasting with the BL cultivar, which was the largest and the only one with an angular shape and prominent ribbing. Additionally, the CA variety displayed the significantly highest eccentricity coefficient.

Figure 1. *Pedrosillano*, *Castellano* and *Blanco Lechoso* cultivars (from left to right).

3.1.2. Proximate Composition

The proximate composition of the three studied Spanish chickpea varieties is shown in Table 3. As was expected, there are barely significant differences among varieties, standing out the differences found in fat and total sugar contents. Particularly, the BL variety displayed the highest values in these two parameters, in contrast to the PE variety, which demonstrated the lowest concentrations of fat and total sugar. Furthermore, a clear inverse relation between moisture and ash content is evident. Interestingly, although not statistically significant, it is noteworthy that despite being the smallest chickpea, the PE variety exhibited the second highest protein level, and the highest carbohydrate and fiber content, which might be all associated with its lower fat content.

Table 3. Proximate composition of the Spanish chickpea varieties analyzed.

Seed Parameters	Chickpea variety			Signification
	PE *	CA *	BL *	
Moisture (g/100g)	10.40±1.61	8.62±0.26	8.08±0.97	0.742
Ash (g/100g)	2.96±0.04	2.85±0.03	3.09±0.09	0.095
Protein (g/100g)	20.27±0.36	20.41±0.56	18.80±1.10	0.519
Fat (g/100g)	5.91±0.09 b	6.24±0.13 ab	6.73±0.40 a	0.016
Starch (g/100g)	45.21±0.50	44.94±0.40	46.65±2.05	0.631
Fiber (g/100g)	23.68±0.42	22.92±0.85	20.00±0.20	0.089
Total sugar (g/100g)	3.80±0.07 c	4.67±0.10 b	5.29±0.10 a	< 0.001
Carbohydrates (g/100g)	71.61±1.25	70.43±1.58	70.87±1.79	0.145
Soluble Solids (°Brix)	6.55 ± 0.14	7.19 ± 0.24	7.13 ± 0.68	0.061

Data are mean±sd. Within a row, means followed by different letter indicate significantly differents (P < 0.05) according to Tukey's test. * PE: *Pedrosillano*, CA: *Castellano*, and BL: *Blanco lechoso*.

3.1.3. Aminoacidic Profile

Figure 2 illustrates the amino acid profile, including individual amino acids, essential amino acids (EAAs), and branched-chain amino acids (BCAAs) of the three Spanish chickpea varieties studied. As depicted, the CA variety demonstrated the highest level in every amino acid, except for

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tyrosine, where the PE variety exhibited higher values. Notably, all studied chickpea varieties displayed high levels of EAAs. Glutamate was the amino acid with the highest concentrations across all three chickpea varieties, followed by aspartate. Conversely, tryptophan exhibited the lowest concentrations in all three chickpea varieties, together with the sulphur-containing amino acids cysteine and methionine.

