



Compositional characterization of three traditional Spanish chickpea (*Cicer arietinum* L.) varieties

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Abstract

This study presents an integrated compositional characterisation of three traditional Spanish chickpea (*Cicer arietinum* L.) cultivars -Pedrosillano (PE), Castellano (CA) and Blanco Lechoso (BL)- together with an evaluation of a selected line (Garabito, GA) derived from Pedrosillano and an assessment of soil-type effects on PE seeds. Marked differences among cultivars were primarily associated with morphometric traits. BL showed the largest seeds, with a 100-seed weight of 58.9 g, compared with 47.0 g for CA and 29.8 g for PE, and the highest lipid (6.73 g/100 g fw) and total sugar contents (5.29 g/100 g fw). Despite its smaller size, PE exhibited high protein (20.3 g/100 g fw), fibre (23.7 g/100 g fw) and carbohydrate contents. All varieties exhibited high levels of essential amino acids. In addition, glutamate and aspartate were the predominant amino acids in the overall profile. Mineral composition differed moderately, with potassium ranging from 10.07 to 11.80 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 (N), whereas CA exhibited the greatest hardness. The GA selected line showed compositional characteristics closely comparable to those of its parental PE ecotype, indicating that selection preserved the original compositional profile. Furthermore, no significant differences were observed in PE seeds grown under contrasting soil types.

Keywords *Cicer arietinum* · Castellano · Pedrosillano · Blanco Lechoso · Traditional varieties · Nutritional composition

Introduction

Legumes are among the most nutrient-rich food sources, and their consumption has been linked to numerous health benefits [1, 2]. Within the *Fabaceae* family, chickpea (*Cicer arietinum* L.) is a traditionally cultivated legume grown in many regions worldwide, which supports its widespread use as whole seeds and as flour. Chickpeas are commonly classified into two main types: Desi, characterised by smaller seeds and a darker seed coat, and Kabuli, which typically has larger seeds and a beige seed coat [3]. Moreover, chickpea cultivation generally requires less water and shows

greater resilience to drought than many other crops [4], offering considerable potential for the cultivation of marginal or lower-quality land [5].

From a nutritional perspective, chickpeas have high nutritional value. Carbohydrates are the most abundant constituents, accounting for approximately 60% of the seed composition, largely due to their starch content [6]. Chickpeas are also valued as an excellent protein source (approximately 20–25% of the seed), not only because of their protein concentration but also owing to their relatively high bioavailability [7]. Although the amino acid profile is well balanced, chickpeas are limited in sulphur-containing amino acids, such as methionine and cysteine [8]. Chickpeas are rich in dietary fibre (22 g/100 g in raw chickpea seed) [8] and contain bioactive compounds [9], with total phenolic compound concentrations reported to range from 0.5 to 6.8 mg catechin equivalents/g [10]. Reported mineral contents include calcium (160 mg/100 g), phosphorus (384 mg/100 g) and magnesium (138 mg/100 g), with comparatively high iron levels (5.0 mg/100 g) relative to other legumes [8, 11]. Despite a low lipid content (1–7%), chickpeas rank among the legumes with the highest lipid content,

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mainly comprising unsaturated fatty acids such as linoleic and oleic acids [12].

Chickpeas also contain anti-nutritional factors that may reduce the bioavailability of certain nutrients. For example, tannins can interact with proteins, reducing digestibility and solubility [13]; phytic acid can inhibit mineral absorption [14]; and trypsin inhibitors can interfere with protein digestion [15]. Chickpea composition varies among cultivars, with Kabuli cultivars often reported to have higher protein contents [16]. Desi chickpeas, for instance, show substantial variation in antioxidant activity among indigenous Pakistani varieties (Balksar 2000, CM 98, Dasht and Winhar 2000) [17]. Studies on Ethiopian chickpeas have also reported marked variability in nutritional and non-nutritional components among cultivars [12]. In addition, environmental conditions, climate, agronomic practices, stressors and soil properties can influence chickpea composition [18]. For example, the growing medium has been shown to affect starch, amylose and protein concentrations in desi cultivars, but not in Kabuli cultivars grown in Canada [19]. Drought and high temperatures have also been reported to influence seed weight and sucrose and starch contents [20].

Chickpea ranks third globally among legumes in terms of production, behind beans, with average annual production exceeding 11.5 million tonnes [21], most of which is concentrated in India. However, annual production has fluctuated in recent years, with climate change cited as a major contributing factor [22]. Within the European Union, Spain is the leading country for chickpea cultivation, with over 38,200 ha and production exceeding 45 thousand tonnes, as reported by the Spanish Ministry of Agriculture, Fisheries and Food. Despite Spain's prominence, chickpeas are predominantly grown under rainfed conditions, resulting in relatively low yields. Chickpeas are an integral component of the Spanish diet and are used in a wide range of traditional dishes. Spain also maintains substantial varietal diversity, reflected in differences in seed size, colour and flavour, shaped by disparate edaphoclimatic and geographical conditions and the development of locally adapted materials. Most chickpeas cultivated in Spain belong to the kabuli type, characterised by beige or cream-coloured seeds [23]. According to del Moral et al. [24], three traditional cultivars are widely grown in Spain: *Blanco Lechoso* (BL), *Castellano* (CA) and *Pedrosillano* (PE). Andalucía is a focal region for chickpea cultivation, where PE and BL are prominent, whereas Castilla y León and Castilla-La Mancha favour the CA cultivar. From these traditional cultivars (BL, PE and CA), new selections have been developed to improve uniformity, yield and resistance to fungal pathogens. One such example is the cultivar *Garabito* (GA), developed in Salamanca (Castilla y León), which was selected to increase yield and refine key traits of the PE ecotype [25].

Despite the increasing interest in chickpea as a sustainable and health-promoting crop, comparative evidence that integrates morphometric traits with proximate composition, amino acid profile, mineral composition and cooking-related technological properties across traditional Spanish cultivars remains limited and fragmented. In addition, there is limited information on whether local, on-farm selections derived from traditional ecotypes preserve the nutritional and technological profile of the original material while improving agronomic uniformity, and on the extent to which contrasting soil types may modulate key morphometric and nutritional attributes within the same cultivar under comparable cultivation conditions.