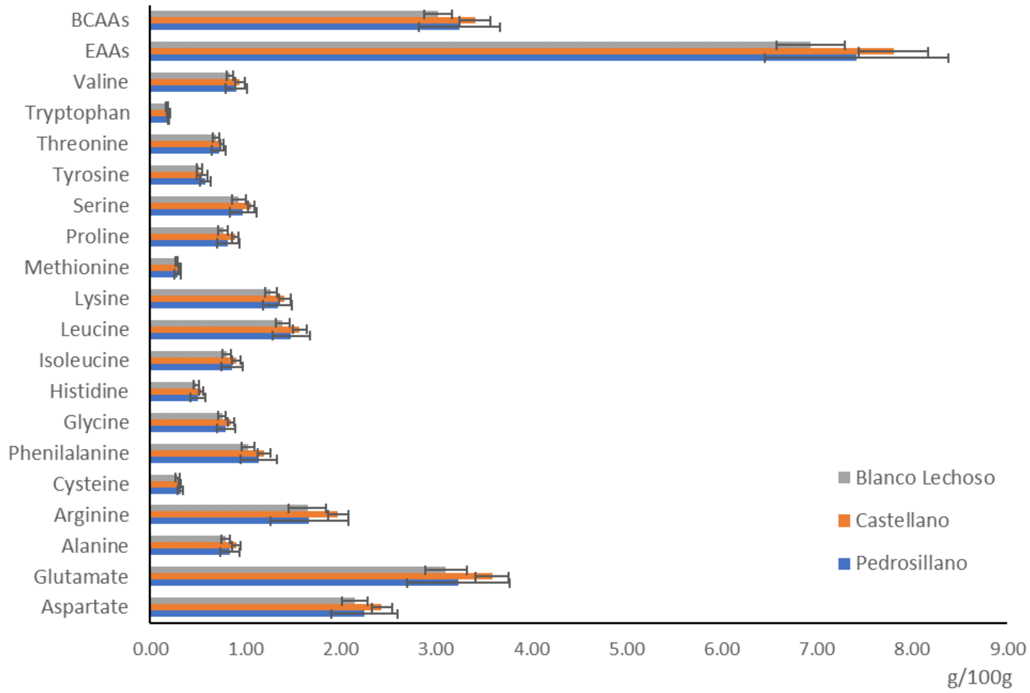

Figure 2. Average content in individual amino acids, essential amino acids (EAAs) and branched-chain amino acids (BCAAs) of the different chickpea varieties tested.

3.1.4. Mineral Content

The mineral content of each of the Spanish chickpea varieties studied is presented in Table 4. The BL variety demonstrated the highest concentration of all analyzed mineral elements. However, significant differences were only observed in the cases of K and Ca contents. Specifically, the PE variety exhibited the significantly highest concentration of Ca, alongside the BL variety. Additionally, the CA variety did not exhibit the highest value in any of the studied mineral element concentrations; instead, it displayed the lowest values in Na, Ca, and Fe, which is related to its low ash content observed in Table 3.

Table 4. Mineral element concentration of the Spanish chickpea varieties analyzed.

Mineral element	Chickpea variety			Signification
	PE *	CA *	BL *	
Na (g/Kg)	3.56±0.12	3.26±0.12	3.59±0.27	0.350
Mg (g/Kg)	1.28±0.01	1.29±0.03	1.35±0.02	0.359
P (g/Kg)	3.38±0.05	3.63±0.11	3.64±0.05	0.053
K (g/Kg)	10.07±0.08 b	10.47±0.17 b	11.80±0.25 a	< 0.001
Ca (g/Kg)	1.38±0.05 a	1.11±0.07 b	1.36±0.13 a	0.018
Fe (mg/Kg)	74.50±4.23	74.30±5.96	80.46±22.9	0.927

Data are mean±sd. Within a row, means followed by different letter indicate significantly differents (P < 0.05) according to Tukey's test. * PE: *Pedrosillano*, CA: *Castellano*, and BL: *Blanco lechoso*.

3.1.5. Soaking and Cooking Behaviour

The response of the three studied Spanish chickpea varieties to soaking and cooking processes is detailed in Table 5. Water absorption during soaking did not show significant differences among the varieties, with all of them absorbing over 200%. However, significant differences were observed in the amount of water absorbed after cooking. Specifically, the BL variety absorbed the highest amount of water post-cooking, while the CA variety absorbed the lowest quantity. Furthermore, the cooking process also led to significant differences in seed hardness, with the CA seeds being the hardest, followed by the PE seeds, and ultimately the BL seeds. These results display an inverse correlation with the earlier water absorption values. Finally, significant variations among the varieties were noted in soluble solids content, with the BL variety exhibiting the highest value and the PE variety showing the lowest, after the cooking process.

Table 5. Mean values (±standard deviation) of soaked and cooked chickpeas.