Based on prior evidence that genotype is a primary determinant of seed size and seed coat traits, and that genotype $\times$ environment interactions may influence composition, we formulated the following hypotheses: (H1) The cultivars BL, PE and CA can be differentiated by their morphometric and technological profiles using multivariate analysis whereas proximate composition varies only slightly among cultivars. (H2) GA, as a selection derived from PE, shows morphometric improvements while maintaining a nutritional and technological profile similar to PE; and (H3) Under conditions of moderate edaphic contrast, soil type has a limited effect on the nutritional and morphometric traits of the PE cultivar. Accordingly, the objectives of this study were threefold: (1) to provide an integrated characterisation of three traditional Spanish chickpea cultivars (BL, PE and CA) combining morphometric, nutritional and technological traits; (2) to benchmark GA against its source ecotype PE to determine whether selection modified morphometric traits while preserving nutritional and technological quality; and (3) to assess the influence of two contrasting soil types on the morphometric and nutritional traits of PE.

This work aligns with the goal of preserving genetic diversity and promoting sustainable food systems, as articulated in the European 2030 Agenda. Characterising local cultivars is important not only to encourage farmers to maintain their cultivation but also to inform policies that support their production.

Materials and methods

Plant material, growing conditions and edaphoclimatic context

A total of 38 chickpea samples (each consisting of a 2 kg homogenised batch) representing three cultivars were analysed: *Pedrosillano* (PE; desi type; $n=26$, including 4 samples from the GA selected line), *Castellano* (CA; kabuli type; $n=10$) and *Blanco Lechoso* (BL; kabuli type; $n=4$).

All samples were obtained from rainfed crops grown in the province of Salamanca (Spain). The number of samples per cultivar was proportional to the cultivated area of each cultivar within the province.

The cultivation area corresponds to a typical rainfed agricultural landscape in the northern sub-plateau of the Iberian Peninsula, characterised by plains and peneplains. The edaphoclimatic conditions are representative of a continental Mediterranean climate, with scarce and irregular rainfall, prolonged hot summers occasionally interrupted by storms, and long cold winters. Samples were sown in the second week of March 2022 (following a severe winter) and harvested in August 2022. During the growing season, soils were managed under conventional tillage and no pesticides or fertilisers were applied.

Although meteorological conditions are broadly consistent across the chickpea cultivation area, slight differences in soil characteristics were observed. All CA and BL samples, together with half of the PE samples, were cultivated on a slightly alkaline clay-loam soil (pH 7.8; 41° 4' 20.2" N, 5° 38' 26.9" W), classified (USDA-NRCS Soil Taxonomy) as a *Rhodoxeralf*. The remaining PE samples ($n=13$) were grown on a slightly acidic sandy-loam soil (pH 6.5; 40° 58' 11.6" N, 5° 9' 20.6" W), classified as a *Xerochrept*. The edaphic and meteorological conditions during the study period are summarised in Table 1.

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

Morphometric and physical seed characteristics

Colour, colour intensity, shape and ribbing were assessed on 70 seeds per sample following the International Union for the Protection of New Varieties of Plants descriptor guidelines (UPOV TG/143/5) [26]. Seed weight was determined in triplicate by weighing 100 dried seeds.

Seed volume, eccentricity coefficient and surface area were calculated using the methodology and equations described by Chenoll et al. [27]. Volume (mm^3) and surface area (mm^2) were calculated assuming an ellipsoidal shape. The eccentricity coefficient quantifies the similarity of an ellipsoid to a sphere, with a value of 0 indicating perfect spherical symmetry. Measurements were taken on 70 seeds per sample using a digital calliper (Atrium Enterprises GmbH, Lünen, Germany).

Proximate composition

Moisture content was determined according to the gravimetric method AOAC 925.10 [28] 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 [29] by incineration at 550 ± 10 °C in a Thermolyne muffle furnace (Thermo Scientific, Madrid, Spain). Protein content was measured by the Kjeldahl method (AOAC 950.36) [30]. Total lipid was determined by Soxhlet extraction (AOAC 935.38) [31] using petroleum ether. Starch content was determined enzymatically (AOAC 996.11) [32], and total dietary fibre was measured according to AOAC 991.43 [33] using an ANKOM analyser (ANKOM Technology, Macedon, NY, USA). Total carbohydrates were calculated by difference as:

$$\text{Carbohydrates} = 100 - (\text{Ash} + \text{Protein} + \text{Total Fat} + \text{Fibre} + \text{Moisture})$$

Total sugars were determined by volumetric analysis using the Luff–Schoorl method following acid inversion with hydrochloric acid, and results were expressed as glucose equivalents. All determinations were carried out in triplicate and results are reported as g/100 g fresh weight (fw).

Soluble solids were estimated from the refractive index (°Brix) measured with a portable refractometer (RSD500,

Table 1 Edaphic and meteorological conditions for the 2022 growing season (March–August) and long-term (30-year) averages in the cultivation area in the central plateau of the Iberian Peninsula

Parameter	Growing season		Long term (1992–2022)	
	1 March 2022–31 August 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	
	Clay Loam Soil		Sandy Loam Soil	
	(41° 4' 20.2"N, -5° 38' 26.9"W)		(40° 58' 11.6"N, -5° 9' 20.6"W)	
pH	7.8		6.5	
Organic Matter (%)	1.4		1.2	
N (%)	0.12		0.10	
P (ppm)	27.3		23	
K (ppm)	490		125	
Ca (ppm)	3700		1200	
Mg (ppm)	703		350	

Data were 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.)

0–85% °Brix, 0.1% resolution; Labbox, Barcelona, Spain) in an aqueous extract prepared by mixing 1 g of chickpea with 5 mL of distilled water. The mixture was stirred and allowed to stand for 5 min, then centrifuged at 10,000 rpm; °Brix was measured in the supernatant.

Amino acid analysis

Amino acids were determined by liquid chromatography using a Biochrom amino acid analyser (Pharmacia, UK), following the method described in Commission Regulation (EC) No 152/2009 [34]. Cysteine and methionine were quantified after oxidation with performic acid/phenol, and were measured as cysteic acid and methionine sulphone, respectively; tyrosine was determined in the non-oxidised hydrolysate. Oxidised and non-oxidised samples were hydrolysed with hydrochloric acid for 23 h (pH 2.20). Total tryptophan was determined after alkaline hydrolysis with saturated barium hydroxide and heating at 110 °C for 20 h. Separation was performed on a 200 mm × 4.6 mm column (Pharmacia Biotech). Photometric detection was carried out at 570 nm for all amino acids except proline (440 nm). Tryptophan was quantified using spectrofluorometric detection (excitation 280 nm; emission 356 nm). Results are reported as g/100 g fw. Cysteic acid was converted to cysteine using a molecular weight of 120.15 g/mol, and methionine sulphone was converted to methionine using a molecular weight of 149.21 g/mol.

The amino acid score (AAS) was calculated as [mg of amino acid per g of test protein / mg of amino acid in the reference requirement pattern] × 100 [35]. FAO/WHO recommendations [36] were used as the reference pattern for three age groups: infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents and adults. The first limiting amino acid was defined as the amino acid with the lowest AAS.

Mineral analyses

Mineral content was determined by ICP-MS following Absi et al. [37], with minor adaptations. Briefly, 0.200 g of milled sample was subjected to closed-vessel microwave digestion with HNO₃ (Milestone system). After digestion, extracts were made up to volume with ultrapure water and analysed using an Agilent 7800 ICP mass spectrometer (Agilent, Santa Clara, CA, USA). Quantification was performed using multi-element certified standards (1 g/L; Pan-reac, Spain). Reagent blanks were included. Analyses were performed in duplicate and mineral contents are reported as mg/kg fresh weight.

Soaking behaviour

Soaking behaviour was assessed according to Bidkhor and Mohammadpour [38], using a seed: water ratio of 1:30 (w/w). Whole seeds (6.0 g) were soaked in tap water at 25 °C for 10 h in covered containers. Seeds were then drained and gently blotted with paper towel for 30 s, and soaked weight was recorded. Water absorption (%) was calculated as $[(W_1 - W_0)/W_0] \times 100$, where W_0 is the initial seed weight and W_1 is the soaked seed weight.

Cooking behaviour

Chickpeas (without prior soaking) were boiled for 2.5 h at 100 °C. This cooking time was selected based on recommendations from the Spanish Agency for Food Safety and Nutrition (AECOSAN) [39]. It was decided to study the cooking process separately in order to evaluate the influence of soaking and cooking independently. After cooking, seeds were blotted with paper towel and cooled to 25 °C. Cooking water uptake was determined from the weight gain after cooking, and the number of detached seed coats was counted. Soluble solids remaining after cooking were estimated as °Brix in an aqueous extract prepared by homogenising 1.000 ± 0.002 g of cooked chickpea with 5 mL of distilled water in a Falcon® tube using an Ultra-Turrax (IKA Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany). After centrifugation (Sigma 4K15, Osterode am Harz, Germany) for 5 min at 10,000 rpm, °Brix was measured in the supernatant using a portable refractometer (ZUZI 300; Barcelona, Spain).

Texture of cooked chickpeas was measured using a TA-XT2i texture analyser (Texture Technologies Corp., Stable Micro Systems, Godalming, UK). A penetration test based on Yildirim et al. [40] with minor modifications, was used under the following conditions: pre-test speed 2 mm s⁻¹, test speed 1 mm s⁻¹, post-test speed 2 mm s⁻¹, and penetration distance 4 mm. Measurements were performed at 25 °C using a 2 mm diameter probe and a 5 kg load cell, with 10 replicates per sample.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 27 (IBM, Armonk, NY, USA). For each of the three objectives of this study -characterisation of the three Spanish chickpea cultivars, comparison of PE with the GA selected line, and evaluation of soil-type effects on PE- differences among groups were assessed using one-way analysis of variance (ANOVA). It was selected as a standard approach and is generally considered reasonably robust in unbalanced designs when deviations from assumptions are not severe. Prior to ANOVA, assumptions were evaluated

Table 2 Morphometric parameters and seed weight (mean $\pm$ SD) of the Spanish chickpea cultivars analysed

Seed parameters	Chickpea variety			Significance p -level
	PE *	CA *	BL *	
Colour**	Yellow (2.1)	Greyed brown (2.9)	Whitish (1.2)	<0.001
Intensity of colour	Medium	Light	Medium	–
Shape**	Round (1.1)	Round to angular (1.9)	Angular (3.0)	<0.001
Ribbing**	Weak (3.2)	Medium (5.1)	Strong (7.0)	<0.001
Seed weight (g)***	29.80 $\pm$ 0.52 ^c	47.00 $\pm$ 1.01 ^b	58.89 $\pm$ 1.56 ^a	<0.001
Volume (mm ³)	268.28 $\pm$ 3.83 ^c	403.31 $\pm$ 11.70 ^b	522.37 $\pm$ 3.11 ^a	<0.001
Eccentricity coefficient	0.57 $\pm$ 0.01 ^b	0.64 $\pm$ 0.01 ^a	0.63 $\pm$ 0.01 ^{ab}	<0.001
Surface (mm ²)	715.90 $\pm$ 64.91 ^c	951.26 $\pm$ 109.39 ^b	1154.89 $\pm$ 91.37 ^a	<0.001

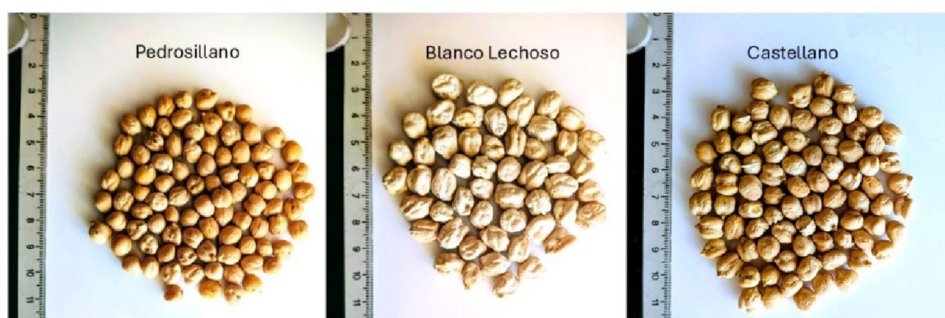
Within a row, means with different superscript letters differ significantly ($p < 0.05$). No superscript letters are shown when no significant differences were found ($p \geq 0.05$)

*PE Pedrosillano, CA Castellano, BL Blanco lechoso

** Based on the methodology of the International Union for the Protection of New Varieties of Plants (UPOV), Significance according to χ^2 test

*** Weight of 100 seeds determined in triplicate

Fig. 1 Seed morphology of the Spanish chickpea cultivars Pedrosillano (small, yellow, round), Blanco Lechoso (large, whitish, angular), and Castellano (medium-sized, greyed-brown, round to angular)



using standard diagnostics, including inspection of residual Q-Q plots and Shapiro-Wilk tests for residual normality, and Levene's test for homogeneity of variances. As these checks did not indicate major violations affecting interpretation, one-way ANOVA was retained as the primary approach; for brevity, diagnostic outputs are not reported. Means and standard errors of the mean (SEM) were calculated for all variables. Statistical significance was set at $\alpha = 0.05$, using Snedecor's F as the test statistic. Where appropriate, homogeneous subsets were identified using Tukey's honestly significant difference (HSD) post hoc test [41].