	Chickpea variety			**Signification**
	PE *	**CA ***	**BL ***	
Soaking				
Water absorption (%)	210.61±2.23	206.12±2.24	214.73±5.94	0.413
Cooking				
Water absorption (%)	112.08±0.05 ab	79.24±0.07 b	137.85±0.10 a	0.001
Hardness of seed (N)	696.12±14.45 ab	836.06±28.42 a	679.96±36.48 b	< 0.001
Peeled skin (%)	0.04	0.00	0.00	0.803
Soluble solids (ºBrix)	0.89±0.02 b	1.01±0.06 ab	1.10±0.10 a	0.022

Data are mean±sd. Within a row, means followed by different letter indicate significantly different ($P < 0.05$) according to Tukey's test. * PE: *Pedrosillano*, CA: *Castellano*, and BL: *Blanco lechoso*.

3.1.6. Discriminant Analysis of the Three Main Spanish Chickpea Varieties

A scatter diagram depicting the discriminant analysis based on three Spanish chickpea varieties is presented (Figure 3), with the positioning of the 38 chickpea samples defined by discriminant functions 1 and 2. The discriminant analysis conducted on the three main Spanish chickpea varieties yielded two discriminant functions, jointly explaining 92.7% of the total variance (81.5% for function 1 and 11.2% for function 2). Specifically, seed weight, volume, surface area, and total sugar were found to have the greatest impact on Function 1, while K, Ca, ash content, and eccentricity coefficient were the primary variables influencing Function 2. Function 1 notably segregates PE samples (in the negative portion) from the rest of the chickpea samples, indicating a clear differentiation based on morphometric characteristics, as PE chickpeas are notably smaller than CA and BL chickpeas. Conversely, Function 2 situates chickpea samples with higher potassium (K), calcium (Ca), and ash content in the negative portion of the axis, reflecting the negative correlation of these variables with Function 2 itself.

Table S1 presents the structure matrix resulting from the discriminant analysis, highlighting the most influential parameters on the distribution of chickpea samples in the two-dimensional space.

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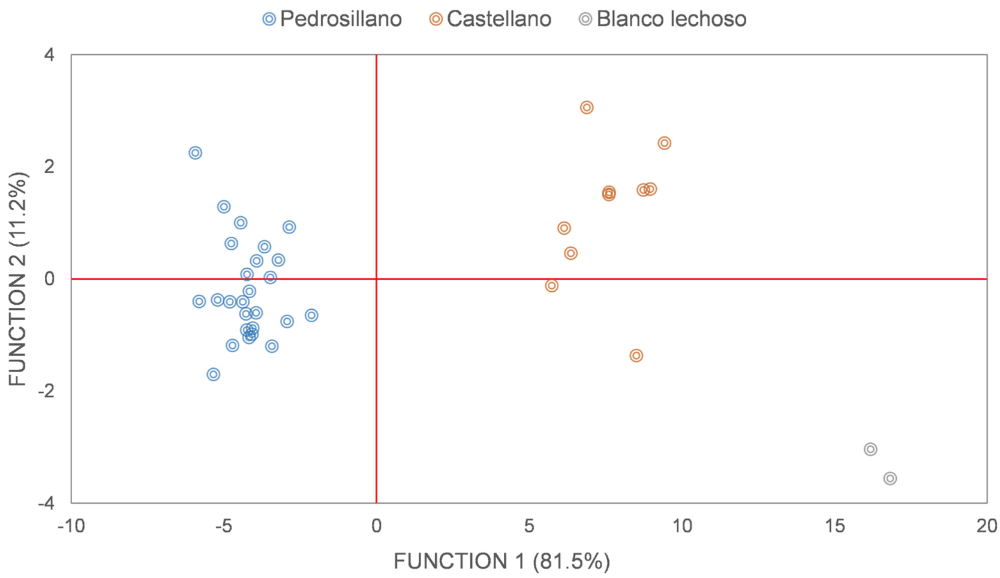

Figure 3. Scatter plot exhibiting discriminant coordinates based on the nutritional and physicochemical characteristics of the three main Spanish chickpea varieties. The percentage of variance explained by the discriminant functions is shown in parenthesis.

3.2. Comparison of GA Selected Cultivar with PE Cultivar

Table 6 presents the physical and morphometric parameters as well as the proximate and mineral composition of GA-selected cultivar and *Pedrosillano*, both desi types, compared to the kabuli chickpeas varieties included in this study.

Table 6. Physical, morphometric, Proximate and mineral composition parameters of *Garabito* selected cultivar and *Pedrosillano* origin cultivar.

Seed parameters	PE *	GA *	Other varieties	Signification
Physical and morphometric parameters				
Color	Yellow	Yellow	Greyed brown	0.101
Intensity of color	Dark	Medium	Medium	0.02
Shape	Round	Round	Round to angular	0.002
Ribbing	Weak	Weak	Medium	< 0.001
Seed weight (g)	29.80±2.66b	29.78±3.04b	48.98±5.50a	< 0.001
Volume (mm³)	215.02±21.00b	212.45±9.21b	338.52±50.00a	< 0.001
Eccentricity coefficient	0.58±0.04b	0.55±0.03b	0.64±0.04a	< 0.001
Surface (mm²)	714.40±50.69b	724.18±43.55b	985.20±112.94a	< 0.001
Proximate composition				
Moisture (g/100g)	8.89±1.12	8.29±1.21	8.53±0.87	0.599
Ash (g/100g)	2.96±0.19	2.85±0.13	2.91±0.16	0.436
Protein (g/100g)	20.17±1.88	20.78±1.84	20.14±1.79	0.821
Fat (g/100g)	5.90±0.47b	5.96±0.18b	6.32±0.44a	0.043
Starch (g/100g)	45.12±2.73	45.69±0.85	45.23±1.58	0.902
Fibre (g/100g)	23.87±2.22	22.63±1.62	22.43±2.69	0.208
Total Sugar (g/100g)	3.81±0.38b	3.72±0.29b	4.78±0.38a	< 0.001
Soluble solids (°Brix)	6.54±0.74	6.64±0.55	7.18±0.74	0.060
Mineral content				
Na (g/Kg)	3.60±0.65	3.35±0.42	3.31±0.38	0.332
Mg (g/Kg)	1.28±0.06	1.29±0.07	1.30±0.08	0.672
P (g/Kg)	3.42±0.24ab	3.19±0.13b	3.63±0.31a	0.011
K (g/Kg)	10.13±0.39ab	9.76±0.40b	10.69±0.72a	0.003

Ca (g/Kg)	1.40±0.24a	1.28±0.32a	1.16±0.22b	0.059
Fe (mg/Kg)	77.14±22.35	59.99±6.44	75.33±19.79	0.321

Data are mean±sd. Within a row, means followed by different letter indicate significantly different (P < 0.05) according to Tukey's test. * PE: *Pedrosillano*, GA: *Garabito.*

The comparison between the *Garabito* (GA) selected cultivar and its parental *Pedrosillano* (PE) line shows that both exhibit an almost identical physical and morphometric profile. No significant differences were observed in seed weight, volume, surface area, or eccentricity coefficient, confirming that the selection process preserved the characteristic small size and shape of the original PE ecotype. Likewise, qualitative descriptors such as color, shape, and ribbing remained unchanged, demonstrating that GA maintains the traditional morphological identity associated with *Pedrosillano*-type chickpeas.

Similarly, the proximate composition of GA did not differ significantly from that of PE, with both cultivars showing comparable levels of protein, fat, fiber, starch, and total sugars. This indicates that nutritional attributes were not altered during the selection process, and that GA retains the biochemical profile typical of the *Pedrosillano* variety.