Associations among categorical variables (colour, colour intensity, shape and ribbing) were tested using the chi-squared (χ^2) test, assuming equal expected frequencies, with significance assessed at $\alpha = 0.05$. To visualise potential separation between PE and GA samples, a discriminant coordinate analysis based on Mahalanobis distances was performed [42]. Different sample sizes per cultivar were used for the PE-GA comparison and for the soil-type assessment. In the province of Salamanca, the distribution of sampled cultivars reflected their relative cultivated area. Given this unequal sample sizes, statistical power to detect small differences involving BL is reduced; therefore, non-significant results were interpreted conservatively, especially for comparisons including BL.

Results

Characterization of the three main Spanish chickpea cultivars

Physical and morphometric parameters

The main qualitative and quantitative physical and morphometric characteristics of the Spanish chickpea cultivars studied are shown in Table 2; Fig. 1.

Significant differences ($p < 0.05$) were observed among cultivars for all measured variables. The cultivars differed in colour and colour intensity. PE showed the smallest seed size, with lower 100-seed weight (29.8 g), surface area (715.90 mm²) and volume (268.28 mm³), whereas BL had the largest seeds (volume 522.37 mm³), the most angular shape and the strongest ribbing. CA showed the highest eccentricity coefficient (0.64).

Proximate composition

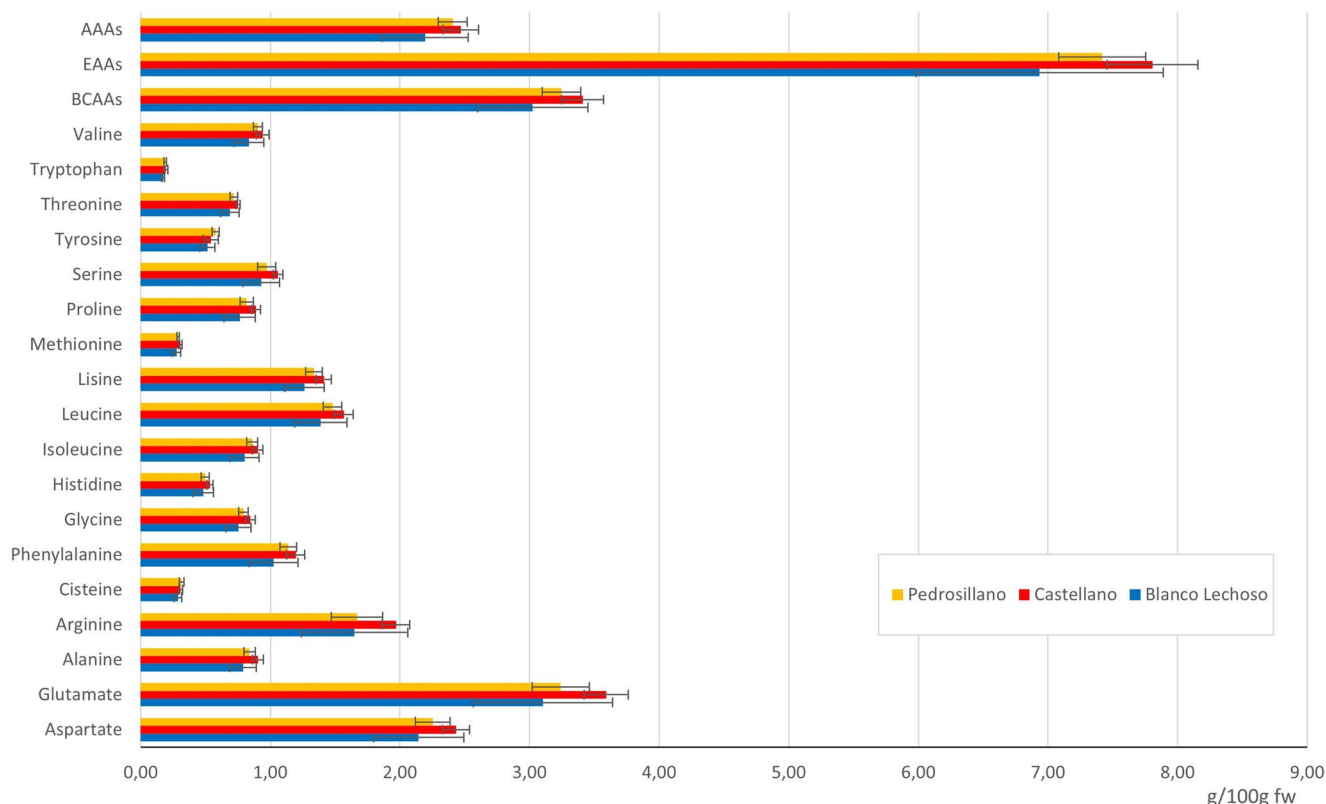
The proximate composition of the three Spanish chickpea cultivars is presented in Table 3. As expected, few significant differences were observed among cultivars, with the clearest differences occurring for total lipid and total sugars.

Table 3 Proximate composition (mean±SD) of the Spanish chickpea cultivars analysed, expressed on a fresh-weight basis

Within a row, means with different superscript letters differ significantly ($p < 0.05$). No superscript letters are shown when no significant differences were found ($p \geq 0.05$)

*PE Pedrosillano, CA Castellano, BL Blanco lechoso

Seed parameters	Chickpea variety			Significance p -level
	PE*	CA*	BL*	
Moisture (g/100 g)	10.40±1.61	8.62±0.26	8.08±0.97	0.742
Ash (g/100 g)	2.96±0.04	2.85±0.03	3.09±0.09	0.095
Protein (g/100 g)	20.27±0.36	20.41±0.56	18.80±1.10	0.519
Lipid (g/100 g)	5.91±0.09 ^b	6.24±0.13 ^{ab}	6.73±0.40 ^a	0.016
Starch (g/100 g)	45.21±0.50	44.94±0.40	46.65±2.05	0.631
Fibre (g/100 g)	23.68±0.42	22.92±0.85	20.00±0.20	0.089
Total sugar (g/100 g)	3.80±0.07 ^c	4.67±0.10 ^b	5.29±0.10 ^a	<0.001
Carbohydrates (g/100 g)	39.49±1.67	38.10±2.68	39.66±3.39	0.281
Soluble Solids (°Brix)	6.55±0.14	7.19±0.24	7.13±0.68	0.061