The discriminant analysis between the Garabito (GA) selected cultivar, its parental *Pedrosillano* (PE) and the other varieties (Kabuli type) yielded two discriminant functions, jointly explaining 90.4% of the total variance (78,3.5% for function 1 and 12.1% for function 2). Figure 4 reveals that separation among the *Pedrosillano* (PE) variety, its *Garabito* (GA) selected cultivar, and the other Spanish chickpea varieties is driven primarily by morphometric traits rather than compositional parameters. In particular, as indicated in Table S2, seed weight, volume, and surface area show the strongest correlations with Function 1, indicating that size-related variables account for most of the variability (78.3%). The fact that GA and PE cluster closely for these parameters suggests that the selection process leading to the GA cultivar preserved the characteristic small seed phenotype of the original PE variety but exhibiting greater homogeneity. In Table 6, lower standard deviation values can be observed for most parameters in the case of *Garabito* compared to the deviations in *Pedrosillano*.

Function 2, which explains a smaller proportion of the variance (12.1%), is more strongly associated with mineral content—especially Fe, P, K, and Ca—and with the eccentricity coefficient (Table S2). These parameters contribute to differentiating the Kabuli traditional varieties from PE and GA (Desi type), rather than separating GA from PE. The limited influence of proximate composition variables (protein, fat, starch) on both functions underscores the overall nutritional similarity between the GA selection and its parental cultivar, as well as the minor divergence with respect to proximate composition between these varieties and the Kabuli type.

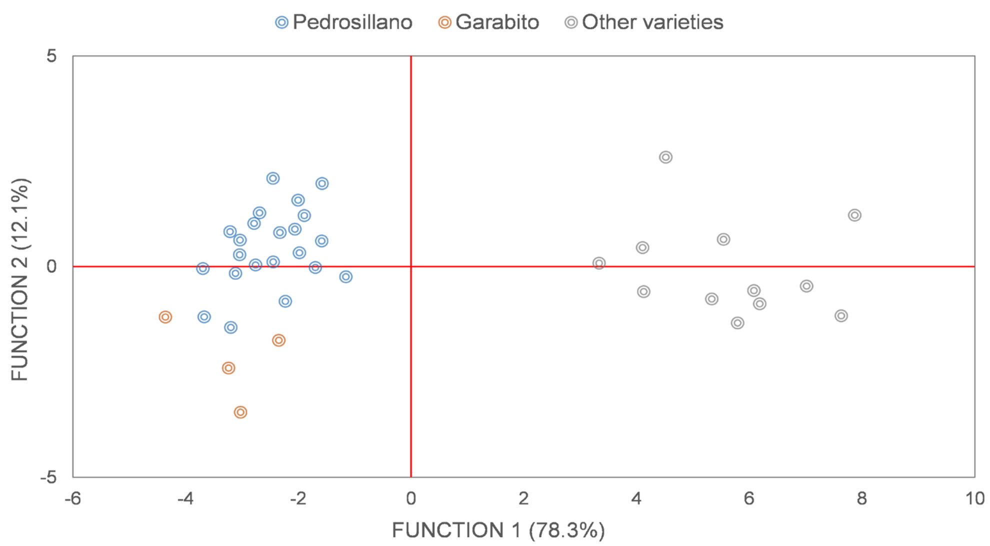

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Figure 4. Scatter plot exhibiting discriminant coordinates based on the nutritional and physicochemical characteristics of the *Pedrosillano* variety, the *Garabito* selected cultivar and of the rest of the studied varieties. The percentage of variance explained by the discriminant functions is shown in parenthesis.

3.3. Influence of the Type of Soil on the Characteristics of the PE Cultivar

Results related to physical and morphometric parameters and the proximate and mineral composition of *Pedrosillano* cultivar grown in different types of soil are shown in Table 7.

Table 7. Means and standard deviations for physical, morphometric, proximate and mineral composition parameters of *Pedrosillano* cultivar grown in different types of soil.

	Clay-loam soil (pH 7.8)	**Sandy-loam soil (pH 6.5)**	**Signification**
Physical and morphometric parameters			
Colour	Yellow	Yellow	0.973
Intensity of colour	Medium	Medium	0.228
Shape	Round	Round	0.059
Ribbing	Weak	Weak	0.241
Seed weight (g)	29.32±0.65	30.15±0.77	0.443
Volume (mm^3)	213.28±4.17	215.61±6.01	0.770
Eccentricity coefficient	0.57±0.01	0.58±0.01	0.592
Surface (mm^2)	712.69±12.96	718.26±14.02	0.781
Proximate composition			
Moisture (g/100g)	8.46±0.31	11.82±2.76	0.331
Ash (g/100g)	2.98±0.06	2.94±0.05	0.577
Protein (g/100g)	20.35±0.53	20.20±0.51	0.838
Fat (g/100g)	5.96±0.08	5.88±0.14	0.662
Starch (g/100g)	45.96±0.75	44.66±0.65	0.203
Fibre (g/100g)	22.92±0.43	24.23±0.64	0.127
Total Sugar (g/100g)	3.78±0.11	3.81±0.10	0.811
Soluble solids (°Brix)	6.47±0.17	6.61±0.21	0.634
Mineral content			
Na (g/Kg)	3.50±0.19	3.60±0.17	0.689
Mg (g/Kg)	1.29±0.02	1.27±0.02	0.382
P (g/Kg)	3.34±0.05	3.42±0.07	0.398
K (g/Kg)	10.01±0.14	10.11±0.09	0.551
Ca (g/Kg)	1.33±0.06	1.41±0.07	0.412
Fe (mg/Kg)	66.62±4.39	80.28±6.29	0.112

The comparison of PE samples grown on slightly alkaline clay-loam soil versus slightly acidic sandy-loam soil shows no statistically significant differences across all measured physical, nutritional, and mineral variables. This suggests a remarkable stability of the PE cultivar across these different edaphic environments (found in the legume-growing areas of Salamanca Province) under similar climatic and growing management conditions. Morphometric traits including seed weight, volume, surface area, and eccentricity show minimal variation between soil types, indicating that seed development is largely genetically determined in this variety and not strongly modulated by soil texture or pH under the studied conditions. Likewise, proximate composition variables (protein, fat, fiber, starch, sugars) exhibit minor, non-significant fluctuations, confirming that macronutrient accumulation in PE is robust to these soil differences. Mineral composition also remains statistically unchanged between these soils.

4. Discussion

The present study provides an integrated characterization of three traditional Spanish chickpea cultivars (*Pedrosillano*, PE; *Castellano*, CA; and *Blanco Lechoso*, BL), along with an evaluation of the *Garabito* (GA) selected line derived from *Pedrosillano*, and an assessment of soil-type effects on PE seeds. Overall, the findings demonstrate that seed morphology is the principal discriminant attribute among cultivars, while nutritional composition exhibits comparatively limited variability -consistent with previous literature reporting a relatively narrow compositional range among type [12,13,42].

The three Spanish chickpea varieties showed pronounced differences in physical and morphometric traits, with BL displaying the largest seed size and strongest ribbing, CA positioning in an intermediate state, and PE characterized by the smallest and most spherical seeds. These observations align with previous typification of Spanish germplasm, where PE types are consistently described as small-seeded ecotypes [29] concordant to Desi type. The PE chickpeas are categorized as small, wrinkled, and dark-colored chickpeas whereas BL and CA are kabuli varieties (large, smooth-coated, and white to cream-colored) [43]. Based on our results, CA showed a greyed-brown coloration and an intermediate size relative to PE and BL. According to Brun et al. [42], BL could be gathered as a second sub-group of the kabuli seeds, as they present the highest seed size and weight (> 50 g), in agreement with the macrocarpa kabuli-type descriptors. Since seed size and external morphology serve as primary descriptors in chickpea classification [30], the marked differentiation observed here supports the genetic distinctiveness of these cultivars and reinforces the value of conserving such local germplasm.