**Fig. 2** Average content in individual amino acids, essential amino acids (EAAs) and branched-chain amino acids (BCAAs) of the different chickpea cultivars tested (Pedrosillano, Castellano and Blanco Lechoso)

BL showed the highest lipid and total sugar contents (6.73 and 5.29 g/100 g fw, respectively), whereas PE showed the lowest values for both parameters. Despite being the smallest-seeded cultivar, PE did not differ significantly from the other cultivars in protein (20.27 g/100 g fw), carbohydrates (71.61 g/100 g fw) or fibre (23.68 g/100 g fw). This pattern may be related to its lower lipid content (5.91 g/100 g fw).

Amino acid profile

Figure 2 shows the amino acid profile (individual amino acids, essential amino acids (EAAs) and branched-chain amino acids (BCAAs)) of the three Spanish

chickpea cultivars. No statistically significant differences were observed for any amino acid among cultivars. All cultivars exhibited high levels of EAAs. Glutamate was the most abundant amino acid across cultivars (3.1–3.6 g/100 g fw), followed by aspartate (2.1–2.4 g/100 g fw). In contrast, tryptophan showed the lowest concentrations, together with the sulphur-containing amino acids cysteine and methionine. The leucine: isoleucine ratio was 1.7:1 in all cultivars, within the FAO/WHO recommended range [43].

Table 4 Mineral element concentration (mean $\pm$ SD) of the Spanish chickpea varieties analysed

Mineral element	Chickpea variety			Significance <i>p</i> -level
	PE *	CA *	BL *	
Na (g/kg fw)	3.56 $\pm$ 0.12	3.26 $\pm$ 0.12	3.59 $\pm$ 0.27	0.350
Mg (g/kg fw)	1.28 $\pm$ 0.01	1.29 $\pm$ 0.03	1.35 $\pm$ 0.02	0.359
P (g/kg fw)	3.38 $\pm$ 0.05	3.63 $\pm$ 0.11	3.64 $\pm$ 0.05	0.053
K (g/kg fw)	10.07 $\pm$ 0.08 ^b	10.47 $\pm$ 0.17 ^b	11.80 $\pm$ 0.25 ^a	<0.001
Ca (g/kg fw)	1.38 $\pm$ 0.05 ^a	1.11 $\pm$ 0.07 ^b	1.36 $\pm$ 0.13 ^a	0.018
Fe (mg/kg fw)	74.50 $\pm$ 4.23	74.30 $\pm$ 5.96	80.46 $\pm$ 2.29	0.927

Within a row, means with different superscript letters differ significantly ($p < 0.05$). No superscript letters are shown when no significant differences were found ($p \geq 0.05$)

*PE *Pedrosillano*, CA *Castellano*, BL *Blanco lechoso*

Table 5 Mean values ($\pm$ SD) of soaked and cooked chickpeas

	Chickpea variety			Significance <i>p</i> -level
	PE*	CA*	BL*	
Soaking				
Water absorption (%)	210.61 $\pm$ 2.23	206.12 $\pm$ 2.24	214.73 $\pm$ 5.94	0.413
Cooking				
Water absorption (%)	112.08 $\pm$ 0.05 ^{ab}	79.24 $\pm$ 0.07 ^b	137.85 $\pm$ 0.10 ^a	0.001
Hardness of seed (N)	696.12 $\pm$ 14.45 ^{ab}	836.06 $\pm$ 28.42 ^a	679.96 $\pm$ 36.48 ^b	<0.001
Peeled skin (%)	0.04	0.00	0.00	0.803
Soluble solids (°Brix)	0.89 $\pm$ 0.02 ^b	1.01 $\pm$ 0.06 ^{ab}	1.10 $\pm$ 0.10 ^a	0.022

*PE *Pedrosillano*, CA *Castellano*, BL *Blanco lechoso*

Mineral content

Mineral contents are reported in Table 4. Blanco Lechoso showed significantly higher K content (11.80 g/kg) content than PE and CA ($p < 0.001$). Calcium content was significantly higher in PE (1.38 g/kg) and BL (1.36 g/kg) than in CA ($p = 0.018$), which is related to its low ash content. No significant differences were detected for Na, Mg, P or Fe.

Soaking and cooking behaviour

The responses of the three cultivars to soaking and cooking are shown in Table 5. Water absorption during soaking did not differ significantly among cultivars ($p \geq 0.05$), with all absorbing $> 200\%$. In contrast, significant differences were observed after cooking ($p = 0.001$): CA absorbed the least water (79.24%), whereas BL absorbed the most (137.85%). Cooking also resulted in significant differences in seed hardness ($p < 0.001$), with CA exhibiting the greatest hardness and BL the lowest (836 N and 680 N respectively); this pattern mirrored the inverse relationship with post-cooking water uptake. Soluble solids (°Brix) also differed significantly among cultivars after cooking ($p = 0.022$), with BL showing higher values than PE (1.1 °Brix vs. 0.89 °Brix).

Comparison of GA selected line with the PE cultivar

Table 6 summarises the physical and morphometric traits, together with proximate and mineral composition, of the GA selected line and its parental cultivar *Pedrosillano* (both

Desi type), compared with the Kabuli cultivars included in this study (BL and CA). GA samples were analysed separately from the remaining PE samples.

The comparison between the GA selected line and its parental PE cultivar showed an almost identical physical and morphometric profile. No significant differences were observed in 100-seed weight (both 29.8 g), volume (215 and 212 mm³, respectively), surface area or eccentricity coefficient, indicating that selection preserved the characteristic small size and shape of the original PE ecotype. Likewise, qualitative descriptors such as colour, shape and ribbing were unchanged, supporting the conclusion that GA maintains the traditional morphological identity associated with *Pedrosillano*-type chickpeas.

Similarly, proximate composition did not differ significantly between GA and PE. Both showed comparable levels of protein (20.8–20.2 g/100 g), lipid (5.96–5.90 g/100 g), fibre (22.63–23.87 g/100 g), starch (45.69–45.12 g/100 g) and total sugars (3.72–3.81 g/100 g). These results indicate that compositional attributes were not altered during the selection process and that GA retains the biochemical profile typical of the PE cultivar.