The discriminant analysis further confirmed that seed weight, surface area, and volume were the dominant contributors to cultivar separation, explaining over 80% of the variance. These results again highlight the morphological robustness of these traits and match findings in other studies where size-related parameters outperform chemical composition in discriminating chickpea varieties [31,42,43].

Although proximate composition showed fewer significant differences across cultivars, subtle patterns were detected. BL exhibited the highest fat and total sugar contents, whereas PE showed the lowest values for both. Similar variability in fat and sugar profiles among cultivars has been reported elsewhere [21], suggesting genetic bases for these traits.

Interestingly, despite its smaller seed size, PE showed comparatively high levels of protein, carbohydrates, and fiber -even surpassing BL for some parameters. Aguilera et al. [1] reported that the total dietary fiber content of chickpeas was the highest (18-22 g/100 g) among the other pulses showing the Kabuli type of chickpea has a lower content of total dietary fiber and insoluble fiber as compared to the Desi type. This could be due to the thinner hulls and seed coat in the Kabuli type compared to the desi type. Since fat content was lowest in PE, reciprocal increases in carbohydrate and fiber are consistent with the nutrient partitioning trends reported in chickpeas [11]. These attributes may consider the functional value of PE as a nutrient-dense cultivar.

The amino-acid profile revealed that CA generally contained the highest concentrations of individual amino acids, whereas glutamate and aspartate dominated across all varieties. These results are consistent with previously established amino-acid patterns in chickpeas [13,42], reinforcing the notion that this legume provides a protein of high nutritional quality. According to Sots et al. [44], the amino-acid composition of desi chickpea is more enriched than that of kabuli, particularly with respect to essential amino acids (EAAs) such as lysine, isoleucine, valine, leucine, and phenylalanine. Our results (Figure 2) showed no significant differences in EAA content, with considerable variability observed in the PE chickpea content among the samples analysed. Similarly, Wang et al. [45] and Ipekesen et al. [46], found no significant differences in essential amino acid content between desi and kabuki chickpeas.

Mineral composition showed more cultivar-specific variation with BL exhibited the highest concentration for most minerals. PE and BL shared the highest Ca content. Although previous studies [46] had suggested that desi chickpeas contain higher concentrations of Ca, according to our results, the BL chickpea (kabuli type) also contained high levels of Ca per 100 grams of wet weight as a result of its lower water content (Table 3). CA observed low level in Ca indicates a distinctive mineral

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uptake profile that may be nutritional relevant. These findings highlight significant genotype-dependent variability in mineral accumulation [17,44].

Soaking behavior was similar among cultivars, but significant differences arose during cooking: BL absorbed the most water and produced the softest cooked seeds, whereas CA remained the hardest. These patterns coincide with previous findings relating larger seed size to greater hydration capacity and softer cooked texture [39,43]. PE displayed intermediate cooking behavior, consistent with its smaller size and lower water uptake. These results have culinary relevance, as BL may be preferred in gastronomic contexts requiring rapid softening, whereas CA may resist disintegration better in long-cooked traditional dishes. Significant differences in soluble solids content were found among the varieties, with the PE variety showing the lowest value after cooking, this represents losses of more than 86% of soluble solids during the cooking process. On the other hand, BL was the variety that showed the lowest losses (84.5%). The soluble solids lost during cooking are mainly vitamins and minerals [47]. These results highlight the significant nutrient losses that occur when chickpeas are consumed after discarding the cooking water.

Regarding assessment of the *Garabito* (GA) selected cultivar, the comparison of GA with its parental PE ecotype demonstrates that selection efforts preserved the morphometric profile characteristic of PE, including small seed size, weak ribbing, and similar eccentricity. Proximate and mineral composition also showed no significant differences between the two. This stability aligns with breeding objectives aimed at improving agronomic performance while maintaining traditional quality traits [29]. Overall, these results confirm that GA is nutritionally and morphologically equivalent to PE, suggesting that agronomic improvements such as yield (indicated by growers) were achieved without modifying the essential characteristics of the original cultivar. The preservation of nutritional traits despite selection indicates that GA improves uniformity and production consistency without compromising the compositional identity of PE. This finding highlights that agronomic selection did not introduce major biochemical divergence but instead primarily enhanced morphological uniformity, as evidenced by the lower standard deviations observed for most parameters. This aspect is crucial in the breeding process, as reported by Shahnaz et al. [48].

The discriminant analysis confirmed the close affinity between GA and PE clustered together, and their separation from CA and BL was driven by the same morphological variables described earlier.

With respect to the Influence of Soil Type on *Pedrosillano* chickpeas, the analysis of PE grown in two distinct soil types (slightly alkaline clay-loam and slightly acidic sandy-loam) showed no significant differences in any morphological, nutritional, or mineral parameter. This result suggests a high degree of environmental stability for the PE genotype under rainfed Mediterranean conditions, with a total precipitation of 158 mm recorded during the cropping cycle and an average temperature of 16.3 °C (Table 1). The results indicate that maintaining under similar climatic and growing management conditions with (traditional tillage) the quality characteristics of *Pedrosillano* chickpeas could be conservated. While soil type is known to modulate nutrient uptake and seed composition in many legumes [24], the absence of significant changes here indicates that PE maintains its compositional integrity across contrasting edaphic environments as long as the soil is able to supply the crop with the necessary nutrients. Given that mineral uptake is often highly sensitive to soil pH, cation exchange capacity, and texture, the absence of significant effects highlights an efficient nutrient acquisition strategy in the PE genotype. This finding could be relevant for increasing the area included under the quality label "Garbanzo *Pedrosillano*" in the province of Salamanca, which is currently restricted to the "*Armuña*" place and could potentially be extended to other areas of the province. The overall stability of PE reinforces its suitability for cultivation across diverse marginal and dryland environments in Spain, supporting broader sustainability and food security objectives [7,9].

Overall, these findings underscore the importance of preserving and promoting traditional chickpea germplasm as a valuable genetic resource for sustainable agriculture, regional food heritage, and future breeding programs. Further research should deepen the understanding of genotype-

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environment interactions, particularly under climate change scenarios, and explore the technological and sensory implications of the physicochemical traits identified in this work.

5. Conclusions

This study provides a comprehensive physicochemical and nutritional characterization of three traditional Spanish chickpea cultivars (*Pedrosillano, Castellano, and Blanco Lechoso*), together with an evaluation of the *Garabito* selected line and the influence of soil type on the *Pedrosillano* variety. The results demonstrate that seed morphology particularly seed weight, volume, and surface area is the main discriminator among cultivars, whereas proximate and mineral compositions show comparatively limited variability. *Blanco Lechoso* stands out for its larger seed size, high fat and sugar contents, and superior hydration and softening capacity after cooking, while *Castellano* exhibits a distinct mineral profile and the greatest post-cooking hardness. *Pedrosillano*, despite its small seed size, presents a nutritionally robust profile with high protein, fiber, and carbohydrate contents.

The *Garabito* selected cultivar proved to be highly similar to its parental *Pedrosillano* line in both morphology and composition, indicating that the selection process successfully enhanced uniformity and agronomic traits without altering the nutritional identity of the traditional variety. Moreover, the stability observed across soil types for *Pedrosillano* highlights its adaptability to different edaphic conditions within Mediterranean dryland environments.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org., Table S1: Structure matrix resulting from the discriminant analysis based on chickpea variety in which correlations between variables and discriminant functions are shown.; Table S2: Structure matrix resulting from the stepwise discriminant analysis which shows correlations between variables and discriminant factors.

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