Discriminant analysis including GA, PE and the pooled Kabuli cultivars (BL+CA) yielded two discriminant functions, jointly explaining 90.4% of the total variance (78.3% for Function 1 and 12.1% for Function 2). Figure 3 shows that separation among PE/GA and the other Spanish cultivars is driven primarily by morphometric traits rather than by compositional variables. In particular, seed weight, volume and surface area showed the strongest correlations with

Table 6 Physical, morphometric, proximate (fw) and mineral composition (fw) parameters of the Garabito selected line and its parental Pedrosillano cultivar

Seed parameters	PE*	GA*	Other varieties (BL+CA)	Significance
Physical and morphometric parameters				
Colour**	Yellow (2.2)	Yellow (2.1)	Greyed brown (2.9)	0.101
Intensity of colour	Dark	Medium	Medium	–
Shape**	Round (1.0)	Round (1.2)	Round to angular (2.2)	0.002
Ribbing**	Weak (2.9)	Weak (3.2)	Medium (4.9)	<0.001
Seed weight (g)***	29.80±2.66 ^b	29.78±3.04 ^b	48.98±5.50 ^a	<0.001
Volume (mm ³)	215.02±21.00 ^b	212.45±9.21 ^b	338.52±50.00 ^a	<0.001
Eccentricity coefficient	0.58±0.04 ^b	0.55±0.03 ^b	0.64±0.04 ^a	<0.001
Surface (mm ²)	714.40±50.69 ^b	724.18±43.55 ^b	985.20±112.94 ^a	<0.001
Proximate composition				
Moisture (g/100 g)	8.89±1.12	8.29±1.21	8.53±0.87	0.599
Ash (g/100 g)	2.96±0.19	2.85±0.13	2.91±0.16	0.436
Protein (g/100 g)	20.17±1.88	20.78±1.84	20.14±1.79	0.821
Lipid (g/100 g)	5.90±0.47 ^b	5.96±0.18 ^b	6.32±0.44 ^a	0.043
Starch (g/100 g)	45.12±2.73	45.69±0.85	45.23±1.58	0.902
Fibre (g/100 g)	23.87±2.22	22.63±1.62	22.43±2.69	0.208
Total Sugar (g/100 g)	3.81±0.38 ^b	3.72±0.29 ^b	4.78±0.38 ^a	<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±0.24 ^{ab}	3.19±0.13 ^b	3.63±0.31 ^a	0.011
K (g/kg)	10.13±0.39 ^{ab}	9.76±0.40 ^b	10.69±0.72 ^a	0.003
Ca (g/kg)	1.40±0.24	1.28±0.32	1.16±0.22	0.059
Fe (mg/kg)	77.14±22.35	59.99±6.44	75.33±19.79	0.321

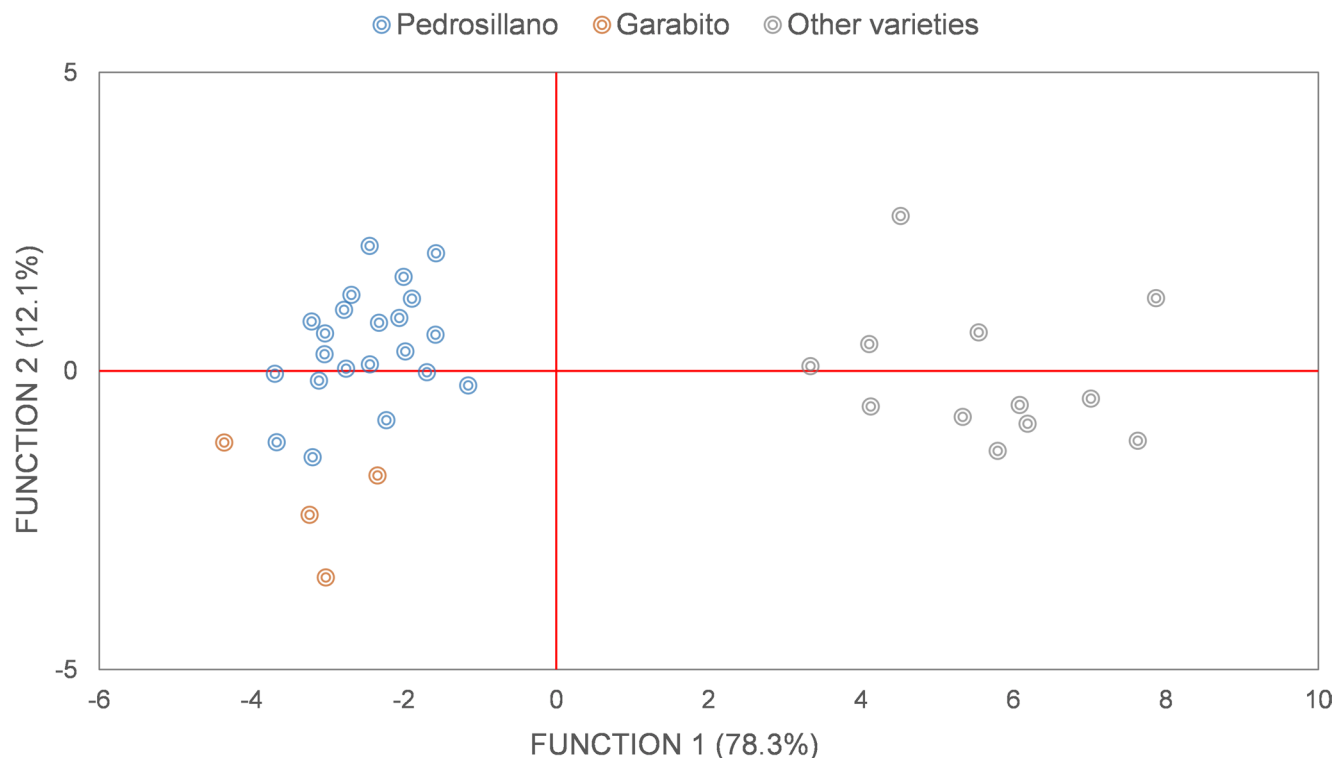
Within a row, means with different superscript letters differ significantly ($p<0.05$). No superscript letters are shown when no significant differences were found ($p\geq0.05$)

For categorical data significance level is according to χ^2 test

*PE Pedrosillano, GA Garabito, Other varieties include Castellano and Blanco Lechoso

**Based on the methodology of the International Union for the Protection of New Varieties of Plants (UPOV), Significance according to χ^2 test

***Weight of 100 seeds determined in triplicate

**Fig. 3** Scatter plot showing discriminant coordinates based on the compositional and physicochemical characteristics of Pedrosillano, the Garabito selected line and the other cultivars included in the study

(Castellano and Blanco Lechoso). Percentages in parentheses indicate the variance explained by each discriminant function

Table 7 Means and standard deviations for physical, morphometric, proximate (fw) and mineral (fw) composition parameters of the Pedrosillano cultivar grown on different soil types

	Clay-loam soil (pH 7.8)	Sandy-loam soil (pH 6.5)	Significance
Physical and morphometric parameters			
Colour*	Yellow (2.1)	Yellow (2.0)	0.973
Intensity of colour	Medium	Medium	–
Shape*	Round (1.1)	Round (1.1)	0.059
Ribbing*	Weak (3.1)	Weak (3.2)	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/100 g)	8.46±0.31	11.82±2.76	0.331
Ash (g/100 g)	2.98±0.06	2.94±0.05	0.577
Protein (g/100 g)	20.35±0.53	20.20±0.51	0.838
Lipid (g/100 g)	5.96±0.08	5.88±0.14	0.662
Starch (g/100 g)	45.96±0.75	44.66±0.65	0.203
Fibre (g/100 g)	22.92±0.43	24.23±0.64	0.127
Total Sugar (g/100 g)	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

*Based on the methodology of the International Union for the Protection of New Varieties of Plants (UPOV), Significance according to χ^2 test

**Weight of 100 seeds determined in triplicate

Function 1 (Table S1), indicating that size-related variables account for most of the variability (78.3%). The close clustering of GA and PE for these parameters suggests that selection preserved the small-seeded phenotype of PE while improving homogeneity, as reflected by the generally lower standard deviations observed for GA than for PE in Table 6.

Function 2 explained a smaller proportion of the variance (12.1%) and was more strongly associated with mineral variables -particularly Fe, P, K and Ca- and with the eccentricity coefficient (Table S1). These variables contributed primarily to differentiating the pooled Kabuli cultivars (BL and CA) from PE and GA (desi type), rather than separating GA from PE. The limited contribution of proximate composition variables (e.g., protein, lipid and starch) to both functions further supports the overall compositional similarity between GA and its parental cultivar, and the relatively small divergence in proximate composition between the Desi and Kabuli materials analysed.

Influence of soil type on the characteristics of the PE cultivar

Results for physical and morphometric traits, together with proximate and mineral composition, of the *Pedrosillano* cultivar grown on two contrasting soil types are presented in Table 7.

The comparison of PE samples grown on slightly alkaline clay-loam soil (pH 7.8) and slightly acidic sandy-loam soil (pH 6.5) showed no statistically significant differences for any measured morphometric, proximate or mineral variable (Table 7). Morphometric traits, including 100-seed weight, volume, surface area and eccentricity coefficient, differed only marginally between soil types, suggesting that seed development in this cultivar is largely genotype-driven and not strongly modulated by soil texture or pH under the conditions studied. Similarly, proximate composition variables (protein, lipid, fibre, starch and sugars) showed only minor, non-significant variation between soils, indicating that macronutrient accumulation in PE is robust across these edaphic environments. Mineral composition also remained statistically unchanged between soils.

Discussion

The present study provides an integrated characterisation of three main traditional Spanish chickpea cultivars (*Pedrosillano*, *Castellano* and *Blanco Lechoso*), together with an evaluation of the *Garabito* selected line derived from *Pedrosillano*, and an assessment of soil-type effects on PE seeds. Overall, the findings show that seed morphology is the principal discriminant attribute among cultivars,

whereas nutritional composition displays comparatively limited variability, consistent with previous reports of a relatively narrow compositional range within chickpea types [7, 8, 44]. In this context, the preservation of morphologically and compositionally distinct local chickpea materials may contribute to the maintenance of regional food heritage and crop diversity. Moreover, the diversification of cropping systems is increasingly considered important for improving the resilience and sustainability of Mediterranean agriculture under climate-change scenarios [4, 22].

The three Spanish cultivars showed pronounced differences in physical and morphometric traits: BL displayed the largest seed size and the strongest ribbing, CA was intermediate, and PE was characterised by the smallest and most spherical seeds. These observations are consistent with previous typification of Spanish germplasm, in which PE types are described as small-seeded ecotypes [25], broadly concordant with the Desi type. *Pedrosillano* is typically described as small-seeded, wrinkled and darker-coloured, whereas BL and CA are Kabuli cultivars (larger, smoother-coated, and white to cream-coloured) [45]. In the present study, CA showed a greyish-brown colouration and an intermediate size relative to PE and BL. According to Brun et al. [44], BL can be considered a second subgroup within kabuli seeds, characterised by a particularly large seed size and weight (> 50 g), consistent with macrocarpa kabuli-type descriptors. As seed size and external morphology are primary descriptors in chickpea classification [26], the clear differentiation observed here supports the genetic distinctiveness of these cultivars and reinforces the value of conserving local germplasm. This is expected because size- and shape-related traits are strongly genotype-controlled, whereas proximate composition in chickpea typically shows a narrower range and is more sensitive to year-to-year and environmental variation, thereby reducing its discriminatory power.

Consistent with this, seed weight, volume and surface area had the greatest influence on cultivar characterisation, clearly separating PE (smaller seeds) from CA and BL. These results highlight the robustness of morphometric traits and agree with studies in which size-related parameters outperform chemical composition in discriminating chickpea cultivars [27, 44–46].

Although proximate composition showed fewer significant differences among cultivars, subtle patterns were evident. BL exhibited the highest lipid and total sugar contents, whereas PE showed the lowest values for both. Similar variability in lipid and sugar profiles among cultivars has been reported elsewhere [16], suggesting a genetic basis for these traits.

Notably, despite its smaller seed size, PE showed comparatively high protein, carbohydrates and fibre, in some

cases exceeding BL. Aguilera et al. [1] reported that chickpeas display among the highest total dietary fibre contents (18–22 g/100 g) of pulses and that Kabuli chickpeas tend to have lower total and insoluble fibre contents than Desi types, potentially due to differences in hull and seed coat characteristics. In addition, because lipid content was lowest in PE, reciprocal increases in carbohydrates and fibre are consistent with nutrient partitioning trends reported for chickpea seeds [6]. Collectively, these attributes support the functional value of PE as a nutrient-dense cultivar. Such inverse trends may reflect compositional trade-offs during seed filling, whereby increases in one storage fraction occur at the expense of others, and differences in seed coat proportion that can influence apparent fibre levels across morphotypes.

The amino acid profile showed no statistically significant differences among cultivars for any of the amino acids analysed. This agrees with previously reported amino acid patterns in chickpeas [8, 44] and reinforces the view that chickpea provides protein of high nutritional quality. Sots et al. [47] reported that desi chickpeas may be enriched relative to kabuli chickpeas in several essential amino acids (EAAs), including lysine, isoleucine, valine, leucine and phenylalanine. In the present study, EAA contents did not differ significantly among cultivars (Fig. 2), although variability was observed among PE samples. Similarly, Wang et al. [48] and Ipekesen et al. [49] reported no significant differences in essential amino acid contents between desi and kabuli chickpeas. Based on the FAO/WHO (2011) amino acid scoring pattern [36], tryptophan was the limiting amino acid for young children (6 months to 3 years), in 10% of samples, whereas valine was limiting in the remaining 90%. For older children, adolescents and adults, valine was the limiting amino acid in all samples. These findings are consistent with results reported for other legumes, highlighting the importance of selecting a specific amino acid reference standard in order to assess the protein quality of plant proteins for inclusion in a properly balanced diet [35].

Mineral composition showed some cultivar-specific variation. In particular, BL had significantly higher K content than PE and CA ($p < 0.001$), while Ca content was higher in PE and BL than in CA ($p = 0.018$) (Table 4). From a nutritional perspective, this variability is of interest given the recognised roles of potassium in electrolyte homeostasis and neuromuscular function and of calcium in skeletal structure and cellular signalling. Although previous studies [49] have suggested that desi chickpeas may contain higher Ca concentrations, our results show that BL (kabuli type) also contained high Ca values when expressed on a wet-weight basis (Table 4), likely influenced by differences in moisture content. The lower Ca content observed in CA may reflect cultivar-specific differences in mineral uptake

and partitioning. Overall, these findings support genotype-dependent variability in mineral accumulation [12, 47, 50]. Differences in K and Ca among cultivars may reflect genotype-specific uptake and partitioning, as well as contrasting binding of Ca to cell-wall components and seed coat fractions, which may vary with morphotype and tissue proportions.

Soaking behaviour was similar among cultivars; however, significant differences emerged during cooking. BL absorbed the most water and produced the softest, cooked seeds, whereas CA remained the hardest. These patterns are consistent with previous reports linking larger seed size to greater hydration capacity and softer cooked texture [38, 45, 51, 52]. PE showed intermediate cooking behaviour, consistent with its smaller size and lower water uptake. These results have culinary relevance, as BL may be preferred in contexts requiring faster softening, whereas CA may better resist disintegration during long-cooked traditional dishes. Significant differences in soluble solids were observed after cooking: PE showed the lowest value, corresponding to losses of > 86% of soluble solids during cooking, whereas BL showed slightly lower losses (84.5%). As soluble losses during cooking may include water-soluble vitamins and minerals [53], these results emphasise the potential for nutrient losses when chickpeas are consumed after discarding the cooking water.

With respect to the GA selected line, comparison with its parental PE ecotype indicates that selection preserved the morphometric profile characteristic of PE, including small seed size, weak ribbing and similar eccentricity. Proximate and mineral composition also did not differ significantly between GA and PE. This stability aligns with breeding objectives aimed at improving agronomic performance while maintaining traditional quality traits [25]. Overall, GA appears compositionally and morphologically equivalent to PE, suggesting that agronomic improvements such as yield (as indicated by growers) were achieved without altering key cultivar characteristics. The preservation of nutritional traits despite selection suggests that GA may improve uniformity and production consistency without compromising the compositional identity of PE. This is further supported by the generally lower standard deviations for several parameters observed for GA relative to PE, as also highlighted in breeding studies [54].

Discriminant analysis confirmed the close affinity of GA and PE, which clustered together, and their separation from CA and BL was driven by the same morphometric variables described above.

Regarding soil type, PE grown on two distinct soils (slightly alkaline clay-loam and slightly acidic sandy-loam) showed no significant differences in any morphometric, compositional or mineral variable. This suggests a high

degree of environmental stability of the PE genotype under rainfed Mediterranean conditions (158 mm precipitation during the cropping cycle; mean temperature 16.3 °C; Table 1). Under similar climatic conditions and comparable crop management (conventional tillage), the quality attributes of *Pedrosillano* chickpeas appear to be preserved. Although soil type can modulate nutrient uptake and seed composition in many legumes [19, 50], the lack of differences here indicates that PE maintains its compositional integrity across contrasting edaphic environments, provided that these soils, in this study, supply adequate nutrients, as was the case in this study. Because mineral uptake is often sensitive to soil pH, cation exchange capacity and texture, the absence of soil effects may reflect an efficient nutrient acquisition strategy in the PE genotype. Although this statement has been obtained based on a single season and location and should be corroborated over several growing seasons, this finding could be relevant for expanding the production area eligible for the “*Garbanzo Pedrosillano*” quality label in the province of Salamanca, which is currently restricted to the *Armuña* area and could potentially be extended to other parts of the province. 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 programmes [4, 55].

Conclusions

This study provides a comprehensive compositional characterization of three traditional Spanish chickpea cultivars (*Pedrosillano*, *Castellano*, and *Blanco Lechoso*), as well as an evaluation of the *Garabito* selected line and the potential influence of soil type on the *Pedrosillano* cultivar. Seed morphology was the main trait differentiating cultivars, whereas proximate composition and most mineral contents showed limited variation. *Blanco Lechoso* presented the largest seeds, higher hydration capacity, and greater softening after cooking, while *Castellano* retained higher hardness after cooking. Despite its smaller seed size, *Pedrosillano* showed a nutritionally relevant profile with relatively higher protein, fibre, and carbohydrate contents.

The *Garabito* line was closely similar to its parental *Pedrosillano* ecotype in both morphological and compositional traits, indicating that selection improved uniformity while preserving the original characteristics of the traditional cultivar.

Overall, the results highlight the value of traditional chickpea germplasm as a source of compositional and technological diversity with potential applications in sustainable production and breeding programs. Further

multi-environment studies are needed to confirm the stability of these traits and their relationship with sensory and cooking properties.

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Data availability The raw data supporting the conclusions of this article will be made available by the authors on request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Not applicable.

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